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SURVEY OF THE WORLD SORGHUM COLLECTION FOR OPAQUE AND SUGARY LINES

Rameshwar Singh and John D. Axtell

REVIEW OF LITERATURE

Genetic Control of Protein Quality and Quantity

Published information concerning genetic improvement of protein quality and quantity is not available for sorghum to date. However, a high lysine variety (160-Cernum) was reported by Virupaksha and Sastry (1968) which showed lysine values of 2.13 and 3.14% of protein in two determinations at 17.68% protein level. No genetic information concerning the inheritance of increased lysine content of this variety is available and confirmation of its lysine and protein content has not been reported.

Swaminathan, Naik, Kaul and Austin (1969, 1970), using the Osborn-Mendel technique of protein fractionation, have shown that the prolamine (alcohol-soluble) fraction of sorghum protein was extremely poor in lysine, tryptophan, histidine and arginine and rich in glutamic acid and leucine. The albumin, globulin and glutelin fractions were higher in lysine, which is the first limiting amino acid for nutritional quality in sorghum. On the basis of these results they suggested that, similar to corn, protein quality of grain sorghum can be improved by developing new varieties low in prolamine content and high in the albumin, globulin and glutelin protein fractions. Jambunathan and Mertz (1972) also reached similar conclusions. They fractionated endosperm and whole kernel proteins of

four sorghum varieties by using the procedure of Landry and Moureaux (1970). Fractions II and III were very poor in quality of protein, while fraction I was of good quality. Fractions IV and V were slightly inferior in quality of protein than fraction I. Also, they noted that the tryptophan content of the whole kernel of all four samples was more than 1%, which indicates that sorghum grain may not be deficient in tryptophan.

Collins and Pickett (1972) reported a range of protein from 9.2 to 18.2% and lysine from 1.74 to 2.74 g/100 g protein in a nine line diallel study. They found general and specific combining ability (GCA and SCA) important for both characters. Additive gene action was more important for protein and less important for lysine. No positive heterosis was observed for lysine. They noted a negative correlation between protein and lysine (expressed as percent of protein).

Opaque-2 was the first mutant gene found to change the normal amino acid composition of maize endosperm (Mertz, Bates and Nelson, 1964). An increase in lysine content of opaque-2 kernels was caused by a reduction in alcohol soluble protein or zein, along with an increase in albumin, globulin and glutelin fractions. The opaque-2 gene, which is inherited as a simple recessive, affects only the amino acid composition of endosperm with no effect on the embryo (Nelson, 1969).

A second mutant gene affecting lysine content (floury-2 ($\underline{fl}_2$)) was reported in 1965 (Nelson, Mertz and Bates, 1965). Unlike opaque-2 it has a higher methionine content than normal corn. Both $\underline{o}_2$ and $\underline{fl}_2$ have nearly double the normal content of tryptophan. Lysine and tryptophan are the co-limiting amino acids in normal corn in the nutrition of monogastric animals, including man (Nelson, 1970).

The genetic restrictions on changes in protein composition and the genetic control of protein structure has been discussed by Nelson (1969, 1970).

The reduction in zein content of opaque-2 seed is accompanied by some variations from normal in the relative amounts of the various electophoretic components according to Mosse (1966), while Jimenez (1968) found some components completely absent. Floury-2 produced several proteins with altered electrophoretic mobilities. Based on these results, Nelson (1969) suggested that $\underline{o}_2$ may be a regulator gene for zein snythesis, and that the reduction of zein synthesis is the primary effect of the gene.

It has been noted that the ribonuclease activity of endosperm tissue is markedly increased over that of normal (Wilson and Alexander, 1967; Dalby and Davies, 1967). However, Tsai (1973) observed that there are at least four enzymes which are not changed in 22-day $\underline{o}_2$ samples. These are sucrose synthetase, glucose-6-phosphate isomerase, ADP-glucose pyrophosphorylase and UDP-glucose pyrophosphorylase. No effect of the $\underline{fl}_2$ gene on ribonuclease activity has been observed.

The $\underline{o}_2$ gene has been associated with higher levels of potassium (Goodsell, 1968), lower grain yield and kernel weight (Lambert, Alexander and Dudley, 1969; Sreeramulu and Bauman, 1970), higher oil content, higher grain moisture and higher percentage of cracked kernels (Lambert et al., 1969).

Wolf, Khoo and Seckinger,(1967) and Wolf, Zuber and Helm (1969) studied the subcellular structure of the $\underline{o}_2$ and $\underline{fl}_2$ maize endosperms. Using the electron microscope they observed a major decrease in the size of the

protein bodies in the endosperm tissue of these mutants. Protein bodies of normal corn endosperm were nearly 20 times the diameter of protein bodies of o_2 corn. They were able to dissolve these protein bodies with 80 percent ethanol, which supports Duvick's (1961) suggestion that the granules (protein bodies) are the main diet of zein storage.

Opaque-2 kernels partially opaque and partially translucent in phenotype have been observed (Paez, Holm and Zuber, 1969). These modified opaque kernels have a higher test weight than opaque kernels on the same ear with only a slight reduction in lysine content (Bauman and Aycock, 1970).

Glover and Crane (1972) noticed that endosperm protein of brittle-2 (bt_2), brittle-1 (bt_1), sugary-1 (su_1) and shrunken-2 (sh_2) had notable increases in the concentration of lysine and tryptophan. In addition, all showed decreases in the concentration of leucine. Also bt_2 and su_1 had higher concentrations of methionine.

High lysine varieties and mutants have been reported in barley. Munck, Karlson and Hagberg (1969) reported a high lysine high protein barley line called "Hiproly." It was selected from the world barley collection using dye-binding capacity (DBC) as a screening technique. A recessive gene was responsible for the approximately 20-30 percent increase in lysine content. Many high lysine (Hily) segregates have been identified from crosses between Hiproly and a high yielding barley variety.

Doll (1970) found two barley mutants with increased lysine content from EMS (ethyl methane sulfonate) treated material. Recently Ingversen,

Anderson, Doll and Koie (1972) identified an ethyleneimine-induced high lysine mutant - Riso 1508 in barley. Up to a 51 percent increase in lysine as compared to the mother variety was observed. The increase in lysine was due to a decrease of the lysine deficient prolamine fraction with an increase in the lysine-rich albumin and globulin fraction at a constant level of glutelin.

Genetic Control of Carbohydrate Quality and Quantity

Normal sorghum endosperm contains about 60 to 75 percent starch. Of the starch about 25 percent is amylose and 75 percent is amylopectin. Sucrose is a major donor of glucose units that eventually are converted into starch. The pathway between sucrose and starch is quite complex. A number of carbohydrate mutants which affect the amount of starch synthesis in the endosperm as well as the proportions of amylose and amylopectin in the starch are known in cereals. Their effect on the overall nutritional quality of grain has been well studied in corn (Holder, 1971; Barbosa, 1971; Glover and Crane, 1972; Mertz, 1972; Kramer, Pfahler and Whistler, 1958; Creech, 1965; Laughnan, 1953; Nelson and Rines, 1962; Cameron and Teas, 1954; and Nelson, 1967). Sugary endosperm in sorghum was first described by Patel and Patel (1928) in India. They described a variety "Vani" which had wrinkled seeds pitted at the tips and were somewhat flinty. It was used mostly for parching in the green or immature condition.

A mutation from normal to sugary endosperm was recognized at the Chillicothe station by Quinby in 1934 (Karper, Quinby and Jones, 1934). Karper and Quinby (1963) reported that mature seeds with sugary endosperm

contained about twice as much total sugar as mature seeds with normal starchy endosperms. The sugary endosperm character is controlled by a single recessive gene su (Karper, et al., 1934; Gorbet and Weibel, 1972). No linkage was found to exist between the su gene and waxy (wx), or between su and yellow seedling, (y_2) (Karper and Quinby, 1963). Gorbet and Weibel (1972) reported on the inheritance of two dented sorghum lines. They noted a simple recessive mode of inheritance for the dent character.

Jones and Sieglinger (1951) reported a defective endosperm character in sorghum having very shrunken seed of low germination. They reported that this character was inherited as a simple recessive.

Karper (1933) reported that waxy endosperm (wx) in sorghum is inherited as a simple recessive. Since waxy endosperm starch is almost 100 percent amylopectin, the endosperm of waxy kernels stain reddish-brown with iodine rather than purple which is characteristic of normal sorghum endosperm.

The sugary-1 (su_1) gene in corn conditions a translucent wrinkled kernel phenotype and causes a substantial increase in reducing sugars, sucrose and water-soluble polysaccharides (WSP) (Culpepper and Magoon, 1924, Barbosa, 1971). Sugary-2 (su_2) has a slightly tarnished phenotype with about 40 percent amylose in the endosperm starch (Kramer and Whistler, 1949; Dvonch, Kramer and Whistler, 1951). The normal endosperm tissue of corn consists of about 25 percent amylose and 75 percent amylopectin. Shrunken-2 (sh_2) kernels of corn contain a high percentage of sucrose and reducing sugars, but the starch content is only about 30 percent of normal (Holder, 1971; Creech, 1965). Shrunken-1 (sh_1), on the other hand, is very low in sucrose and reducing sugars, but is very high in starch

content as compared to shrunken-2 (Barbosa, 1971; Creech, 1965).

Watson and Yahl (1967) found that the endosperm of o_2 kernels contained more reducing sugars and sucrose, less starch and similar WSP content when compared to normal.

The waxy (wx) gene in corn increases sugars and water soluble polysaccharides (WSP) in su_1 background and also alone (Andrew, Brink and Neal, 1944).

Many double mutant combinations of various carbohydrate mutants as well as combinations with opaque-2 and floury-2 have been developed (Glover and Crane, 1972; Barbosa, 1971; Creech, 1965; Laughnan, 1953; Kramer et al., 1958). Some of the double mutants have nearly normal kernel phenotypes and may have better yield potential than single mutant lines (Glover and Crane, 1972).

Nutritional Studies

Sorghum protein has been reported to be first limiting in lysine and threonine (Shelton, Couch, Hole, Jones, Leighton, Lyman and Briggs, 1951). It has been reported (Howe, Jansen and Gilfillian, 1965; Benton, Harper, Spivey and Elvehjem, 1956) that leucine in excess of the normal requirement can interfere with the utilization of isoleucine and valine. The presence of relatively high concentrations of leucine in sorghum has been suggested as a possible factor in the development of pellagra in population groups subsisting principally on this crop (Gopalan and Srikantia, 1960). Morton (1970) has tentatively linked higher incidences of esophageal cancer with consumption of brown colored sorhum rich in tannins.

Howe et al., (1965) compared the proteins of rice, wheat, corn, millet, sorghum, barley, rye and oat as such, and with lysine and threonine added, in the nutrition of albino rats. Casein at 9.05 percent protein was used as a check. Four week weight gains of rats and protein efficiency ratios (PER) of different diets showed that millet and sorghum were the lowest in nutritional quality. Corn protein was more than twice as high as sorghum as far as weight gain and PER ratios were concerned. The casein diet was about twice as good as corn and four times better than sorghum at similar levels of protein fed. Trible (1971) also noted significantly lower digestibility of sorghum protein over corn or wheat protein. However, with the discovery of opaque-2 and floury-2 mutants of corn, it has been possible to elevate its protein efficiency ratio (PER) to the levels of casein or soybean meal (Mertz, Nelson, Bates and Veron, 1966; Mertz, 1972). Similar mutants in sorghum would be of great practical significance, both as human food and for animal feed.

Many sorghum lines contain polyphenolic compounds (tannins) present in the pigmented testa and pericarp of the mature sorghum grain. Oswalt (1973) observed wide variations in _in vitro_ dry matter disappearance (IVDMD) of various commercial grain sorghum hybrids. Part of the differences in IVDMD percentages were associated with the presence or absence of these polyphenolic compounds expressed as catechin equivalents (CE). A significant negative correlation exists between CE and IVDMD of fourteen-day weanling rat weight gains. Further, he noted that among rations from grain sorghum lines containing similar concentrations of crude protein and amino acid profiles, the high CE rations (more than 1.00) produced

significantly lower weanling rat weight gains than those obtained with rations of low CE lines. He suggested, therefore, that high tannin lines must be studied separately from low tannin lines to identify amino acid deficiencies relative to nutritional quality.

Environmental Effects on Protein Quality and Quantity

Munck (1972) has described various environmental factors that affect the ultimate nutritional quality of barley seed. He reported that the lysine rich fraction (albumins and globulins) is synthesized at a faster rate during early stages of seed formation, whereas lysine poor storage proteins such as prolamins dominate later stages of protein synthesis. Glutelins which are intermediate in lysine are formed at a linear rate with increasing maturation. Thus, factors like seed size and seed maturation will greatly affect the overall amino acid composition of seed. Gustafson (1969) in a phytotron experiment observed that temperature and day length have no significant effect on the concentration of lysine *per seed* in barley.

Campbell and Pickett (1968), studying the effects of soil fertility on protein and amino acid composition of sorghum strains, found that nitrogen fertilization increased protein content by 0.6 percent, with no significant effect on lysine content. Schneider, Early and DeTurk (1952) showed that the zein fraction in corn increased more than any other fraction as a result of nitrogen fertilization.

MATERIALS AND METHODS

The hypothesis being tested was that similar to corn, higher nutritional quality of sorghum would also be associated with opaque, floury or sugary endosperm phenotypes, and that these types might be present in world sorghum germplasm. With this view, over 9,000 lines from the world sorghum collection were surveyed during the spring of 1972. Kernel and endosperm phenotypes of each line were examined. Kernels were sectioned longitudinally into two halves. A small polyethylene bag containing sectioned as well as a few whole kernels was stapled on a 5 x 8 inch index card bearing the India Sorghum (IS) number or other identification.

Based on the endosperm phenotype the lines were classified as vitreous, floury, waxy, sugary, yellow or hollow types. Kernels with shrunken endosperm or large germ size were also separated. However, the scope of this study is confined only to an evaluation of the floury and sugary groups. Defatted whole kernel samples of each floury and sugary line were analyzed for nitrogen content by the micro-Kjeldahl procedure and converted to percent protein by multiplying nitrogen values by 6.25. Lysine was determined by ion-exchange column chromatography. Protein is expressed as percent of dry sample and lysine as percent of protein as well as percent of dry sample.

Whole kernel samples of possible high lysine and sugary (su) mutant lines along with appropriate normal checks were analyzed for reducing sugars, sucrose, water-soluble polysaccharides (WSP), starch and amylose. Total carbohydrate content was calculated by adding values for reducing sugars, sucrose, WSP and starch. The procedure used for the determination

of reducing sugars, sucrose and WSP was essentially similar to one described by Shannon (1968). Amylose and starch were determined by the procedure of Shuman and Plunkett (1964).

RESULTS AND DISCUSSION

Sixty-two floury and five sugary endosperm lines (Table 1) were identified with the world sorghum collection on the basis of endosperm phenotype. Floury lines have a soft, chalky white endosperm[1]. All sugary lines have a characteristic glassy, translucent endosperm except IS 4526. IS 4526 has a partially floury and partially translucent endosperm. A great variation exists in seed size and extent of dimpling among sugary lines. Dimpling ranges from extremely shriveled to almost completely plump. Variation also exists in the protein and lysine content of the floury lines (Table 1). Two lines, IS 11167 and IS 11758, of Ethiopian origin and with slightly dented kernels were extremely high in lysine content. Lysine content (expressed as percent of sample) was almost double the average for world collection sorghums in the case of IS 11758. One line (IS 11168) was noted with extremely low protein content. The unusually low level of protein in this line needs further verification. The variation in protein and lysine contents among floury lines of sorghum parallels the situation in corn where many genes express floury/opaque endosperm phenotypes but only opaque-2, opaque-7 and floury-2 have a pronounced effect on increased lysine content (Nelson et al., 1965; McWhirter, 1971).

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[1] Use of fully mature kernels is very important for this classification since immature seed of even vitreous types may appear floury.

Table 1. Protein and lysine content of defatted whole kernels of floury and sugary endosperm lines selected from the part of the world sorghum collection maintained at Purdue.

I.S. No.	Protein (%)	Lysine content (% of protein)	Lysine content (% of sample)	Origin
		Floury endosperm lines		
2151*	11.0	2.43	.267	---
2164*	12.3	2.20	.271	Kafirisoll, Nigeria
2660	13.8	1.80	.248	Uganda
2663	13.5	1.74	.235	Teso, Uganda
3053*	10.9	2.52	.275	Jimma, Ethiopia
3061	16.6	1.70	.282	Alamiayhu, Ethiopia
5177	14.1	2.08	.293	Andhra Pradesh, India
5244	11.8	2.02	.238	Andhra Pradesh, India
5729	11.8	2.13	.251	Bihar, India
6018	16.5	1.71	.282	Punjab, India
6325	14.6	1.90	.277	Punjab, India
7177	11.8	2.04	.241	Nyasaland
8283*	9.5	2.26	.215	IARI, India
8368	12.0	1.89	.227	Nebraska, U.S.A.
8613	11.4	1.98	.226	Kenya and Uganda
8617	13.3	2.05	.273	Uganda
8633*	12.5	2.24	.280	Farafara, Nigeria
8794	14.8	1.73	.256	Rhodesia thru Uganda
8883	12.1	1.95	.236	Uganda thru Jowett
8884	10.8	1.77	.191	Uganda thru Jowett
8891	10.4	2.08	.216	Uganda thru Jowett
8941	14.9	1.77	.264	Kenya thru Uganda
8945	11.7	1.89	.221	Kenya thru Uganda
9054	12.1	1.90	.230	Kenya thru Uganda
9097*	8.5	2.42	.206	Kenya thru Uganda
9099	11.4	1.78	.203	Kenya thru Uganda
9183*	7.4	2.72	.201	Sudan thru Uganda
9999	16.8	1.82	.306	Debraziet, Ethiopia
10730	13.3	1.89	.251	Indiana, U.S.A.
10785	10.1	2.17	.219	Dirguigui, Chad
11074	13.5	2.52	.340	Shoa, Ethiopia
11086	15.9	1.69	.269	Shoa, Ethiopia
11108	14.6	1.82	.266	Shoa, Ethiopia
11130	13.0	1.43	.186	Shoa, Ethiopia
11131	17.4	1.48	.257	Shoa, Ethiopia
11132*	9.2	2.70	.248	Shoa, Ethiopia
11134	12.1	1.90	.230	Shoa, Ethiopia
11140*	13.0	2.11	.274	Shoa, Ethiopia
11157	15.0	1.68	.252	Wollo, Ethiopia
11165	16.5	1.64	.272	Wollo, Ethiopia
11167*	12.0	3.16	.379	Wollo, Ethiopia
11168*	5.4	3.18	.172	Wollo, Ethiopia
11171	13.6	1.95	.265	Wollo, Ethiopia

Table 1, cont.

11174	15.4	1.65	.254	Wollo, Ethiopia
11573	13.5	2.07	.279	Ethiopia
11581*	13.9	2.22	.308	Dejen, Ethiopia
11582*	12.1	2.43	.294	Dejen, Ethiopia
11610	15.3	1.99	.304	Ethiopia
11611	16.5	1.78	.287	Ethiopia
11653	16.0	2.02	.323	Harrar, Ethiopia
11677	17.3	1.61	.278	Harrar, Ethiopia
11758	18.9	3.12	.590	Harrar, Ethiopia
12135	14.5	1.67	.242	Ethiopia
12139	13.0	1.88	.244	Ethiopia
12207*	10.1	2.26	.228	Kampala, Uganda
12214*	10.3	2.49	.256	Uganda
12218	11.2	2.04	.228	Uganda
12224	15.3	1.66	.254	Uganda
12231	12.7	1.93	.245	Kihihi, Uganda
12233	13.1	1.74	.228	Uganda
12237	11.5	2.09	.240	Mbende, Uganda
12242	14.8	1.37	.203	Wollega, Ethiopia
		Sugary endosperm lines		
4526*	15.3	2.21	.338	Maharashtra, India
4668*	13.4	2.45	.328	Maharashtra, India
5376*	17.3	2.24	.387	Madras, India
5614*	15.5	2.32	.360	---
5623*	12.4	2.42	.300	Mysore, India
Average sorghum**	12.6	2.14	.270	---

* Lines were grown at Purdue Agronomy Farm during summer 1972 as part of study 204.

** Weighted average of 522 sorghum lines (Srinivasan, Axtell and Jambunathan, 1972).

On the basis of these preliminary observations it was hypothesized that IS 11167 and IS 11758 may be the result of a gene mutation which alters the normal amino acid pattern as evidenced by the increased lysine content. Similar gene mutations have already been discovered in corn and barley (Mertz et al., 1964; Munck, Karlsson, Hagberg and Eggum, 1970).

A sugary (su) mutation has been identified in sorghum and was reported to be simply inherited (Patel and Patel, 1928; Karper and Quinby, 1963; Gorbet and Weibel, 1972). However, no information is available regarding the nutritional quality of sugary lines.

Both high lysine lines had a floury endosperm and dented kernels, while the endosperm of the sugary lines is partly or fully glassy and translucent with a great range of variation in the dimpling of the kernels (Table 2).

These preliminary observations provided the basis for further chemical and biological evaluations, as well as for the inheritance study of these high lysine and sugary lines.

Table 2. Phenotypic characters of high lysine and sugary (su) lines.

Purdue identification	IS number	Kernel and endosperm phenotypes
High lysine lines		
204066	11167	Dented, floury
204067	11758	Dented, floury
Sugary lines		
204058	4526	Plump, major part of endosperm floury with a thin outer layer of glassy endosperm.
204068	4668	Moderately shriveled, endosperm glassy and translucent.
204069	5376	Shriveled, endosperm glassy and translucent.
204070	5614	Shriveled, endosperm glassy and translucent.
204064	5623	Shriveled, endosperm glassy and translucent.

Carbohydrate Analysis of Whole Kernel Samples of High lysine and Sugary Lines

Reducing Sugars, Sucrose and Total Sugars

Table 3 presents data with percent of reducing sugars, sucrose and total sugars in whole kernels of high lysine, sugary and normal sorghum lines. Total sugar values reported are the sum of reducing sugars plus sucrose. Sugary lines IS 4668, 5376 and 5623 were extremely high in reducing sugars ranging from 2.52 to 2.69% as compared to 0.34% in normal sorghum. The reducing sugar content of both high lysine lines was equivalent to normal lines.

IS 4526 and IS 5614 were low in reducing sugars, but were very high in sucrose content. IS 5623 was high in both types of sugars. The sucrose content in sugary lines ranged from 0.68% to 2.1% in comparison to 1.03% in normal sorghum. Both high lysine lines were high in sucrose content. The total sugar content of sugary lines ranged from 1.96% to 4.39%. IS 4526 had the lowest sugar content while IS 5623 had the highest value. Normal sorghum IS 8313 had 1.34% total sugars. The high lysine line IS 11167 was nearly two and half times higher while IS 11758 was nearly twice as high in total sugars as normal sorghum.

Starch Content in Whole Kernels and Amylose in Starch

The starch content was substantially reduced in all sugary lines except IS 4526 which was similar to normal sorghum in starch content. Starch content in the normal sorghum line was 60.8%. Four of the sugary

Table 3. Carbohydrate analysis of whole kernel samples of high lysine and sugary lines. (Expressed as percent of dry wt.)

Entry	Reducing sugars	Sucrose	Total sugars	WSP	Starch	Amylose (% of starch)	Total carbohydrates
High lysine lines							
IS 11167	0.38	3.08	3.46	0.91	58.9	25.0	63.27
IS 11758	0.32	2.61	2.93	1.01	57.8	26.2	61.74
Sugary lines							
IS 4526	0.26	1.70	1.96	1.27	58.5	40.6	61.73
IS 4668	2.52	0.68	3.20	29.40	34.0	30.4	66.60
IS 5376	2.78	1.06	3.84	34.20	25.4	31.7	63.44
IS 5614	0.57	2.10	2.67	39.60	22.2	37.4	64.47
IS 5623	2.69	1.70	4.39	35.70	26.3	33.3	66.39
Normal check							
IS 8313	0.34	1.03	1.34	1.11	60.8	25.0	63.25

lines, IS 4668, 5376, 5614 and 5623 ranged in starch content from 22.2% to 34.0%. Both high lysine lines had starch contents comparable to the normal line.

The amylose content of starch among sugary lines was 22 to 62% higher than the normal lines. IS 4526 had the highest amylose content of all the lines tested. High lysine lines IS 11167 and 11758 were equivalent to normal sorghum in amylose content.

Water-Soluble Polysaccharides

Extremely high amounts of water-soluble polysaccharides were associated with the four sugary lines IS 4668, 5376, 5614 and 5623. The range was from 29.4% to 39.6% in these as compared to 1.1% in normal sorghum lines The WSP content of sugary line IS 4526 and the two high lysine lines is very similar to that of normal sorghum.

The carbohydrate content of four sugary lines (IS 4668, 5376, 5614 and 5623) is totally different from normal lines. All these lines have excessive amounts of sugars and WSP. The starch content of these lines is reduced to approximately 40% of the normal sorghum, and there is an increase in the amylose content of starch. These results compare very well with the su_1 mutants in corn (Barbosa, 1971; Creech, 1965). IS 4526 resembles normal sorghum in reducing sugars, WSP and starch but has 65% higher sucrose content and 62% higher amylose content than normal. Similar carbohydrate composition has been reported for su_2 mutants of corn (Barbosa, 1971; Creech, 1965).

The carbohydrate analysis of both high lysine lines compares closely

to normal sorghum except for their higher sucrose content. This suggests that the high lysine content of these lines is not due to a pleiotropic effect of a block in carbohydrate synthesis.

Based on the carbohydrate analysis IS 4668, 5376, 5614 and 5623 could be classified as sugary mutants beyond any doubt. IS 4526, however, closely resembles normal sorghum except that it has a higher amylose content. On this basis it could either be classified as sugary endosperm (similar to su_2 in corn) or as a high amylose sorghum.

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BIOLOGICAL VALUE OF HIGH LYSINE (hl) AND SUGARY (su) MUTANTS

Rameshwar Singh and John D. Axtell

Four rat feeding experiments* were performed on seeds grown in Lafayette, Indiana and Puerto Rico to compare the biological value of high lysine and sugary lines as compared to average sorghum lines.

Experiment 1

Seventy-eight male weanling rats of Wistar breed with nearly similar initial weight were used for evaluation of 13 different diets. Rats were arranged in groups of 6 to make their average weight close to their overall mean weight and were placed in 6 individual wire-mesh cages. Experimental diets were fed to each rat for 21 days except for diet five which lasted for 12 days only.

Individual rat weight gains and their feed consumption were measured on the 7th, 14th and 21st day except for diet five, in which case 7th and 12th day weights were taken. The use of special feed cups minimized the feed spill over to a practically negligible level. Rats were offered food and water ad libitum. Water was changed on alternate days. Rat weight gain, feed consumption, feed efficiency ratio (FER, feed consumed per gram of body weight gain) and protein efficiency ratio (PER, weight

*Rat experiments 1, 2, and 3 were conducted by utilizing facilities of the Biochemistry Department of Purdue University. Experiment 4 was conducted at Shuman Chemical Laboratory, Battle Ground, Indiana.

gain per gram of protein consumed) data were calculated and statistically analyzed. Per day weight gain and feed consumption were also calculated using the last measurements.

Preparation of Diets

Grain samples used for this feeding trial were grown at the Purdue Agronomy Farm during 1972. One high lysine and five sugary mutants were used for this feeding trial along with six normal sorghum lines as check. Crude protein (N x 6.25) level of these lines ranged between 13.3 and 16.2% with a mean of 15.3%. A casein (ANRC casein-HCl) diet was also fed for comparison. Grain samples were ground twice in a coffee grinder. All feeds were brought to approximately 12.5% protein level by the addition of corn starch. Thus each 100 g of basal diet consisted of varying amounts of sorghum grain or casein and corn starch plus 4 g of mineral mixture* and 2 g of vitamin mix **. Protein and lysine contents were determined for both grain and prepared diets. Oil content was determined for grain only.

Experiment 2

This experiment was planned to test the reproducibility of the previous data. Seed of high lysine and sugary mutants was obtained from the 1972-73 Puerto Rico nursery. All high lysine and sugary mutants were fed along with two normal sorghum lines and casein diet. Feed protein was

* Hawk-Oser salt mixture number three, Nutritional Biochemicals, Inc. Cleveland, Ohio

** Vitamin supplement, General Biochemicals, Chagrin Falls, Ohio.

made iso-nitrogenous at the 10% protein level as differences between protein qualities tend to disappear at higher levels of protein intakes (McLaughlan and Campbell, 1969). Other experimental conditions similar to experiment 1 except that the span of the feeding trial was four weeks.

Experiment 3

The high lysine mutant lines are much higher in crude protein than average sorghum lines in addition to their higher lysine contents. Average protein content of the lines of world sorghum collection is 12.6% (Srinivasan, Axtell and Jambunathan, 1972). When protein level of high lysine mutant is diluted down from 16 - 18% to 10 - 12%, they are put at a disadvantage to express their full biological value in terms of weight gain. This view may be criticized on the basis that PER values would hardly change whether they are evaluated at a higher protein level or lower. Morrison and Campbell (1960) have reported that with casein the highest PER value was found at 7% level of protein but plant proteins tend to have higher PER's at 15%, particularly at 2 - 4 weeks. Also, as in most practical feeding uses, grain sorghum is used without any dilution, therefore, it is important to see its biological usefulness as such. This experiment was planned, therefore, to compare the feeding value of high lysine sorghum, normal sorghum, normal corn and opaque-2 corn at their inherent protein levels with 4% mineral mix and 2% vitamin mix supplementation. Our present best nutritional quality line IS 2319 and another high protein (14%) line IS 1486 were used as check. A casein

diet at 17% protein (same as protein level of high lysine sorghum feed) was also used as check. The experiment was carried out for 4 weeks.

Experiment 4

Experiment 4 was conducted to confirm the biological value of high lysine sorghum under different laboratory conditions. The trial was performed at Shuman Chemical Laboratory, Battle Ground, Indiana. High lysine mutant IS 11758 was fed along with one sugary line IS 5376 and two normal checks IS 2319 and IS 2520. All feeds were supplemented with 4% mineral mixture and 2% vitamin mixture. Eight rats were used per diet. The experiment was terminated after 2 weeks.

Experimental Design

Experiments 1, 2 and 3 were considered as randomized complete block design with six replications. Design of experiment 4 was a completely random design. Data obtained were put to appropriate methods of statistical analysis as given by Cochran and Cox (1957).

RESULTS AND DISCUSSION

Experiment 1

Based on their improved amino acid composition, the high lysine and sugary sorghum lines should have better biological value than normal sorghum. To test the above hypothesis, this trial was planned to compare the feeding value of high lysine (IS 11758) and sugary sorghum (IS 4526, 4668, 5376, 5614 and 5623) lines with six normal sorghum lines (IS 2319,

5568, 2520, 0057, 8313 and 6901) and a casein diet. Lack of enough seed supply of the second high lysine line (IS 11167) prevented its inclusion in this trial. IS 2319 has good nutritional quality and is often used as the best check in nutritional studies at Purdue. All diets were diluted to approximately 12.5% protein. However, protein analysis data (Table 1) showed some differences in protein levels of various diets.

Data in Table 2 presents the initial weight of rats and weekly and per day weight gain results. There was no significant difference in the initial weights of rats fed on different diets. The 3 week weight gain of rats on the high lysine sorghum diet was 3 times higher than the mean value for the normal sorghum lines. Rat weight gain on IS 2319 was only 54% of the high lysine line. Differences between weight gain on each normal line and the high lysine line were all highly significant ($P<.01$). Weekly gains in weight were quite consistent during the 3 week trial.

Three sugary lines (IS 4526, 5614 and 5623) were significantly better than the average of the normal lines. However, when compared to IS 2319, only IS 4526 gave significantly ($P<.05$) higher rat weight gain. Rats fed on the casein diet had significantly higher weight gains than any of the sorghum lines.

Feed consumption data presented in Table 3 shows that rats fed on nutritionally better (based on rat weight gain) high lysine and sugary sorghum (IS 11758, 4526 and 5614) consumed significantly higher amounts of feed than those fed on normal sorghum lines or nutritionally inferior sugary lines (IS 4668 and 5376).

Table 1. Protein, lysine and oil contents of whole grain, and protein and lysine contents of feed. Experiment 1

Genotype	Composition of grain				Composition of feed		
	% protein	lysine % of protein	lysine % of sample	% oil	% protein	lysine % of protein	lysine % of sample
High lysine mutant							
IS 11758	16.0	3.00	.480	6.97	13.1	2.59	.339
Sugary mutants							
Is 4526	16.2	2.71	.439	5.00	11.5	2.60	.299
IS 4668	14.9	2.18	.325	3.38	12.5	1.97	.246
IS 5376	16.3	2.27	.370	4.64	12.1	2.28	.276
IS 5614	14.8	2.65	.392	4.90	12.7	2.51	.319
IS 5623	15.1	2.73	.412	5.06	12.1	2.51	.304
Normal lines							
IS 2319	14.2	2.34	.332	4.70	12.3	2.17	.267
IS 5568	14.4	1.87	.269	3.75	12.1	1.71	.207
IS 2520	13.3	1.85	.246	3.50	11.8	1.75	.206
IS 0057	14.3	2.26	.323	3.05	11.6	2.21	.256
IS 8313	17.7	1.75	.310	4.64	11.6	1.80	.209
IS 6901	16.0	2.02	.323	2.94	10.4	1.95	.203
Mean of normal lines	15.0	2.01	.301	3.76	11.6	1.93	.225
Casein	91.0	8.00	7.280	--	15.3	7.05	1.079
Grand mean	21.1	2.73	.883	4.38	12.2	2.55	.324

Table 2. Comparison of rat weight gain. Experiment 1

Genotype	Initial weight of rats (g)	Weight gain (g)			
		7 days	14 days	21 days	per day
High lysine mutant					
IS 11758	49.8	11.7	24.5	37.2	1.77
Sugary mutants					
IS 4526	50.8	9.5	17.8	26.7	1.27
IS 4668	51.5	6.0	10.8	16.3	0.78
IS 5376	51.3	6.8	11.8	16.7	0.79
IS 5614	51.0	9.3	17.7	25.8	1.23
IS 5623	50.0	8.0	15.3+	--	1.27+
Normal lines					
IS 2319	50.8	6.0	13.2	20.0	0.95
IS 5568	49.5	5.3	9.5	13.3	0.63
IS 2520	49.7	2.2	3.8	7.3	0.35
IS 0057	50.0	4.7	10.2	13.7	0.65
IS 8313	50.8	2.0	5.2	6.8	0.32
IS 6901	49.7	4.2	9.2	12.7	0.60
Mean of Normal lines	50.1	4.1	8.5	12.3	0.58
Casein	50.2	28.8	60.2	90.8	4.32
Grand mean	50.4	8.0	16.2	23.9	1.15
C.V.%	8.1	30.7	24.6	24.3	23.90
S.E. of mean	1.7	1.0	1.6	2.4	0.11
LSD. 5%	N.S.	3.4	4.5	6.7	0.31
1%	N.S.	3.8	6.1	8.9	0.42

+ 12 days result

Table 3. Weekly feed consumption of rats. Experiment 1.

Genotype	Feed consumed (g)		
	7 days	14 days	21 days
High lysine mutant			
IS 11758	49.0	107.0	175.7
Sugary mutants			
IS 4526	46.3	100.5	168.5
IS 4668	40.2	86.0	140.3
IS 5376	40.7	84.2	136.5
IS 5614	48.2	105.3	170.3
IS 5623	46.2	81.2+	--
Normal lines			
IS 2319	43.8	90.5	148.0
IS 5568	42.8	85.2	137.5
IS 2520	37.3	71.3	113.5
IS 0057	43.5	87.7	139.0
IS 8313	38.8	78.8	125.3
IS 6901	43.3	87.8	144.5
Mean of normal lines	41.6	83.5	134.6
Casein	59.0	134.3	227.7
Grand mean	44.5	92.9	151.9
C. V. %	10.1	10.8	9.9
S. E. of mean	1.8	4.1	6.1
LSD. 5%	5.1	11.6	17.3
1%	6.9	15.5	23.0

+ 12 day result

Feed consumption per gram of body weight gain expressed as the feed efficiency ratio (FER) in Table 4 was nearly 3 times higher for the normal sorghum lines than for the high lysine line. The FER for casein was lower than the high lysine line but was not significantly different. Three nutritionally better sugary lines (IS 4526, 5614 and 5623) had significantly ($P<.01$) lower FER values than the average of the normal sorghum lines.

Protein efficiency ratio (grams of weight gain per gram of protein consumed) of high lysine line was more than twice as high as the value for normal sorghum lines (Table 4). The PER of the casein diet was significantly higher than that of all sorghum diets. All 3 nutritionally superior sugary lines had significantly ($P<.01$) higher PER values than that of the average sorghum lines.

Experiment 2

Experiment 2 was planned to verify the reproducibility of the data obtained in experiment 1 using high lysine and sugary sorghum seeds grown under tropical conditions (where sorghum is mostly grown) of Puerto Rico. All high lysine and sugary lines were compared with three normal sorghum lines (including IS 2319), and a casein diet, all fed at a 10% protein level.

Table 5 presents protein, lysine and oil content of the whole grain, and also protein and lysine content of the ration fed. The protein content of all sorghum diets ranged from 10.0 to 11.2%. The initial weight of rats and the result of weight gain is presented in Table 6.

Table 4. Comparison of feed efficiency ratio (FER) and protein efficiency ratio (PER). Experiment 1.

Genotype	FER		PER	
	14 days	21 days	14 days	21 days
High lysine mutant				
IS 11758	4.4	4.7	1.74	1.61
Sugary mutants				
IS 4526	6.0	6.5	1.56	1.37
IS 4668	8.3	8.9	1.00	1.04
IS 5376	7.4	8.5	1.15	1.00
IS 5614	6.1	6.7	1.32	1.18
IS 5623	5.4+	-	1.57+	--
Normal lines				
IS 2319	7.2	7.6	1.17	1.10
IS 5568	9.7	10.8	0.92	0.80
IS 2520	22.5	17.1	0.46	0.54
IS 0057	10.4	11.1	1.00	0.85
IS 8313	16.6	21.3	0.57	0.47
IS 6901	10.2	11.7	1.00	0.84
Mean of normal lines	12.8	13.3	0.85	0.77
Casein	2.3	2.5	2.92	2.59
Grand mean	9.2	9.8	1.24	1.12
C.V. %	44.3	40.0	22.60	17.60
S.E. of mean	1.6	1.6	0.11	0.08
LSD. 5%	4.5	4.5	0.31	0.23
1%	6.1	5.6	0.43	0.30

+ 12 days result

Table 5. Protein, lysine and oil content of grain and feeds. Experiment 2.

Genotype	Composition of grain				Composition of feed		
	% protein	lysine % of protein	lysine % of sample	% oil	% protein	lysine % of protein	lysine % of sample
High lysine mutants							
IS 11167	16.6	3.36	.558	5.81	10.1	2.81	.284
IS 11758	18.0	3.38	.608	6.25	10.0	3.15	.315
Sugary mutants							
IS 4526	14.6	2.66	.388	4.72	10.7	2.73	.292
IS 4668	14.3	2.47	.353	3.68	11.2	2.02	.226
IS 5376	13.6	2.37	.322	4.45	10.7	2.35	.251
IS 5614	14.0	2.47	.346	4.00	10.8	2.01	.217
IS 5623	11.8	2.35	.277	3.47	10.7	1.98	.211
Normal lines							
IS 2319	12.7	2.30	.292	4.70	10.9	2.25	.245
IS 2520	13.3	1.85	.246	3.50	11.2	1.76	.197
IS 1269	14.8	2.10	.310	4.21	10.7	2.01	.215
Mean of normal lines	13.6	2.08	.283	4.14	10.9	2.01	.219
Casein	91.0	8.00	7.28	--	13.3	7.36	.979
Grand mean	21.3	3.03	.402	4.48	10.9	2.76	.312

Table 6. Comparison of rat weight gain. Experiment 2.

Genotype	Initial weight (g)	Weight gain (g) 7 days	14 days	21 days	28 days	per day
High lysine mutants						
IS 11167	47.3	6.2	15.2	22.9	34.5	1.23
IS 11758	46.2	9.5	18.8	30.8	48.8	1.75
Sugary mutants						
IS 4526	48.3	5.5	12.9	21.4	31.8	1.14
IS 4668	48.5	0.3	6.5	10.7	16.7	0.60
IS 5376	47.0	4.8	10.0	16.0	26.3	0.94
IS 5614	47.5	2.2	11.0	17.5	25.3	0.91
IS 5623	46.3	4.7	9.2	17.0	27.0	0.96
Normal lines						
IS 2319	48.0	3.7	9.2	15.7	25.3	0.91
IS 2520	46.2	2.0	5.5	7.1	10.3	0.36
IS 1269	46.3	1.5	4.5	10.0	14.0	0.49
Mean of normal lines	46.8	2.4	6.4	10.9	16.5	0.58
Casein	47.7	19.6	43.8	60.3	85.8	3.06
Grand mean	47.2	5.3	13.3	20.9	31.5	1.12
C.V. %	11.1	49.9	35.2	26.5	30.2	30.17
S. E. of mean	2.1	1.1	1.9	2.3	3.9	0.14
LSD. 5%	N.S.	3.1	5.4	6.5	11.1	0.40
1%	N.S.	4.1	7.2	8.6	14.7	0.52

There was no significant difference between initial weight of rats put on different diets. Weight gain results of this experiment were in agreement with those from experiment 1 for all the lines that were repeated here except for IS 5376 which showed better rat weight gain results in this trial. Similar to experiment 1, weight gain on IS 11758 diet was nearly double of IS 2319 and three times higher than the average of normal sorghum lines. Gain in weight of rats on the other high lysine line (IS 11167) was only 71% of IS 11758. However, it was significantly ($P < .01$) superior to the control sorghum lines, and was 36% better than IS 2319 in terms of weight gain. Rat weight gain was highest on IS 4526 among the sugary lines as was noted in experiment 1.

Figures 1 and 2 show a comparison of weekly rat weight gains on high lysine, sugary and average sorghum. A slight increase in the rate of weight gain during the 4th week period is evident from both figures.

The feed consumption and FER and PER ratios are presented in Tables 7 and 8 respectively. These followed the same general pattern described in experiment 1 with the exception that a 15% improvement in PER value of IS 11758 was noted in this experiment over PER value of this line in previous experiment.

Correlation coefficients between percent oil in grain, protein and lysine in feed, weight gain and feed consumption by rats and feed efficiency and protein efficiency ratios were calculated for experiment 2 and are presented in Table 9. For this isonitrogenous feeding trial, both lysine expressed as a percent of protein and as percent of sample were significantly correlated ($P < .01$) with rat weight gain, feed consumption and PER. As expected in this case, the level of protein had

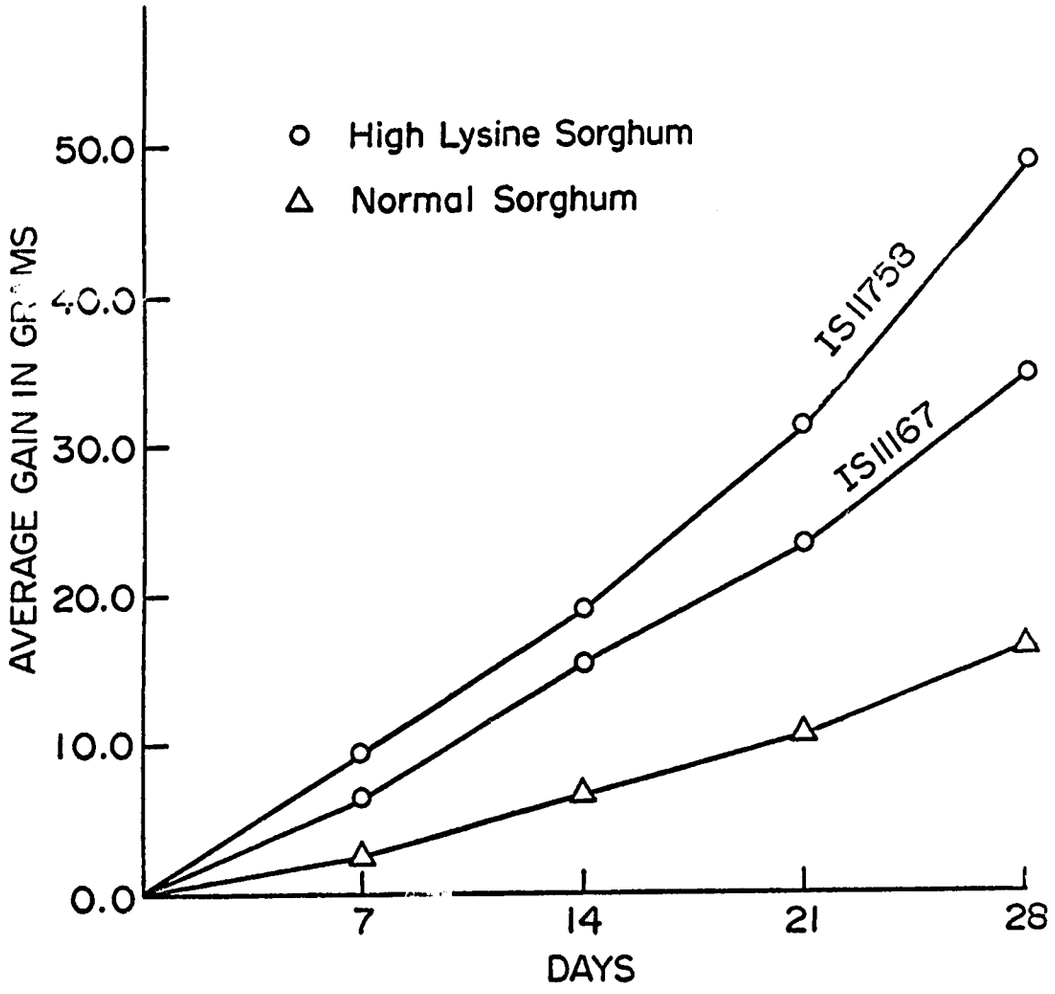

Figure 1. Average weight gain of rats fed on high lysine and normal sorghum at 10% protein level.

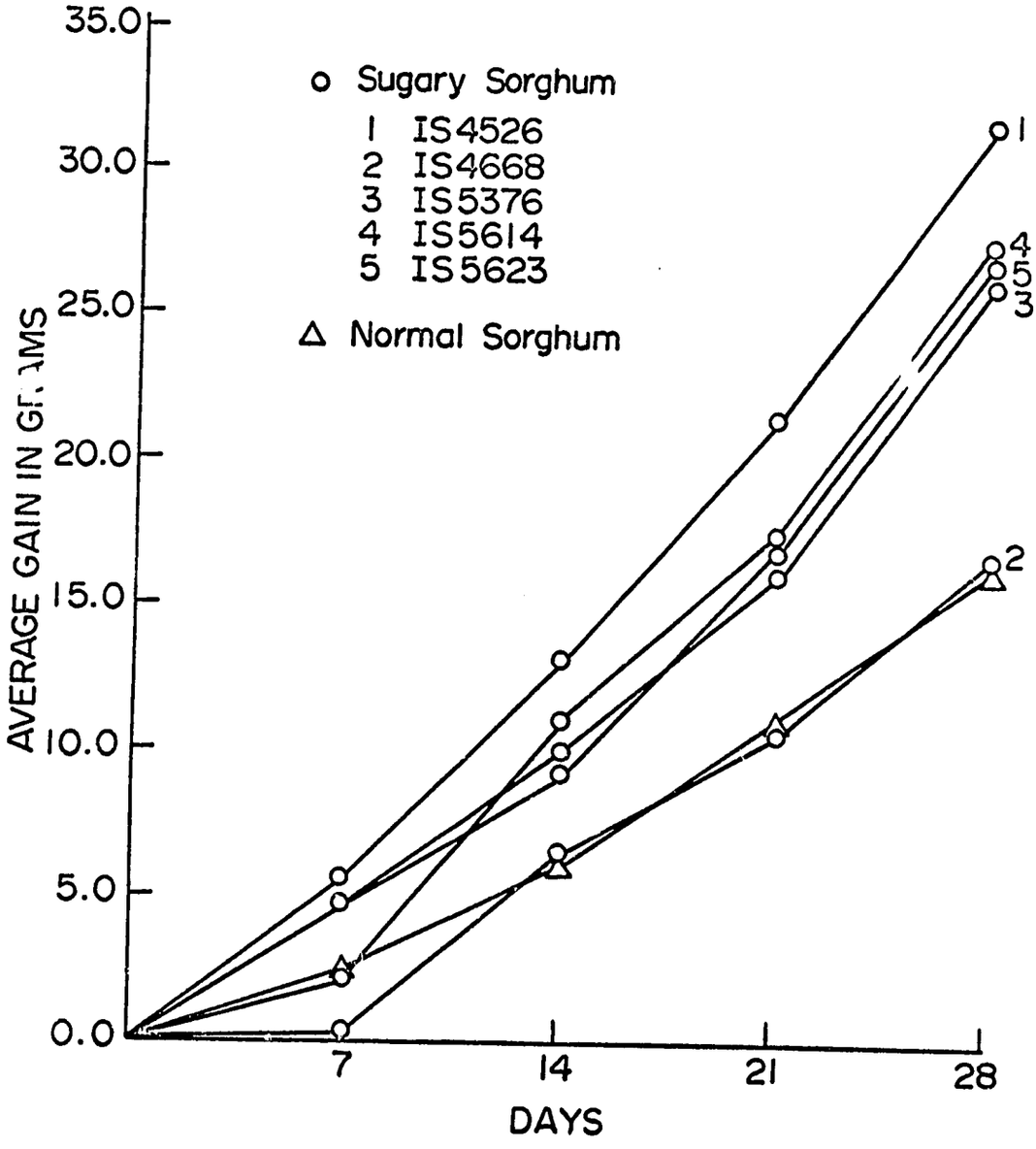

Figure 2. Average weight gain of rats fed on sugary and normal sorghum at 10% protein level.

Table 7. Weekly feed consumption of rats. Experiment 2.

Genotype	Feed consumed (g)			
	7 days	14 days	21 days	28 days
High lysine mutants				
IS 11167	39.8	84.7	137.7	193.0
IS 11758	45.2	98.7	165.0	234.8
Sugary mutants				
IS 4526	41.2	86.5	143.2	200.2
IS 4668	34.2	72.8	118.3	159.2
IS 5376	40.7	82.2	135.3	193.5
IS 5614	37.8	81.0	131.5	179.2
IS 5623	40.7	84.5	139.7	194.3
Normal lines				
IS 2319	37.5	75.8	131.0	187.8
IS 2520	34.7	69.7	109.3	150.0
IS 1269	40.2	83.5	132.2	179.8
Mean for normal lines	37.5	76.3	124.2	172.5
Casein	52.2	120.7	197.7	291.2
Grand mean	40.4	85.4	140.1	196.6
C.V. %	16.4	16.5	16.1	16.9
S.E. of mean	2.7	5.6	9.2	13.5
LSD. 5%	7.7	15.9	26.1	38.4
1%	10.2	21.8	35.0	51.4

Table 8. Comparison of feed efficiency ratio (FER) and protein efficiency ratio (PER). Experiment 2.

Genotype	FER		PER	
	21 days	28 days	21 days	28 days
High lysine mutants				
IS 11167	6.1	5.6	1.64	1.78
IS 11758	5.5	4.9	1.85	2.06
Sugary mutants				
IS 4526	6.9	6.5	1.38	1.45
IS 4668	11.6	9.8	0.80	0.92
IS 5376	9.5	8.0	1.07	1.25
IS 5614	7.9	7.4	1.19	1.30
IS 5623	8.3	7.3	1.14	1.32
Normal lines				
IS 2319	8.4	7.5	1.10	1.24
IS 2520	16.3	13.3	0.67	0.61
IS 1269	14.5	13.3	0.69	0.74
Mean for normal lines	13.1	11.3	0.82	0.86
Casein	3.2	3.5	2.37	2.20
Grand mean	9.9	9.8	1.25	1.35
C.V. %	104.6	157.4	17.89	16.96
S.E. of mean	4.2	6.3	0.09	0.09
LSD. 5%	12.1	N.S.	0.25	0.26
1%	N.S.	N.S.	0.34	0.35

Table 9. Correlation coefficients between percent oil in grain, protein and lysine in feed, weight gain and feed consumption, FER and PER values. Experiment 2.

	% protein in feed	Lysine (% of P) in feed	% lysine in feed	Weight gain	Feed consumed	FER	PER
% oil in grain	-.322	.260	.159	.427	.453	-.585*	.696*
% protein in feed		-.100	-.066	.189	-.283	.180	-.268
Lysine (% of P) in feed			.994**	.955**	.919**	-.629*	.734**
% lysine in feed				.926**	.885**	-.570	.667*
Weight gain					.977**	-.804**	.892**
Feed consumed						-.771**	.875**
FER							-.949**

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

no effect on weight gain, feed consumption, FER, or PER. This indicates that when protein from different diets is fed at a similar level, the differences in weight gain or feed consumption are mainly due to differences in the protein quality.

Experiment 3

High lysine sorghum lines with substantially higher level of protein (besides their higher lysine content) do not express their apparent biological value in terms of weight gain when fed at isonitrogenous level. Most normal sorghum lines with 12 - 14% protein are put to advantage in isonitrogenous trials because they need less dilution with starch to bring their protein level to a specified protein level than is needed

for high lysine lines which have 16 - 18% protein. Further, for most practical purposes, sorghum grain is used without dilution. With these points in mind, experiments 3 and 4 were planned to compare the biological value of high lysine sorghums with other checks without any dilution except the usual supplementation with a 2% vitamin and 4% mineral mixture.

IS 11758 was compared with two normal sorghum lines (IS 2319 and IS 1484), opaque-2 corn, normal corn, and a casein diet. Opaque-2 corn was included in this trial to have a better nutritional quality grain diet as a check.

Protein and lysine contents of the whole grain and feed ration are presented in Table 10. The casein diet had 1.104% lysine in the ration as compared to 1.0% lysine required for optimal growth of weanling rats (Mertz, 1969). The opaque-2 corn diet had 81% lysine present in the high lysine sorghum diet.

It may be mentioned here that due to a shortage of enough seed of high lysine sorghum line IS 11758, some moulded seeds of this line were used for feeding during the fourth week period. The adverse effect of the moulds on feeding quality of grain may be noticed by comparing the weight gain results presented in Table 11. The 28-day rat weight gain on each diet was more than twice the weight gain for 14-day period except for the high lysine sorghum lines. For this reason, it would be appropriate to use the three-week results to compare the feeding quality of different diets in this experiment.

The 21-day rat weight gain results presented in Table 11 and Figure 3 shows that the biological value of IS 11758 was five times higher

Table 10. Protein and lysine content of grain and feeds. Experiment 3.

Genotype	Composition of grain			Composition of feed		
	% protein	lysine % of protein	lysine % of sample	% protein	lysine % of protein	lysine % of sample
High lysine mutant						
IS 11758	18.4	3.38	.622	18.4	3.17	.583
Normal sorghum						
IS 2319	12.7	2.30	.292	12.6	2.23	.281
IS 1484	14.0	1.93	.270	12.6	2.01	.253
Mean for normal sorghum	13.3	2.11	.281	12.6	2.12	.267
Opaque-2 corn	12.5	4.00	.500	12.1	3.89	.471
Normal corn	9.4	2.73	.257	8.6	2.90	.249
Casein	91.0	8.00	7.280	17.2	6.42	1.104

than the average of two normal lines, and four times higher than IS 2319. When compared to the corn diets, IS 11758 was 17% better than opaque-2 corn and three and one-half times better than normal corn. The higher rat weight gain on high lysine sorghum over opaque-2 corn is expected due to comparatively high concentrations of essential amino acids e.g. lysine, threonine and tryptophan and higher protein level in high lysine sorghum diet (Table 14).

Feed consumption (Table 12) was highest on the opaque-2 corn diet, which was nearly 9% higher than feed consumption on the high lysine sorghum diet. Rats consumed 73% more feed on high lysine sorghum ration than on normal sorghum ration. Among these corn and sorghum diets, the feed efficiency ration was lowest for the high lysine sorghum. Opaque-2 corn had a 16% higher feed requirement per unit gain in weight than high

Table 11. Comparison of rat weight gain. Experiment 3.

Genotype	Initial weight (g)	Weight gain (g)				
		7 days	14 days	21 days	28 days	per day
High lysine mutant						
IS 11758	44.8	16.7	48.9	68.0	94.2	3.36
Normal sorghum						
IS 2319	44.3	3.9	11.9	19.0	28.5	1.02
IS 1484	44.7	1.1	7.6	12.3	19.2	0.68
Mean for normal lines	44.5	2.5	9.7	15.7	23.9	0.85
Opaque-2 corn	43.3	15.9	42.0	59.0	91.5	3.27
Normal corn	43.8	5.2	14.2	22.0	30.2	1.08
Casein	43.3	25.0	69.2	111.0	181.2	6.50
Grand mean	44.1	11.3	31.7	48.6	74.1	2.65
C.V. %	7.8	28.5	13.1	17.9	16.7	16.70
S.E. of mean	1.4	1.3	1.7	3.5	5.0	0.18
LSD. 5%	N.S.	3.8	4.9	10.3	14.7	0.52
1%	N.S.	5.2	6.7	14.0	19.9	0.71

lysine sorghum IS 11758.

Experiment 4

Experiment 4 was conducted at a different testing laboratory* to confirm the repeatability of the increased biological value of the high

*Shuman Chemical Laboratory, Inc., Battle Ground, Indiana.

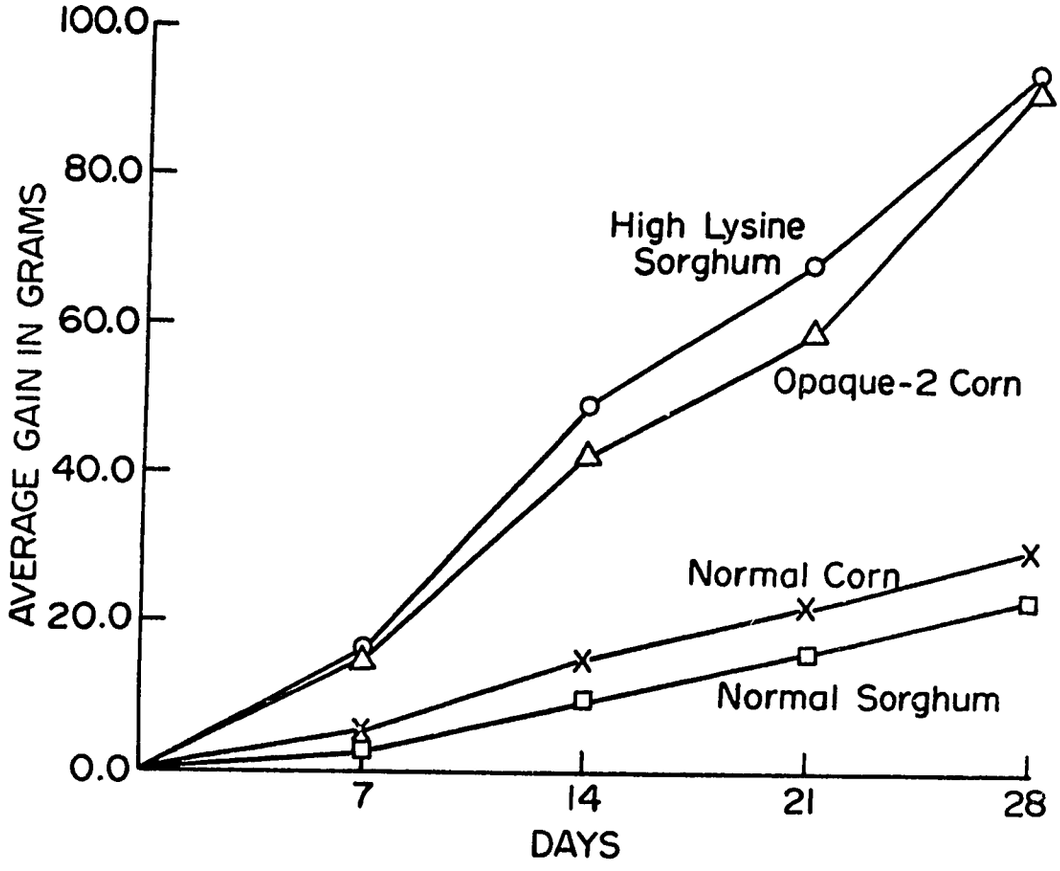

Figure 3. Average weight gain of rats fed on high lysine and normal sorghum and opaque-2 and normal corn.

TABLE 12. Comparison of feed consumption and feed efficiency ratio (FER). Experiment 3.

Genotype	Feed consumed (g)				FER		
	7 days	14 days	21 days	28 days	14 days	21 days	28 days
High lysine mutant							
IS 11758	54.7	133.2	215.3	284.5	2.8	3.2	3.0
Normal sorghum							
IS 2319	44.5	85.8	135.0	188.2	8.1	7.3	6.8
IS 1484	37.2	74.2	113.7	159.3	10.6	9.4	8.5
Mean for normal lines	40.8	80.0	124.3	173.7	9.3	8.3	7.6
Opaque-2 corn	58.3	143.5	235.7	292.2	3.4	3.7	3.4
Normal corn	49.5	102.7	164.3	226.5	7.4	7.5	7.4
Casein	51.3	129.3	222.2	363.0	1.9	2.0	2.0
Grand mean	49.2	111.4	181.0	261.2	5.7	5.6	5.2
C.V. %	15.4	10.7	12.7	12.7	29.0	19.4	16.9
S.E. of mean	3.1	4.9	9.4	13.6	0.7	0.4	0.4
LSD. 5%	9.0	14.2	27.4	39.5	2.0	1.3	1.1
1%	12.2	19.2	37.1	53.5	2.7	1.7	1.5

lysine and sugary sorghum lines. Eight rats were individually fed for 2 weeks on high lysine sorghum (IS 11758), sugary sorghum (IS 5376) and two normal sorghum varieties (IS 2319 and 2520). All rations were composed of 94% sorghum with 6% vitamins and minerals. Two-week results of this experiment compared very well with the 2-week results of experiment 3. The data presented in Table 13, and illustrated in Figure 4, show that the rat weight gain on the high lysine diet was over five times higher than the average for rats fed on the normal sorghum lines. The sugary sorghum line IS 5376 was nearly twice better in rat weight gain than the normal sorghum. The FER values were also quite comparable to the experiment 3 values.

These four experiments demonstrate unequivocally that the two high lysine sorghum lines are substantially superior in biological value to any sorghum line currently available.

There are two aspects of protein improvement in cereals, the quantity of protein in a diet and the balance of amino acids in their proteins. A line could have a lower protein content but higher concentration of essential amino acids in its protein (as is the case with opaque-2 corn), or a higher protein content with reduced essential amino acid concentration in its protein. High lysine sorghum mutants have some of both.

The amino acid pattern of high lysine mutants is greatly changed due to a single gene mutation. It is hard to say at this stage whether the increase in lysine content of both high lysine lines is due to similar gene(s). The dented kernel character may be the effect of a closely linked gene with a high lysine gene or a pleiotropic effect of the high

TABLE 13. Gains of rats on normal, sugary and high lysine sorghum. Experiment 4.

Genotype	Protein and lysine content of feed			Initial wt. of rats (g)	14 days wt. gain (g)	Per day wt. gain (g)	14 days feed consumed (g)	FER
	% protein	lysine % of protein	lysine % of sample					
High lysine mutant								
IS 11758	18.4	3.17	.583	50.8	44.2	3.16	132.2	3.05
Sugary mutant								
IS 5376	13.7	1.97	.270	50.7	14.7	1.05	94.2	6.67
Normal sorghum								
IS 2319	12.6	2.23	.281	50.7	11.9	0.85	96.6	7.79
IS 2520	12.9	1.62	.209	50.7	3.9	0.28	80.1	24.26
Mean for normal lines	12.7	1.92	.245	50.7	7.9	0.56	88.4	16.02
Grand mean	14.4	2.25	.336	50.7	18.7	1.33	100.8	10.66
C.V. %	--	--	--	8.2	32.4	32.48	14.7	100.5
S.E. of mean	--	--	--	1.5	2.1	0.15	5.2	2.5
LSD. 5%				N.S.	6.1	0.45	15.2	7.2
1%				N.S.	8.3	0.60	20.5	9.6

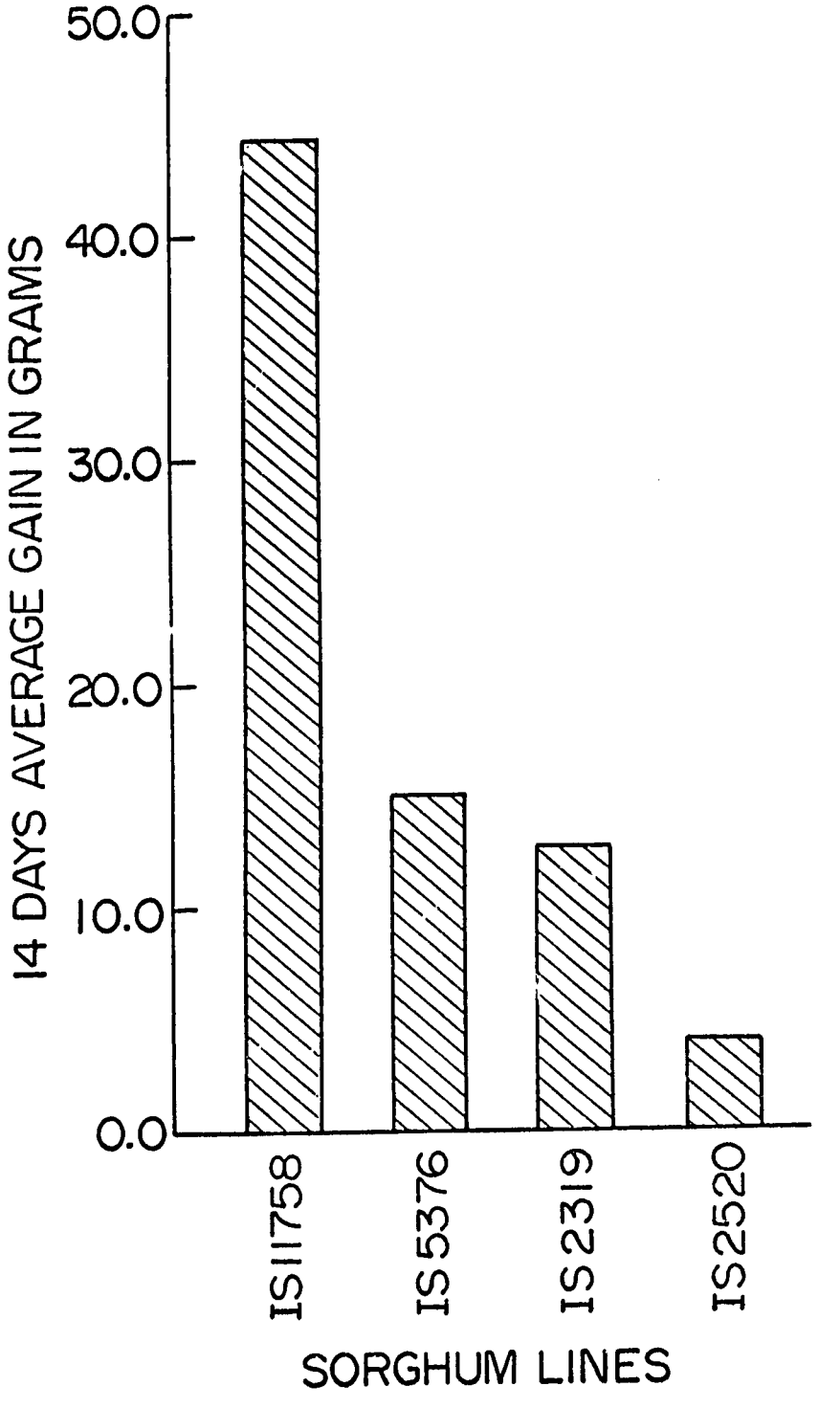

Figure 4. Average weight gain of rats on high lysine (IS 11758), sugary (IS 5376), and normal sorghum (IS 2319, IS 2520).

lysine gene. As noted earlier, the effect of the high lysine gene is not confined to lysine, but it changes the pattern of some other amino acids too as compared to normal.

Taking albino rats as test animals, their essential amino acid requirement is compared with the concentration of these amino acids in high lysine and normal sorghums in Table 14 and Figure 5. These data indicate that besides lysine, sulphur amino acids (methionine and cystine), histidine and threonine are also in short supply in average sorghum as far as the nutrition of rats is concerned. The substantial increase in gain of rat weight on high lysine mutant as compared to average sorghum may be attributed to greatly increased concentration of these amino acids in this line. Lysine content (expressed as percent of sample) in high lysine sorghum is increased to .6% as compared to 27% in average sorghum. The weanling rat requirement for lysine is 1% in the feed at 15% protein level (Mertz, 1969). Similar to $\underline{o}_2$ corn, (Barbosa, 1971) high lysine sorghum is higher in sucrose content as compared to normal corn. It may be expected that this increased sucrose content may also be adding to the overall increased nutritional quality of high lysine sorghum.

IS 11167 was noted to be slightly inferior in biological value to IS 11758. This may be attributed to the lower protein and some of the other essential amino acids content of this line as compared to IS 11758.

Increase in the biological values of sugary lines may also be attributed to their better amino acid balance coupled with more easily digestible carbohydrate (Table 15).

Table 14. Protein and essential amino acid content of average* and high lysine sorghum and opaque-2 corn** and the requirements for weanling rats.*** (Expressed as percent of sample.)

Amino acids	Average sorghum	High lysine sorghum		Opaque-2 corn	Weanling rat requirement
		IS 11167	IS 11758		
Arginine	0.45	0.72	0.83	0.76	0.2
Histidine	0.25	0.34	0.37	0.37	0.4
Isoleucine	0.51	0.58	0.71	0.36	0.5
Leucine	1.80	1.73	2.27	0.98	0.9
Lysine	0.27	0.54	0.58	0.52	1.0
Methionine plus cystine	0.42	0.51	0.66	0.46	0.6
Phenyl alanine plus tryosine	1.22	1.13	1.75	0.90	0.9
Threonine	0.41	0.48	0.55	0.40	0.5
Tryptophan	0.16	0.17	0.20	0.14	0.2
Valine	0.67	0.75	0.90	0.55	0.4
% protein	12.6	14.3	17.3	10.5	16.0

* Average of two determinations on samples from Purdue and Puerto Rico nursery.
** Nelson, 1969.
*** Mertz, 1969.

McLaughlan and Campbell (1969) have emphasized that growth of an animal under defined conditions provides a relatively simple way of measuring the value of dietary proteins. Absence of one or more of the essential amino acids in the diet will reduce or completely stop the growth.

It may be argued whether results of greater biological value of high lysine sorghum as noticed with rats can be translated directly to man. Hegsted and Worcester (1947) and Mitchell (1954) reported that man resembles the growing rat quite closely in his metabolic utilization

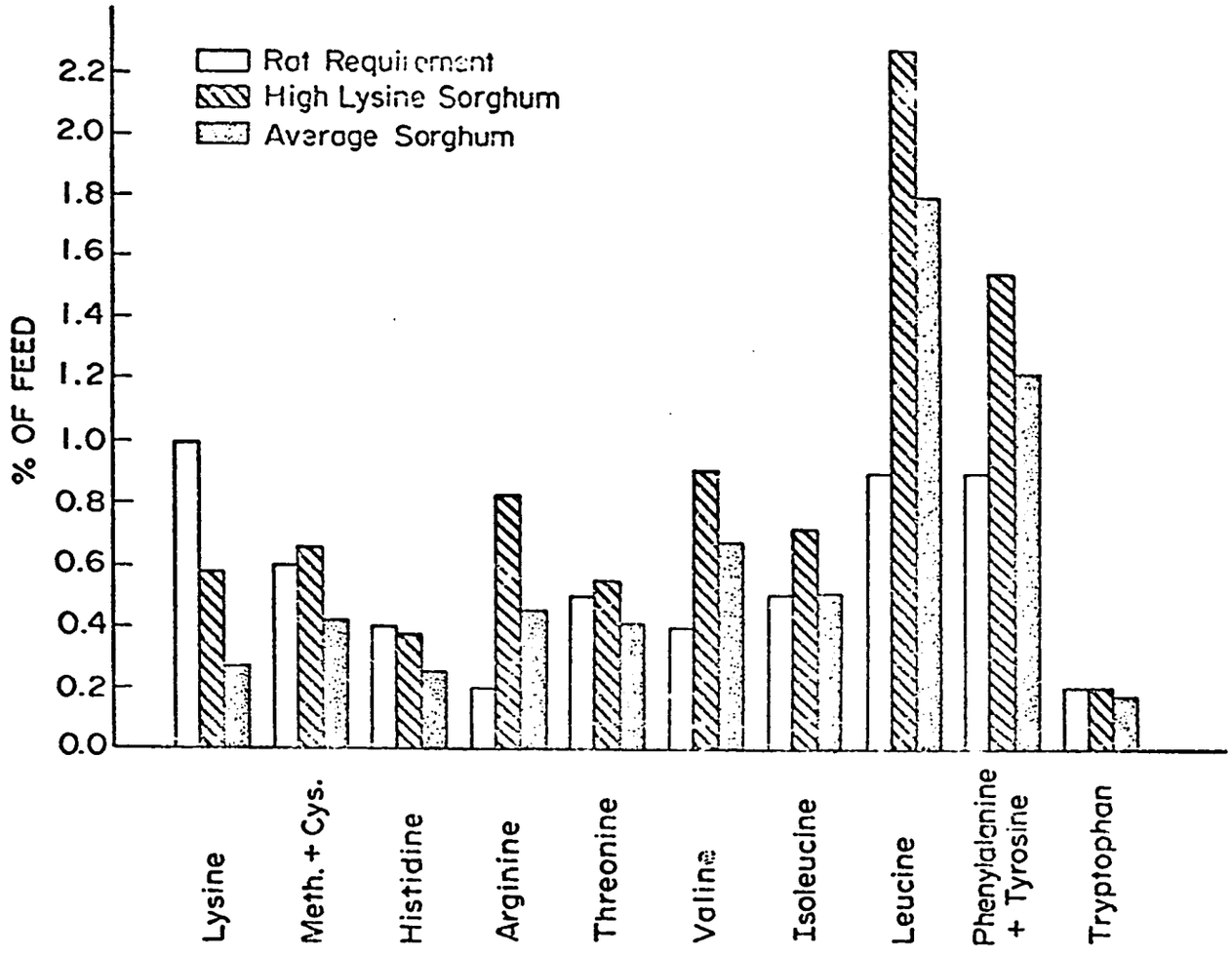

Figure 5. Comparison of weanling rat essential amino acid requirements expressed as percent of feed with high lysine and average sorghum.

TABLE 15. Protein and essential amino acid content of average and sugary* sorghum grain and the requirement for weanling rats. (Expressed as percent of sample.)

Amino acids	Average sorghum	Sugary sorghum line					Weanling rat requirement**
		IS 4526	IS 4668	IS 5376	IS 5614	IS 5623	
Arginine	0.45	0.58	0.49	0.56	0.54	0.50	0.2
Histidine	0.25	0.30	0.29	0.31	0.31	0.30	0.4
Isoleucine	0.51	0.63	0.60	0.60	0.58	0.52	0.5
Leucine	1.80	2.10	2.12	2.16	2.04	1.80	0.9
Lysine	0.27	0.42	0.33	0.35	0.36	0.35	1.0
Methionine plus cystine	0.42	0.61	0.54	0.61	0.66	0.53	0.6
Phenylalanine plus tyrosine	1.22	1.46	1.41	1.47	1.36	1.24	0.9
Threonine	0.41	0.47	0.43	0.47	0.43	0.41	0.5
Tryptophan	0.16	0.17	0.15	0.15	0.14	0.14	0.2
Valine	0.67	0.75	0.71	0.74	0.72	0.66	0.4
% protein	12.6	15.6	14.6	15.0	14.4	13.5	16.0

* Average of two determinations on samples from Purdue and Puerto Rico grow out.
** Mertz, 1969.

of food protein. They reported that the rat growth results were very much applicable to the evaluation of human diets. Howe, Jansen and Gilfillian (1965) also pointed out that since weanling rats are rapidly growing animals, their nutritional requirement may be more stringent than those of men. Therefore, a protein which is performing well in weanling rats will almost certainly prove satisfactorily in the infants. Better nutritive value of opaque corn protein for humans has been demonstrated by Bressani (1966) and Byrnes (1969). Therefore, the greater quality of high lysine and sugary sorghum protein as observed with rats should also be superior to normal sorghum protein in the diets of man and domestic animals. Mertz, Nelson, Bates and Veron (1969), while discussing the nutritional value of $\underline{o}_2$ gene in high protein background, have indicated that 150 g of opaque-2 maize with 20% protein and 3 g lysine/100 g protein should provide the minimum daily lysine requirement of most 70 kg adults. High lysine sorghum line IS 11758, therefore, can be very well used as protein supplement in the diets of humans in the countries where sorghum is a staple food.

Another point of concern is the influence of high lysine (hl) gene in the plants carrying it. At this stage it is hard to speculate on its yield potential. The test weight of IS 11758 is 10% lower than average sorghum, while IS 11167 has a 10% higher test weight than average sorghum. However, a slight decrease in yield could be tolerated on the basis that one would require only about one-third of high lysine sorghum as compared to normal sorghum grain to get similar growth rate. When feed consumption is not taken into consideration, high lysine sorghum would sustain five

times better growth than normal sorghum. PER value for this line is only twice the normal sorghum due to its higher inherent protein level.

Direct use of these high lysine mutant lines in the United States and other mechanized parts of the world may be questionable primarily due to their taller plant height. However, the simple recessive mode of inheritance of the high lysine gene would make it easy to transfer it into the locally adapted varieties. In countries where dual purpose type (for grain as well as fodder) varieties are grown, it could be possible to use these lines as such.

SUMMARY AND CONCLUSIONS

High lysine and sugary mutant lines which have many desirable changes in their amino acid composition were found to have much higher nutritional quality than any sorghum line currently available. The high lysine line was found to have highest nutritional quality. When fed at 12.5% protein level, it was three times better than normal sorghum lines as far as the weight gain of rats was concerned. Weight gain of rats on our currently best nutritional line IS 2319 was only 54% of this line. Among sugary endosperm mutants IS 4526, 5614, and 5623 gave significantly higher ($P < .05$) weight gain of rats over normal lines.

Feed consumption of rats per gram of body weight gain (FER) was only 35% of the normal sorghum lines for the high lysine line IS 11758. Average FER ratio for three nutritionally better sugary lines was 5.8 as compared to 13.3 for normal sorghum lines. Protein efficiency ratio (PER) for

IS 11758 was 1.61 as compared to 0.77 for normal sorghum lines. This represents that the protein quality of high lysine sorghum is more than twice better than the average sorghum lines.

The results of the second experiment confirmed the superior biological value of high lysine line IS 11758 and sugary lines IS 4526, 5614 and 5623. IS 5376, which was noted to show lower rat weight gain, performed similar to the above three sugary lines in this experiment. Rat weight gain on high lysine line IS 11167 was significantly lower ($P < .05$) than IS 11758. This may be due to its lower protein and other essential amino acid contents than IS 11758.

Rat feeding experiment 3 compares the nutritional quality of high lysine sorghum IS 11758 with two normal sorghum lines IS 2319 and IS 1484, opaque-2 and normal corn and a casein diet. The casein diet was fed at a protein level similar to the high lysine sorghum diet. All sorghum and corn samples were fed without any dilution except usual mineral and vitamin supplementation.

Rats gained over three and one-half times more on high lysine sorghum than our current nutritionally best line IS 2319. On the average IS 2319 has been found twice better in nutritional quality as compared to most sorghum lines. When compared to the opaque-2 corn diet, the rats gained 15% more weight on IS 11758. Weight gain of rats on casein diet was significantly higher ($P < .01$) than all diets.

Feed efficiency ratio on IS 11758 was 3.2 as compared to 7.3 for IS 2319, 3.7 for opaque-2 corn and 7.5 for normal corn. This shows that for similar gain in rat weight only about 86% of the amount of opaque-2

corn feed is needed for high lysine sorghum.

Experiment 4, which was conducted under different laboratory conditions, also proved the greater nutritional value of high lysine sorghum.

Based on good correlation between weanling rat protein requirements and that of humans, it is expected that high lysine sorghum, which is superior to any sorghum and corn diet in rats, would have similar value in the diet of man and domestic animals.

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INHERITANCE OF HIGH LYSINE AND SUGARY LINES AND THEIR AMINO ACID COMPOSITION

Rameshwar Singh and John D. Axtell

Selected floury and sugary lines were grown at the Purdue Agronomy Farm and in Puerto Rico during the summer of 1972 and winter of 1972-73 respectively. IS 11167 and 11758 were very late at Purdue. To get mature seeds, headed plants were transplanted from the field to the greenhouse.

Crosses were made on Redlan and Martin male steriles as well as on Purdue population 3R (PP3R$\underline{ms}_3$) genetic male sterile plants during 1972. F_1 progenies were grown in Puerto Rico and fifteen plants were selfed from each row to obtain F_2's. B (non-restorer) and R (restorer) reaction was noted by checking the seed set on selfed heads. PP3R$\underline{ms}_3$ does not segregate for male sterility in F_1 generation and all F_1 plants behave as fertile. Restorer lines produce fertile F_1's when crossed on cytoplasmic male sterile lines while non-restorer produce sterile F_1's. B types were backcrossed, using pollen from the original male parent. F_2 and backcross segregation ratios (normal vs. high lysine and normal vs. sugary) were observed. Chi-square tests were performed to test goodness of fit on these ratios to a theoretical ratio.

Plant height and number of days to 50% flowering were recorded. One hundred seed weight was calculated by weighing 100 random seeds. Germ and endosperm of 100 kernels were separated by hand dissection for estimating their proportion by weight.

Chemical Evaluation

Whole kernel and endosperm samples of two high lysine and five sugary lines were analyzed for crude protein and amino acid composition (g/100 g protein). Oil content of whole kernels was also determined. Amino acid analysis was done with an automatic Beckman Model 120C ion-exchange resin chromatography amino acid analyzer except for methionine, cystine and tryptophan which were analyzed separately.

Methionine and cystine were determined as methionine sulfone and cysteic acid using the method described by Moore (1963). Tryptophan was determined by the method of Slump and Schrender (1969). Percent oil was determined by nuclear magnetic resonance (NMR) at the University of Illinois, Urbana, Illinois.

A more critical test of the affect of the high lysine (hl) and sugary (su) genes on altered amino acid pattern and carbohydrate synthesis was made by analyzing normal and mutant kernels obtained from segregating F_1 heads of + + X hl hl and + + X su su crosses. Genetic male sterile PP3Rms_3 was used as the normal parent. F_2 kernels were separated into normal (vitreous) and floury kernels. Floury endosperm was homozygous for the hl gene (hl hl hl) while normal kernels had none, one or two doses of hl gene (+ + +, + + hl, + hl hl). Similarily kernels from + + X su su F_2's were separated into normal and mutant classes. Here mutant classes were homozygous for the su gene (su su su) while normal kernels had none, one or two doses of su gene (+ + +, + + su, + su su).

RESULTS AND DISCUSSION

Inheritance of High Lysine Character

Preliminary results of the inheritance study described here suggests a simple recessive mode of inheritance for the high lysine character. Both phenotypic classification of kernels and amino acid analysis was carried out on kernels obtained from individual F_2 heads (from normal and high lysine crosses) on which both normal and high lysine kernels were found. The normal line used as female parent was PP3R$\underline{ms}_3$ genetic male sterile.

PP3R$\underline{ms}_3$ has vitreous kernels while high lysine lines IS 11167 and IS 11758 have floury kernels. All F_1 seeds obtained from PP3R$\underline{ms}_3$ X IS 11167 or PP3R$\underline{ms}_3$ X IS 11758 crosses were vitreous. Chi-suare analysis of F_2 endosperm data presented in Table 1 indicates a reasonable fit to a 3 vitreous: 1 floury ratio for both crosses.

Table 1. Classification of F_2 kernels from normal X high lysine crosses with χ^2 and probability values.

Cross		Number of Seeds				
Female	Male	Vitreous	Floury	Total	χ^2	P
PP3R$\underline{ms}_3$ X	11167	3593	1167	4760	0.592*	.25-.50
PP3R$\underline{ms}_3$	11758	2376	815	3191	0.497*	.25-.50

* Based on 3:1 expected ratio

Amino Acid Composition of Segregating Kernels

Table 2 presents the protein and lysine content of vitreous and floury kernels from segregating heads. This provides a more critical test of the affect of the high lysine gene on lysine content. Use of vitreous and floury kernels from the same head eliminates the obvious criticism of comparing kernels from different heads with different gene backgrounds.

None of the vitreous and floury classes from the same segregating head had similar protein content. Protein content of floury kernels was usually higher than vitreous kernels. Average increase in lysine content of floury kernels was 53% in case of PP3R$\underline{ms}_3$ X IS 11167 cross and 43% for PP3R$\underline{ms}_3$ X IS 11758 cross. However, when lysine as percent of sample was compared (which takes into account the differences in protein level also), the average increase in lysine content of floury kernels was 73% for the first cross and 76% for the second cross.

The differences in the lysine content of both kernel classes were more pronounced in the endosperm tissues (Table 3). The average lysine (% of protein) content of the endosperm of vitreous kernels in both crosses was 1.38% as compared to 2.53% for the endosperm of floury kernels. This represents an average increase of 83% in the lysine content of the latter. As noted for whole kernels, the increases were more pronounced when lysine expressed as percent of sample is compared.

Similar to the opaque-2 mutants of corn (Nelson, 1969) the affect of the high lysine gene is confined to endosperm only. Table 4 presents protein and lysine contents of defatted embryo of vitreous and floury

Table 2. Protein and lysine content of defatted vitreous and floury kernels from segregating heads.

Genotype	Kernel Classes	% protein	Lysine (% of protein)	Lysine (% of sample)
PP3Rms$_3$ X IS 11167	Vitreous (1)*	11.5	2.12	.243
	Floury (1)	12.9	3.38	.436
	Vitreous (2)	11.7	2.02	.236
	Floury (2)	13.3	3.03	.403
	Vitreous (3)	10.7	2.11	.226
	Floury (3)	12.0	3.26	.391
	Vitreous (4)	12.1	2.08	.252
	Floury (4)	14.3	3.09	.442
	Vitreous (5)	12.7	2.13	.271
	Floury (5)	14.2	3.21	.456
Mean	Vitreous	11.7	2.09	.246
	Floury	13.3	3.19	.426
PP3Rms$_3$ X IS 11758	Vitreous (1)	11.1	2.60	.287
	Floury (1)	13.1	3.55	.465
	Vitreous (2)	13.3	2.20	.293
	Floury (2)	17.0	2.75	.468
	Vitreous (3)	13.0	1.90	.247
	Floury (3)	15.0	3.00	.450
	Vitreous (4)	11.6	2.02	.234
	Floury (4)	14.9	3.17	.472
Mean	Vitreous	12.2	2.18	.265
	Floury	15.0	3.12	.464
PP3Rms$_3$	---	14.8	1.84	.273
IS 11167	---	16.6	3.36	.558
IS 11758	---	17.5	3.38	.592

*Vitreous and floury classes with same preceding numbers are segregates from same head.

Table 3. Protein and lysine content of defatted endosperm of vitreous and floury classes.

Genotype	Kernel Classes	% protein	Lysine (% of protein)	Lysine (% of sample)
PP3R$\underline{ms}_3$ X IS 11167	Vitreous (1)*	9.8	1.68	.165
	Floury (1)	10.1	2.83	.286
	Vitreous (2)	10.6	1.37	.145
	Floury (2)	11.2	2.24	.251
Mean	Vitreous	10.2	1.52	.155
	Floury	10.6	2.53	.268
PP3R$\underline{ms}_3$ X IS 11758	Vitreous (1)	12.2	1.10	.134
	Floury (1)	14.8	2.29	.389
	Vitreous (2)	10.4	1.36	.141
	Floury (2)	11.9	2.78	.331
Mean	Vitreous	11.3	1.23	.137
	Floury	13.3	2.54	.360
PP3Rms$_3$	---		1.14	.164
IS 11167	---		2.48	.377
IS 11758	---		2.43	.401

* Vitreous and floury classes with same preceding numbers are segregates from same head.

Table 4. Protein and lysine content of defatted embryo and germ proportion of the seed of vitreous and floury classes from same segregating head.

Genotype	Kernel Class	% protein	Lysine (% of protein)	Germ %
PP3R$\underline{ms}_3$ X IS 11167	Vitreous	24.1	5.11	10.2
	Floury	24.4	5.60	16.0

classes, and also germ proportion in these two classes of kernels of PP3Rms_3 X IS 11167 cross. The protein and lysine contents of both classes were practically similar. Germ proportion in floury kernels was 16.0% as compared to 10.2% in vitreous ones.

The amino acid composition of the defatted endosperm tissue and whole kernels of vitreous and floury classes are presented in Tables 5 and 6 respectively. It may be noticed that the contents of lysine, arginine, aspartic acid and glycine are greatly increased in the floury endosperm along with slight increases in the contents of threonine, valine and isoleucine. Greater decreases are seen in glutamic acid, proline, alanine and leucine. Valid comparison of sulphur amino acids (methionine and cystine) is not possible due to oxidation during hydrolysis. The shift in amino acid pattern of normal and floury endosperm classes are very much comparable to those reported for opaque-2 mutant and normal corn (Nelson, 1969) except that histidine content does not seem to increase in the high lysine mutant as was the case with o_2. Besides these changes in the amino acid pattern, slight increase in the protein content of the floury kernels is also noticed.

These results suggest that increased lysine content in IS 11167 and IS 11758 is the result of a single gene mutation. The high lysine gene is inherited as a simple Mendelian recessive and should be present in homozygous condition for its expression. The high lysine gene has been designated hl.

Table 5. Amino acids in the defatted endosperm of vitreous and floury kernels. (Expressed as g/100 g protein.)

Amino acid	PP3Rms$_3$ X IS 11167		PP3Rms$_3$ X IS 11758	
	Vitreous	Floury	Vitreous	Floury
Lysine	1.20	2.18	1.30	2.59
Histidine	1.99	1.91	1.94	2.04
Arginine	2.55	3.66	2.77	4.44
Aspartic acid	5.56	7.49	6.14	7.24
Threonine	2.56	3.01	2.75	3.11
Serine	3.74	4.09	3.84	3.99
Glutamic acid	26.68	23.98	27.99	20.82
Proline	7.92	6.91	8.35	6.39
Glycine	2.17	2.97	2.37	3.40
Alanine	10.15	9.65	10.50	8.68
Cystine*	0.99	0.91	1.15	0.99
Valine	4.35	4.98	4.71	4.78
Methionine*	1.21	1.00	1.42	1.21
Isoleucine	3.90	4.16	4.05	3.99
Leucine	15.72	14.33	15.93	12.62
Tyrosine	4.28	4.32	4.54	4.24
Phenylalanine	5.45	5.45	5.52	5.24
Percent protein	9.75	10.75	9.69	12.06
Leucine/isoleucine	4.03	3.44	3.93	3.16

*Cystine and methionine values are lower than their actual contents due to oxidation during the hydrolysis.

Table 6. Amino acids in the defatted whole kernels of vitreous and floury classes. (Expressed as g/100 g of protein.)

	PP3Rms$_3$ X IS 11167		PP3Rms$_3$ X IS 11758	
Amino acid	Vitreous	Floury	Vitreous	Floury
Lysine	1.68	2.71	1.59	2.94
Histidine	2.07	2.09	1.95	2.08
Arginine	3.41	4.46	3.45	4.95
Aspartic acid	6.21	7.50	7.21	7.69
Threonine	2.79	3.05	2.73	3.05
Serine	3.93	3.84	4.10	4.17
Glumatic acid	25.52	20.91	25.58	22.28
Proline	7.49	6.14	7.86	6.08
Glycine	2.61	3.47	2.52	3.54
Alanine	9.86	8.78	10.75	9.23
Cystine*	0.71	1.07	1.07	1.07
Valine	4.58	5.06	4.56	4.77
Methionine*	1.05	1.15	0.91	1.18
Isoleucine	3.78	3.92	3.98	3.88
Leucine	14.63	12.41	16.39	13.10
Tyrosine	4.21	4.01	4.60	4.30
Phenylalanine	5.24	5.00	5.68	5.38
Percent protein	11.1	13.1	12.4	15.1
Leucine/isoleucine	3.9	3.2	4.12	3.38

* Cystine and methionine values are lower than their actual contents due to oxidation during the hydrolysis.

Amino Acid Composition of Defatted Whole Kernels and Endosperm of Two High Lysine Lines

The amino acid composition of defatted whole kernels and endosperm of IS 11167 and IS 11758 is presented in Tables 7 and 8 respectively. Weighted average of the amino acid composition of whole kernels of 522 sorghum lines (Srinivasan, Axtell and Jambunathan, 1972) is used for comparing whole kernel results, while average amino acid composition of the endosperm of two normal lines is used to compare endosperm results. Since the entire seed is used for most practical purposes, it is important to compare the amino acid composition of the whole kernel of the two high lysine lines while endosperm data would eliminate any artifact of germ size and would present the actual effect of hl gene.

Tables 7 and 8 indicate that lysine content of the whole kernel as well as the endosperm of IS 11167 and IS 11758 is substantially higher than the average lysine content of the whole kernel and endosperm of normal lines. Other desirable changes are increases in methionine and cystine and decrease in leucine content and narrower leucine:isoleucine ratio. These changes in amino acid contents coupled with an increased protein content should make these lines an ideal food and feedstuff for humans and domestic animals.

Protein and Lysine Contents of Lines Phenotypically Resembling to High Lysine Lines

Another obvious point of an argument may be whether the high lysine character is associated with dented or shrunken kernel phenotype. Many

Table 7. Amino acids in the defatted whole kernels of average sorghum and high lysine lines grown at Lafayette and in Peurto Rico. (Expressed as percent of protein.)

Amino acids	Average sorghum*	IS 11167		IS 11758	
		Laf.	P.R.	Laf.	P.R.
Lysine	2.14	3.41	3.38	3.00	3.26
Histidine	2.01	2.35	2.41	2.13	2.14
Arginine	3.59	5.06	5.23	4.68	4.92
Aspartic acid	7.83	7.02	7.88	6.82	7.92
Threonine	3.26	3.38	3.66	3.26	3.06
Serine	4.52	4.21	4.84	4.28	4.16
Glutamic acid	23.22	19.90	23.93	22.35	22.50
Proline	8.16	6.82	6.95	6.91	6.50
Glycine	3.07	4.07	4.15	3.79	3.70
Alanine	9.89	8.43	9.09	9.34	9.05
Cystine	1.50**	1.82	---	1.93	---
Valine	5.35	5.25	5.10	5.10	5.25
Methionine	1.80**	1.75	---	2.05	---
Isoleucine	4.08	4.06	4.32	4.08	4.15
Leucine	14.27	11.96	13.91	13.41	12.96
Tyrosine	4.50	3.99	4.11	4.39	4.17
Phenylalanine	5.19	5.16	5.26	5.52	5.34
Tryptophan	1.31**	1.19	---	1.21	---
Percent protein	12.6	14.30	16.5	16.56	17.80
Leucine/isoleucine	3.50	2.95	3.22	3.29	3.12

* Weighted average for 522 lines (Srinivasan et al., 1972)

** Weighted average for 3 lines.

*** Weighted average for 9 lines.

Table 8. Amino acids in the defatted endosperm of average sorghum and high lysine lines grown at Lafayette and in Puerto Rico. (Expressed as percent of protein.)

Amino acid	Average sorghum*	IS 11167		IS 11758	
		Laf.	P.R.	Laf.	P.R.
Lysine	2.00	3.01	2.46	2.45	2.43
Histidine	2.55	2.19	1.92	1.98	1.85
Arginine	3.60	4.70	4.44	3.82	3.83
Aspartic acid	8.20	7.56	7.41	7.00	6.48
Threonine	3.60	3.49	3.08	3.25	2.96
Serine	4.95	4.35	4.44	4.33	3.97
Glutamic acid	26.95	19.92	23.89	23.32	24.52
Proline	9.05	7.59	6.91	7.71	7.14
Glycine	2.95	4.14	3.29	3.48	3.03
Alanine	10.95	8.41	9.56	9.67	9.38
Cystine	1.75	---	---	---	---
Valine	5.70	5.35	4.90	5.11	4.90
Methionine	1.35	---	---	---	---
Isoleucine	4.60	4.18	4.18	4.31	4.33
Leucine	16.70	12.34	14.60	14.38	14.18
Tyrosine	5.30	4.19	4.38	4.49	4.46
Phenylalanine	6.00	5.22	5.63	5.74	5.58
Percent protein	9.3	12.1	14.1	13.9	16.5
Leucine/isoleucine	4.60	2.95	3.49	3.34	3.27

*Average of two lines (Jambunathan and Mertz, 1972).

lines with kernel phenotype similar to the high lysine lines (IS 11167) and IS 11758 were identified from the world sorghum collection in the course of the initial survey for high lysine and sugary lines and their protein and lysine content is presented in Table 9. None of these lines have lysine values comparable to high lysine lines. The range of lysine as percent of protein was from 1.68 to 2.18 in these lines. Lysine as percent of dry sample varied from 0.240 to 0.321% as compared to 0.558 and 0.592% in high lysine lines. This indicates that the increased lysine content of the high lysine lines is not due to the shrunken or dented kernel phenotype and is not an artifact of reduced endosperm size.

Agronomic Traits of the High Lysine Lines

Besides their unusually higher level of lysine (50-60% higher than the average sorghum), the high lysine lines are 20 to 40% higher in protein content than normal sorghum lines. Though such higher levels of protein are not uncommon in the world sorghum collection (Srinivasan, Axtell and Oswalt, 1972b), the higher percentage of lysine in protein of these lines coupled with the high protein content of grain elevates the overall lysine content of their grain (lysine expressed as percent of sample) to almost double of normal sorghum. The average lysine content (expressed as percent of sample) of 31 genotypes over six locations and two years was 0.258% (Table 10) as compared to 0.524 and 0.540 for IS 11167 and IS 11758 respectively. The oil content of these lines was also nearly double that of normal sorghum (6.34% as compared to 3.32% in normal sorghum). The average

Table 9. Protein and lysine contents of some shrunken lines from world sorghum collection.

Genotype	Endosperm type	Protein (%)	Lysine % of protein	Lysine % of sample
High lysine lines				
IS 11167	floury	16.6	3.36	.558
IS 11758	floury	17.5	3.38	.592
Shrunken endosperm lines				
IS 2861	floury	15.6	2.06	.321
IS 3125	vitreous	16.2	1.68	.272
IS 3551	floury	11.2	2.14	.240
IS 4622	vitreous	16.4	1.84	.302
IS 4960	floury	13.8	2.18	.301
IS 4994	vitreous	12.1	2.18	.264

Table 10. Agronomic traits of high lysine lines grown at Lafayette and in Puerto Rico.

Trait	IS 11167		IS 11758		Normal sorghum*
	Laf.	P.R.	Laf.	P.R.	
Protein (%)	14.80	16.60	16.00	18.40	12.70
Lysine (% of protein)	3.31	3.36	3.00	3.26	2.05
Lysine (% of sample)	.490	.558	.480	.600	.258
Oil (%)	---	5.81	6.97	6.25	3.32
Seed Weight (g/100 seeds)	2.55	3.01	2.50	2.40	2.75
Protein per seed (mg)	3.77	5.00	4.00	4.42	3.53
Lysine per seed (mg)	.125	.168	.120	.144	.071
Percent germ in seed	14.0	15.3	15.4	17.2	10.10**
Height (cm)	395.0	250.0	385.0	245.0	200.0
B and R reaction	---	B	---	B	---

* Average of 31 genotypes over six locations and two years. (Schaffert, 1972)

**Average of 4 lines (Jambunathan and Mertz, 1973)

B Non-restorer

R Restorer

test weight of IS 11167 and IS 11758 was 2.78 and 2.45 g/100 seed respectively as compared to 2.75 g/100 seed for normal sorghum. The protein and lysine contents when calculated on a per seed basis is much higher in these lines as compared to normal sorghum. The average protein per seed for IS 11167 and IS 11758 is 4.38 and 4.21 mg as compared to 3.53 mg for normal sorghum. The average lysine per seed in the high lysine lines is nearly double the average lysine in normal sorghum seed. The germ content of these lines is nearly 55% higher than normal sorghum. Plant height of these two high lysine lines interacts much with the environment. A slight effect of environment was noted on all the characters described above while plant height was substantially reduced under the tropical environment of Puerto Rico. Both lines are non-restorer (B) type. This provides an opportunity for their utilization in hybrid programs by converting them to cytoplasmic male sterile lines.

The inheritance data presented in Tables 1, 2, 3, 4, 5 and 6 suggests a simple recessive mode of inheritance of the high lysine gene. It is noted that floury endosperm and slight indentation of the kernels is associated with the high lysine character. This may be due to a pleiotropic effect of the high lysine gene or the effect of other closely linked gene. However, an extreme variation in the extent of indentation and test weight of kernels carrying the high lysine gene has been observed in various backgrounds. Therefore, it seems likely that it should be possible to get almost plump kernels of high lysine lines in some genetic backgrounds. Besides, the background effect is also evident on the lysine content of mutant (floury) kernels. Table 2 indicates that the lysine

content of floury kernels on PP3R$\underline{ms}_3$ (a segregating population) background varies from 2.75 to 3.55% at an average protein level of 14.1%. The average lysine content of PP3R$\underline{ms}_3$ is 1.84% which is relatively low. Two naturally occurring high lysine mutant lines IS 11167 and IS 11758 have average lysine contents of 3.34 and 3.13% at 15.7 and 17.2% protein level. These mutant lines represent the effect of high lysine gene in high protein background, similar to the effect of $\underline{o}_2$ gene in Illinois High Protein (IHP) maize. These results indicate that it could be possible to get still higher values of lysine (expressed as percent of protein) in proper background besides the possibility of getting a relatively plump kernel as mentioned earlier.

Amino acid analysis data presented in Table 5 indicates that many interesting changes are taking place in high lysine kernels. Lysine content is greatly changed. Contents of other essential amino acids like threonine and isoleucine are also slightly increased. Another desirable change is the reduction in the leucine level and overall reduced leucine to isoleucine ratio. Reduction in leucine content is very important as its excessive concentration in corn and sorghum protein has been indicated as possible reason for pellagra (Gopalan and Srikantia, 1960). Isoleucine has been found to have an antagonistic effect, which partially compensates for excessive leucine.

Another desirable feature of high lysine gene is its effect on increased germ content. Protein in the germ is of superior nutritional quality.

Based on these desirable changes in the high lysine kernels, it is

expected that it should have much greater nutritional quality as compared to the normal sorghum.

Inheritance of the Sugary Gene
Phenotypic Segregation

The F_2 segregation data for three sugary lines IS 4668, 5376, and 5614, and back-cross segregation data for IS 4526 is presented in Table 11. The endosperm phenotype of normal X sugary F_1's were normal in all four cases. Data from classification of F_2 kernels from PP3R$\underline{ms}_3$ X sugary crosses show a good fit to 3 plump to 1 mutant segregation with chi-square probability values of 0.25 to 0.75. Plump and mutant kernel phenotypes are shown in Figure 5. Back-cross data also indicated a reasonable fit to a 1:1 ratio for plump and mutant classes. This supports the simple recessive mode of inheritance of the sugary (su) gene as previously reported (Karper and Quinby, 1963; Gorbet and Weibel, 1972).

Carbohydrate Analysis of the Endosperm of Segregating Kernels

Data presented in Table 12 indicate that the total sugar content in the endosperm of mutant sugary kernels was 2 to 4 times higher than their normal counterpart plump kernels. Change in the WSP content was also very pronounced. The average WSP content of normal endosperms was 0.89%, whereas the WSP content of mutant endosperms was approximately 22%. Starch content of the mutant endosperm was reduced by 71% of the normal endosperm tissue. No difference was noted in the amylose content of the two kernel classes. This carbohydrate analysis confirms the classification

Table 11. Classification of F_2 and backcross seeds from normal X sugary crosses with chi-square (χ^2) and probability values.

Cross		Number of seeds			χ^2	
Female	Male	Plump	Mutant	Total		P
F_2						
PP3Rms_3	X 4668	1355	430	1785	.789*	.25 - .50
PP3Rms_3	X 5376	1573	514	2087	.153*	.50 - .75
PP3Rms_3	X 5614	1439	465	1904	.338*	.50 - .75
Backcross						
Redlan *ms*	X IS 4526	415	435	850	.470**	.25 -.50

* Based on 3:1 expected ratio.
** Based on 1:1 expected ratio.

of these lines in the world sorghum collection as sugary mutants.

Amino Acid Compositions of Defatted Whole Kernels of Sugary Lines

In general all sugary lines were higher in protein content (Table 13) than the average of sorghum lines in the world collection. Amino acid analysis indicates that the content of lysine, methionine and cystine were increased. These changes should add to the improved nutritional quality of sugary lines. It may be noted that lysine content in the 1972-73 crop was slightly reduced in most cases. This was probably due to a higher percentage of outcrossing because of late bagging of heads, and the composite chemical analysis of a mixture of normal and sugary kernels.

TABLE 12. Carbohydrate analysis of the endosperm of plump and mutant kernels of segregating heads. (Expressed as percent of dry wt.)

Genotype	Kernel class	Reducing sugars	Sucrose	Total sugars	WSP	Starch	Amylose (% of starch	Total carbohydrates
PP3Rms$_3$ X IS 4668	plump	0.23	0.08	0.31	0.79	69.1	26.2	70.2
	mutant	0.64	0.69	1.33	22.60	49.1	26.9	73.0
PP3Rms$_3$ X IS 5376	plump	0.29	0.10	0.39	0.99	69.1	27.5	70.4
	mutant	0.44	0.56	1.00	21.30	48.6	25.6	74.2

TABLE 13. Amino acids in the defatted whole kernels of average sorghum and sugary mutants grown at Lafayette and in Puerto Rico. (Expressed as percent of protein.)

Amino acids	Average sorghum*	IS 4526		IS 4668		IS 5376		IS 5614	IS 5623	
		Laf.	P.R.	Laf.	P.R.	Laf.	P.R.	P.R.	Laf.	P.R.
Lysine	2.14	2.71	2.65	2.18	2.47	2.27	2.37	2.47	2.73	2.35
Histidine	2.01	1.97	1.86	2.01	1.99	2.19	1.88	2.12	2.23	2.11
Arginine	3.59	3.85	3.54	3.32	3.39	4.02	3.48	3.74	3.68	3.78
Aspartic acid	7.83	7.31	6.92	6.85	6.75	7.46	6.52	7.36	6.97	6.53
Threonine	3.26	3.30	2.77	3.08	2.81	3.32	2.95	3.01	3.17	2.87
Serine	4.52	4.25	3.70	4.04	3.80	4.31	4.22	4.11	4.09	3.73
Glutamic acid	23.22	23.01	22.15	24.39	24.67	25.03	23.76	25.12	22.21	23.61
Proline	8.16	7.36	6.64	7.90	7.41	7.96	7.56	7.47	7.32	7.35
Glycine	3.07	3.26	2.99	3.02	2.95	3.53	3.13	3.44	3.29	3.20
Alanine	9.89	9.43	8.85	9.82	9.59	10.18	9.50	9.68	9.07	9.25
Cystine	1.50	1.85	--	1.88	--	1.84	--	2.00	1.79	--
Valine	5.35	4.96	4.66	4.91	4.82	5.27	4.54	5.02	4.82	4.92
Methionine	1.80	2.47	--	1.74	--	1.90	--	2.50	1.72	--
Isoleucine	4.08	4.13	3.90	4.14	4.06	4.22	3.86	4.04	3.87	3.97
Leucine	14.27	13.71	13.19	14.63	14.49	14.81	14.01	14.15	13.06	13.71
Tyrosine	4.50	4.29	4.03	4.46	4.21	4.61	4.34	4.27	4.23	4.16
Phenylalanine	5.19	5.35	5.07	5.50	5.21	5.58	5.17	5.18	5.00	5.09
Tryptophan	1.31	1.05	--	1.01	--	0.92	--	1.00	0.93	--
% Protein	12.60	16.20	15.0	14.90	14.30	16.30	13.60	14.00	15.10	11.80

* See Table 10.

Agronomic Traits of Sugary Lines

All five sugary lines had a relatively higher protein content than the average of 31 normal sorghum lines. Lysine expressed as percent of protein and also as percent of sample, as well as oil contents, were also higher than normal lines except for IS 4668 which was not much different from normal sorghum. 100 seed weight of the sugary lines was lower and averaged at 2.11 g as compared to 2.75 g for normal sorghum (Table 14). Some improvement in the test weight of sugary seeds from Puerto Rico was noted which may be partly due to a higher frequency of out-crossing noted previously. Per seed protein and lysine content was not higher than normal sorghum lines. IS 4526, 5614 and 5623 were non-restorer (B-lines) types while IS 4668 and 5376 were restorer (R-lines) types. Having both B and R lines among sugary lines would be desirable for development of a hybrid production program.

SUMMARY AND CONCLUSIONS

A high lysine gene (designated hl) has been found to be present in two sorghum lines of Ethiopian origin. It is inherited as simple Mendelian recessive. The hl gene causes major increases in lysine, arginine, aspartic acid and glycine and slight increases in threonine, valine and isoleucine. Decreases in the contents of glutamic acid, proline, alanine and leucine were also evident.

As reported earlier, the sugary (su) gene was found to be inherited as simple Mendelian recessive. Homozygous recessive condition should

TABLE 14. Agronomic traits of sugary mutants grown at Lafayette and in Puerto Rico.

Trait	IS 4526		IS 4668		IS 5376		IS 5614		IS 5623		Average sorghum*
	Laf.	P.R.	Laf.	P.R.	Laf.	P.R.	Laf.	P.R.	Laf.	P.R.	
Protein (%)	16.2	15.0	14.9	14.3	16.3	13.6	14.8	14.0	15.1	11.8	12.7
Lysine (% of protein)	2.71	2.65	2.18	2.01	2.27	2.37	2.65	2.47	2.73	2.35	2.05
Lysine (% of sample)	.439	.398	.325	.287	.370	.322	.392	.346	.412	.277	.258
Oil (%)	5.00	4.72	3.38	3.68	4.64	4.45	4.90	4.00	5.06	3.47	3.32
Seed weight (g/100 seeds)	1.57	2.36	2.02	2.58	1.61	2.34	1.45	2.49	2.04	2.64	2.75
Protein per seed (mg)	2.54	3.54	3.01	3.69	2.62	3.18	2.15	3.49	3.08	3.12	3.53
Lysine per seed (mg)	.069	.094	.066	.074	.060	.075	.057	.086	.080	.073	.071
Percent germ in seed	8.8	12.5	4.7	7.8	5.7	8.9	5.0	9.2	9.4	9.8	10.10**
Height (cm)	270.	---	275	---	255	---	250	---	300	---	200
Days to 50% flowering	103	---	96	---	98	---	99	---	98	---	---
B and R Reaction	---	B	---	R	---	R	---	B	---	B	---

* Average of 31 genotypes over six locations and two years (Schaffert, 1972).
** Average of 4 lines (Jambunathan and Mertz, 1973).
B Non-restorer
R Restorer

exist for its expression.

The sugary gene was found to be associated with increased protein and lysine (expressed as percent of protein) contents. However, per seed protein and lysine contents were similar to the level of normal sorghum kernels due to the effect of su gene on reduced kernel size. The methionine and cystine content of some sugary lines are higher than normal sorghum. The su gene was also found to be associated with increased amounts of reducing sugars, sucrose and WSP and a decreased content of starch. Though there was no significant difference in the amylose content of normal and sugary starch in the segregating kernels, all sugary lines had higher amylose contents. This point needs further verification in future studies.

Higher protein, lysine and sulphur amino acid contents of high lysine and sugary lines as compared to normal sorghum should have better nutritional value as has been noted in corn. The changed carbohydrate composition of sugary lines should also add to their nutritional quality.

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ESTIMATION OF GENETIC COMPONENTS, HERITABILITY AND GENETIC ADVANCE OF PROTEIN, LYSINE AND OIL CONTENT IN GRAIN SORGHUM

Samwiri Z. Mukuru, W. E. Nyquist and J. D. Axtell

Four crosses were made using the hot water technique among six parental lines that were considered homozygous and unrelated. From each cross random 45 F_2 plants were selected and these were advanced to the third generation in Puerto Rico. From each F_3 progeny row, two random plants were selected and selfed. All the 360 F_3 selections from the four crosses were evaluated in the fourth generation in one replication and two locations. The segregating progenies from each cross are referred to as a population.

The characters on which data were recorded as a single observation for a plot were days to flower, plant height, panicles per plot, grain yield (kg/ha), kernel weight and volume, percent protein, percent lysine of protein, percent oil and catechin equivalent values. All other characters were appropriately computed. Percent heterosis over midparent for kernel weight and volume was negative in all populations but the percent heterosis over midparent for other characters were variable from population to population.

Transgressive segregation occurred in both directions in most populations for most characters except kernel weight and volume where transgressive segregation occurred only towards the lower kernel weight and volume except in population II where no transgressive segregation was indicated. Each population exhibited a wide range of variation for most

characters in the fourth generation.

The analysis of variance indicated significant differences among the F_2 subpopulations and F_3 subpopulations within F_2 subpopulations in all populations for several characters, but for some other characters significance was indicated for F_2 subpopulations only or F_3 subpopulations within F_2 subpopulations. In most populations there were no significant differences for either F_2 subpopulations or F_3 subpopulations within F_2 subpopulations for grain yield, kernel density and percent lysine of sample.

The results suggest significant heterozygosity in the F_2 subpopulations in the fourth generation for kernel weight and volume, percent protein and percent oil. For these characters selection both within and among F_2 families in early generations would be most effective.

Estimates of dominant genetic variances in all populations for all the seed and chemical characters were always larger than those for additive genetic variances. This clearly indicates that, while additive gene action is present in the inhertiance of the seed and chemical characters, dominance gene action appeared to be the most important in these populations.

Heritability estimates for each character were variable from population to population, and the heritabilities among the F_2 and F_3 subpopulations in the fourth generation for each character in a population were equally variable.

Estimates of heritability and genetic progress expected under selection for the chemical and seed characters were not very high but they do give sufficient evidence that substantial improvement can be made, particularly for kernel weight and volume in most populations, percent protein in population III, percent lysine of protein in population III and percent oil in

populations III and IV.

Phenotypic correlations of grain yield with panicles per plot, panicle weight and kernels per panicle were positive and significant in all populations but the correlations between grain yield and kernel weight or kernel volume were nonsignificant in most cases. This implies that panicles per plot, panicle weight and kernels per panicle are the only components of yield.

Kernel weight and kernel volume were highly positively associated in all populations but the associations of kernel weight or volume with days to flower, plant height, kernels per panicle and percent lysine of protein were negative and significant in most populations. The phenotypic correlations between kernel weight and percent oil were negative and significant in population IV and this might be a barrier to simultaneous selection for larger kernels and high percent oil in this population.

In population IV, percent protein and percent oil were positive, significantly correlated but percent protein and percent lysine of protein were negative, significantly correlated in all populations.

In this study no consistent and favorable correlations of chemical traits with any plant or seed characters were found in all populations. However, kernel weight or volume could be used as a good indicator in selecting percent protein in population I and percent oil in population III while percent oil could be used as a good indicator in selecting percent protein in population IV.

EFFECT OF TANNIN CONTENT OF SORGHUM BICOLOR (L.) MOENCH GRAIN ON NUTRITIONAL QUALITY

Donn P. Cummings and John D. Axtell

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 establish what level of tannin in sorghum will cause decreased rat performance using iso-nitrogenous, "isogenic" grain sorghum diets.

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 -penta-hydroxyflavan (Bate-Smith and Rasper, 1969).

Bate-Smith et al. (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, Clark, Rooney and Johnson (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, Johnson and Stacy, 1964; Voigt, 1966; Harris, 1969; Niehaus and Schmidt, 1970; Tipton, Floyd, Marshall and McDevitt, 1970; and McMillian, Wiseman, Burns, Harris and Greene, 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, Van Etten and Dimler, 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 et al, 1962). The role of tannin in weathering resistance is not well understood, although Harris et al (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 Leoti sorghum was characterized by Miller and Kneen (1947) and later identified as a series of condensed tannins by Strumeyer and Malin (1969). Smart, Bell, Stanley and Cope (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 Alumout (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.

Rostagno, Featherston and Rogler (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, Kadirvel, Vohra and Kratzer (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, Hurwood, Burton and Fuelling, 1969; Rostagno et al, 1973a). Chang et al (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, Rostagno et al (1973b) 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, Potter and Brown (1966) concluded that dietary levels of tannin between 0.64 and 0.84 percent (from grain sorghum) are required to show a depressing affect

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 affects of tannin and tannic acid through dietary supplementation with various compounds. Chang et al (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, Kratzer and Joslyn (1966) were not able to reproduce these results and reported that methionine, choline, betaine, or ornithine did not reduce the growth depressing affects of tannic acid. Connor et al (1969) were able to confirm the work of Chang et al (1964) in part. They found that methionine and choline partially alleviated the growth depressing affects of tannic acid, but did not completely eliminate the growth depressing affects 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 et al (1940). McGinty (1968a) has reported that the use of polyethylene glycol alleviated the decrease in digestibility caused by tannin in sorghum. Armstrong, Featherston and Rogler (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. Jambunarhan et al (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 lease three ways: 1) lower digestibility due to reduced protein availability, 2) toxic affects, 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, Oswalt, Mertz, Pickett, Jambunathan and Srinivasan (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.

MATERIALS AND METHODS

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 50 g each were randomly assigned to each treatment 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 Experiment I.

Table 1. Composition of rat diets.

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

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

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 2) 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.

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 3). 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

Table 2. Analyses of grain and diets.

	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

Table 3. 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

efficiency, and protein efficiency occurred above any differences due to dehulling (Table 3 and Figure 1). 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 4 indicates a highly significant affect 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.

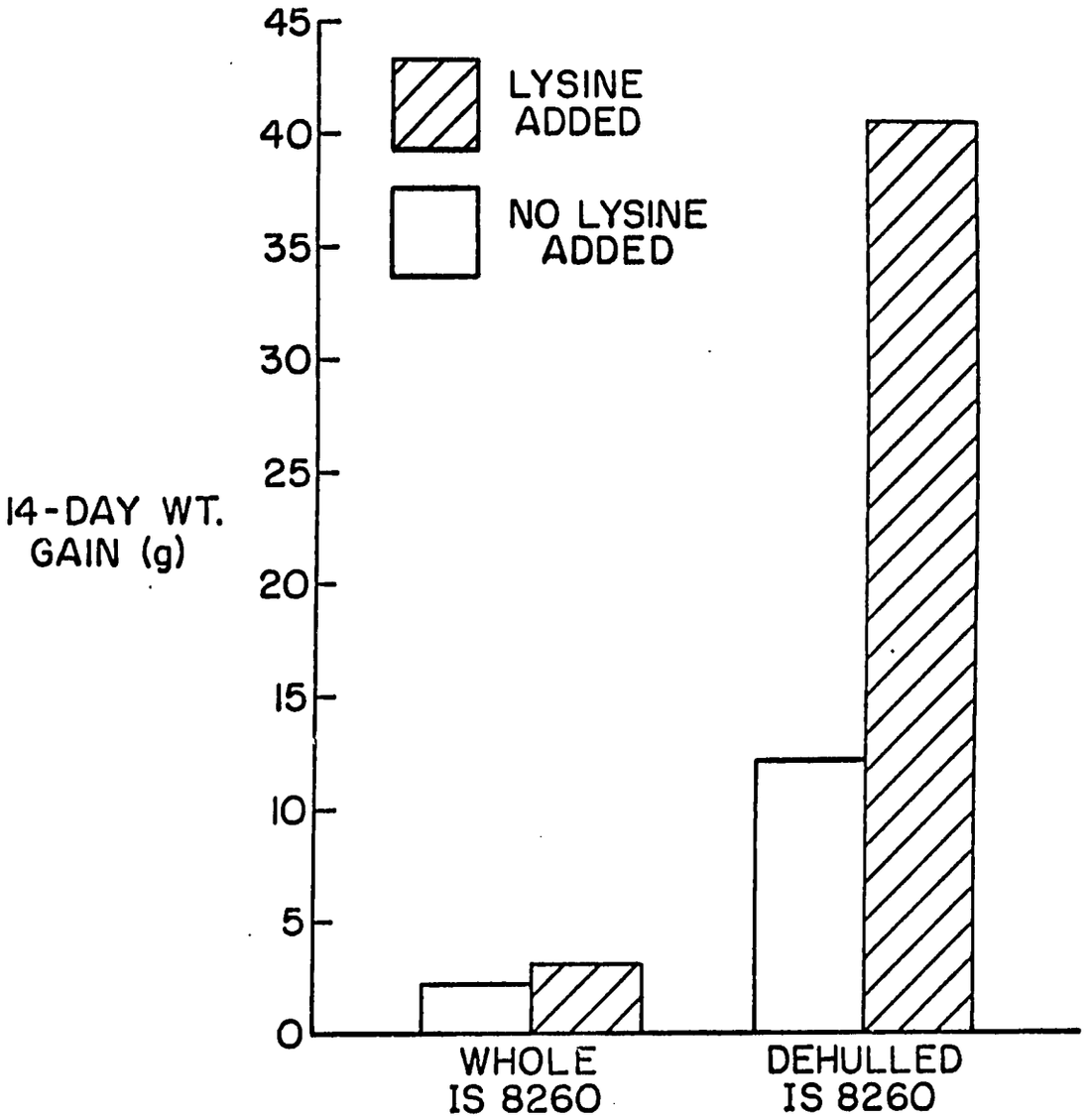

Figure 1. Comparison of 14-day weight gain of rats fed whole and dehulled sorghum diets with and without lysine added.

Table 4. 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.

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 2), 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 2) indicating that tannin may also bind with lysine (Figure 2). Further investigation is needed to determine the mechanisms responsible for these observations.

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 2). 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 5). 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 6), representing an 11-fold increase due to reduced tannin content. Figure 3 illustrates the effects of CE levels on 28-day weanling rat weight gain.

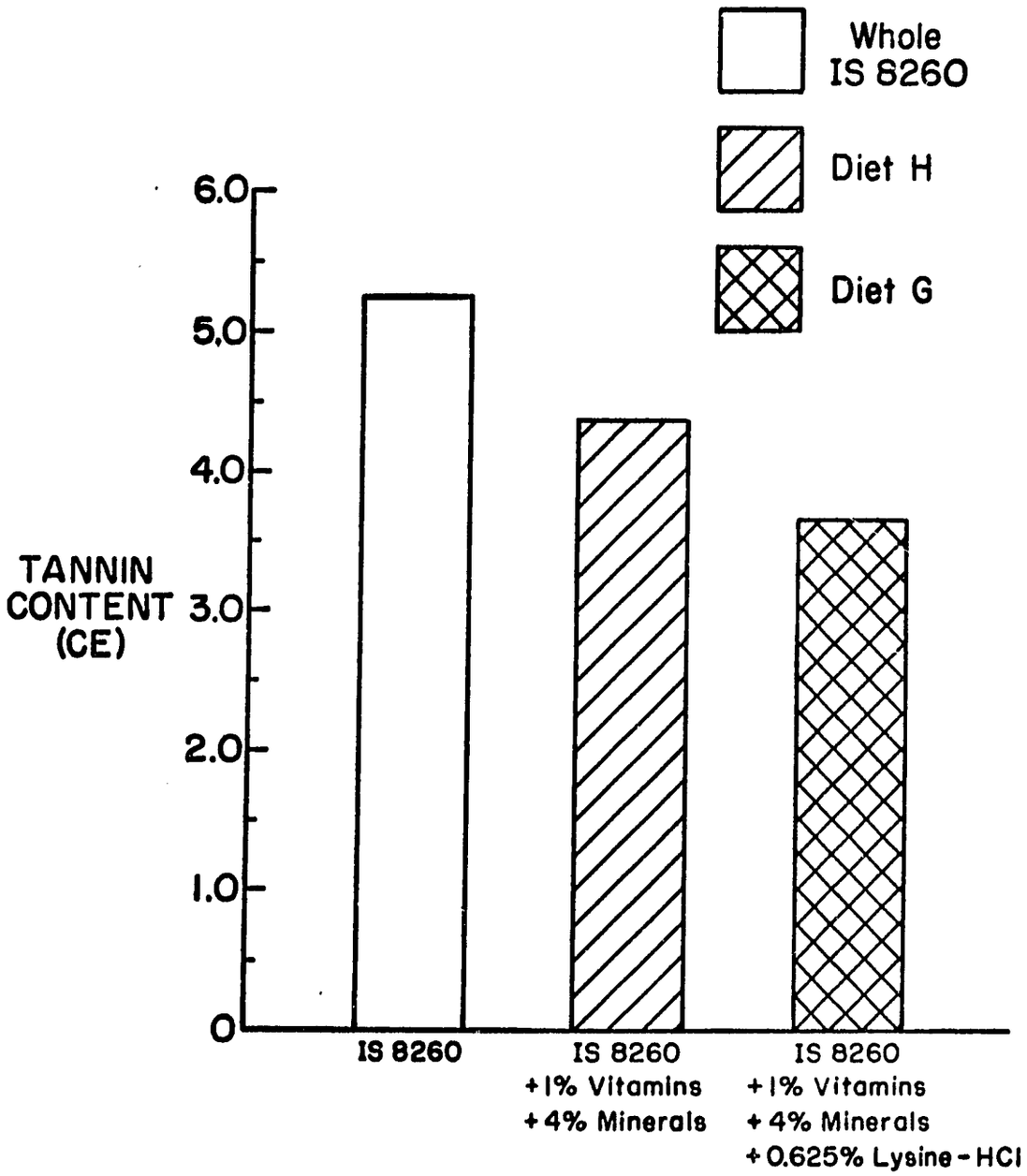

Figure 2. The effect of addition of vitamins, minerals and lysine-HCl on the CE level of sorghum diets.

Table 5. Analysis of Variance (mean squares) for CE levels.

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.

The marked growth-depressing affects 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 6). 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 7).

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

		14-Day Data				28-Day Data			
Diet	CE Level	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	79.4b	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).

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.

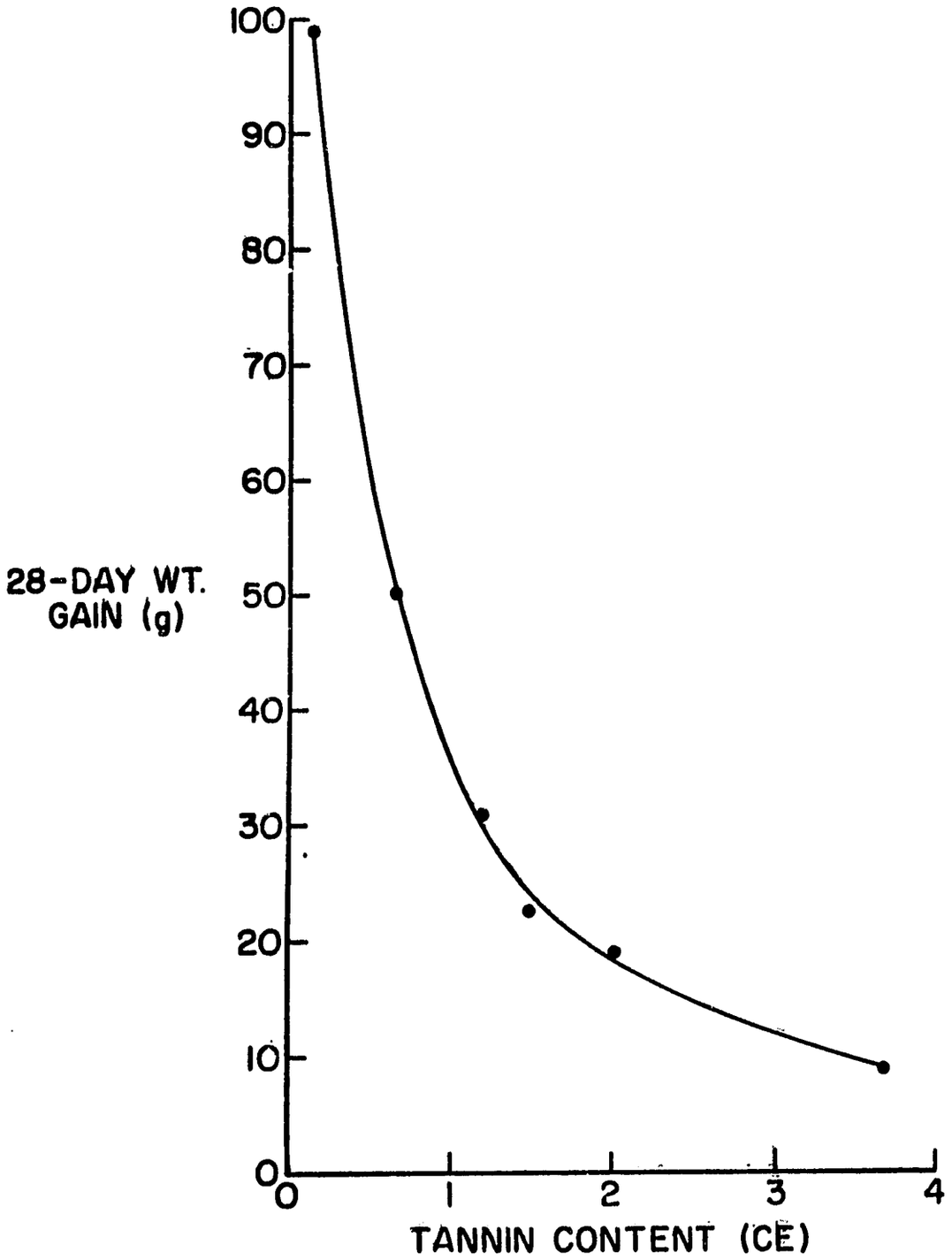

Figure 3. The effect of CE level on 28-day weanling rat weight gain.

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.

SUMMARY

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 only slight 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.

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RELATIONSHIPS OF PIGMENTED TESTA TO NUTRITIONAL QUALITY OF SORGHUM GRAIN

Donn P. Cummings and John D. Axtell

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

[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.

RESULTS AND DISCUSSION

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 1. 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 2).

Table 1. 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 1. cont.

Sample #	Testa	Catechin Equivalents VHCl	MVHCl	Group
089	Present	0.69	2.25	II
090	Absent	0.95	0.89	*
091	Present	3.00	5.35	III
092	Present	0.75	3.90	II
093	Present	0.92	2.92	II
094	Present	1.04	2.53	*
095	Absent	0.31	0.60	I
096	Present	0.52	2.24	II
097	Absent	0.21	0.73	I
098	Present	0.34	1.98	*
099	Absent	0.46	0.31	I
100	Present	0.33	3.25	II
101	Present	0.39	6.68	II
102	Present	0.71	3.84	II
103	Present	0.75	3.19	II
104	Absent	0.28	0.57	I
105	Present	2.08	2.26	III
106	Present	0.95	2.29	II
107	Present	0.47	4.69	II
108	Present	0.50	2.07	II
109	Absent	1.12	0.52	*
110	Present	0.74	3.99	II
111	Absent	0.20	1.33	*
112	Present	1.35	2.20	*
113	Absent	0.30	0.55	I
114	Present	0.42	5.04	II
115	Present	1.05	1.64	*
116	Present	0.62	2.92	*
117	Absent	0.22	0.27	I
118	Present	0.38	2.62	II
119	Present	0.93	2.94	II
120	Present	0.44	3.96	II
121	Present	0.94	3.99	II
122	Present	0.46	3.90	II
123	Present	0.74	4.30	II
124	Present	0.45	4.40	II
125	Present	0.45	2.56	II
126	Absent	1.37	0.57	*
127	Present	2.14	5.77	III
128	Present	2.55	4.99	III
129	Present	3.45	4.92	III
130	Absent	2.47	0.78	*
131	Absent	0.30	0.73	I
132	Absent	0.38	0.72	I
134	Present	2.24	4.92	III
135	Absent	0.26	0.83	I
136	Absent	0.30	0.94	I
137	Present	0.98	3.96	II
138	Absent	0.34	0.94	I
139	Absent	0.22	0.88	I
140	Absent	0.24	0.72	I
141	Present	0.38	3.39	II
142	Present	0.42	3.55	II
143	Present	1.24	3.78	*
144	Present	0.59	3.49	II
145	Absent	0.45	0.90	I
146	Present	0.78	3.49	II
147	Present	3.20	6.26	III
148	Present	5.53	7.84	III
149	Absent	0.59	1.78	*
150	Present	0.59	2.72	II
152	Present	0.50	2.38	II
153	Absent	0.22	0.85	I
154	Present	0.96	3.62	II
155	Absent	0.39	1.21	*
156	Present	0.58	3.22	II
157	Present	0.40	2.71	II
158	Present	1.40	4.04	*
159	Present	0.95	3.57	II
160	Present	0.57	2.71	II
161	Present	1.18	5.56	*
162	Present	1.69	5.84	III
163	Present	1.63	5.44	III
164	Absent	0.34	0.91	I
165	Present	0.51	3.23	II
166	Present	0.54	3.62	II
167	Present	0.31	3.60	II
168	Present	0.35	3.10	II
169	Absent	0.14	0.64	I
170	Present	0.46	3.13	II
171	Present	0.46	3.62	II
172	Present	0.35	2.84	II
173	Absent	0.42	0.85	I
174	Present	2.16	5.07	III
175	Present	2.30	6.05	III
176	Absent	0.30	0.91	I
177	Present	3.07	6.11	III
178	Present	2.15	5.51	III

Table 1. cont.

Sample #	Testa	Catechin Equivalents VHCl	Catechin Equivalents MVHCl	Group	Sample #	Testa	Catechin Equivalents VHCl	Catechin Equivalents 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.

Table 2. Comparison of mean VHCl and MVHCl values for 175 samples of grain sorghum from a segregating F_2 population.

	N	VHCl Mean CE	VHCl sd	MVHCl Mean CE	MVHCl sd	Correlation Coefficient
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.

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. The correlation of these two 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 analyses of the bulked sorghum samples used to make up the three diets are presented in Table 3.

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

Table 3. Analyses of bulked sorghum samples used in preparation of rat diets.

Group	Testa	No. of Samples	Ave. VHCl CE	Ave. 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

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 4). These differences vary with CE level and their effect on rat performance is completely confounded with CE and assumed to be small. Table 4 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

Table 4. Analyses of rat diets and 14-day rat trial data.

Group	Catechin Equivalents	Protein % of Sample	Lysine % of Protein	Lysine % of Sample	Feed Consumption	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

rat feeding trials. This is evident in FER and PER values also (Table 4). Differences in CE values between Group I and Group II diets measured by the MVHCl test were not reflected in the rat growth 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 F_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.

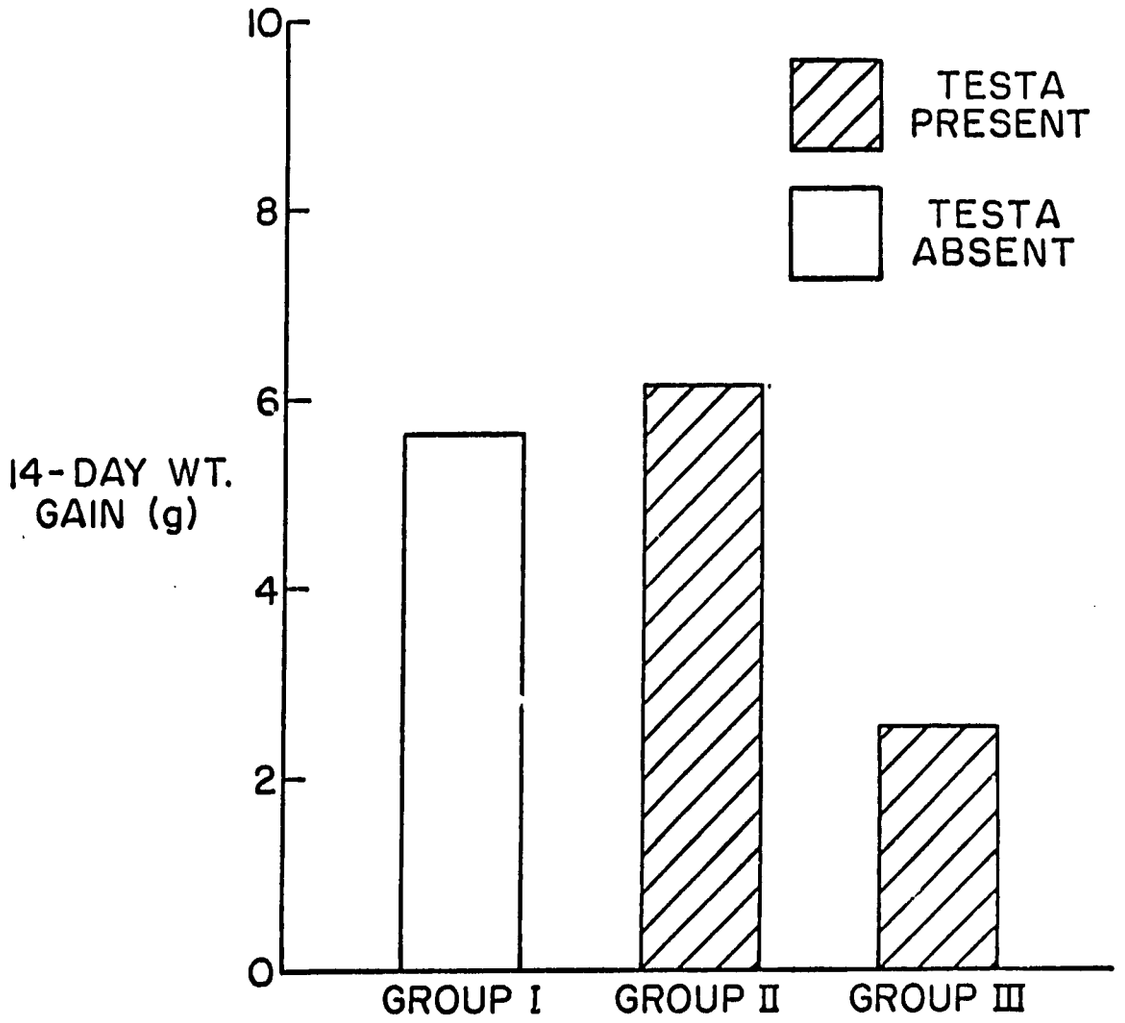

Figure 1. Comparison of 14-day weight gain on three diets

SUMMARY

Differences in catechin equivalent values for the VHCl and MVHCl tests we found. MVHCl values were generally higher and are thought to result from dditional 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.

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EFFECTS OF BIRD RESISTANT SORGHUM GRAIN AND VARIOUS COMMERCIAL TANNINS ON CHICK PERFORMANCE

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Studies were conducted to determine: (1) the effect of protein level on the performance of chicks fed bird resistant sorghum grains; (2) the influence of two levels of DL-methionine in alleviating the detrimental effects of bird resistant sorghum grains; (3) the effects of various sources of commercial tannic acid on chick performance.

Bird resistant sorghum (BR64) produced poorer chick performance than nonresistant sorghum (RS671) in sorghum-soybean meal diets containing 16 and 20% dietary protein (Table 1). Addition of 0.15% DL-methionine to both sorghum grain diets resulted in significant improvements in chick performance, but the magnitude of the response was greater with the bird resistant than with the nonresistant sorghum grain diets. After methionine supplementation, the BR64 diets supported similar chick weight gains as the RS671 diets at both dietary protein levels; however, feed efficiency of chicks fed the BR64 diets remained poorer.

In additional studies nonresistant sorghum RS610 supported significantly better chick performance than either bird resistant sorghum BR64 or sorghum RS610 plus 1% isolated tannic acid. The addition of 0.15% DL-methionine to sorghum RS610 and BR64 diets resulted in significant improvements in chick weight gains with no further increase being noted when 0.30% supplemental DL-methionine was fed. Adding methionine to the RS610 plus tannic acid diet

had little effect on chick weight gain. Supplemental methionine resulted in significant improvements in feed conversion with both the BR64 and RS610 plus tannic acid diets.

Addition of tannic acids of varying molecular weights to a nonresistant sorghum diet resulted in significant depressions in chick performance. The detrimental influence of the tannins did not appear to be closely associated with their molecular weights as all depressions in chick weight gain and feed conversion were roughly similar. The supplementation of 0.15% DL-methionine to diets containing each of the tannic acids had little effect on improving chick performance.

Table 1. The effect of bird resistant (BR64) and nonresistant (RS671) sorghum grains on the weight gain and feed conversion of chicks fed diets containing 16 and 20% dietary protein.

Treatment	Dietary protein	Weight gain (g)	Feed/gain ratio (g)
1. Sorghum RS671	16	227^{c}	1.93^{b}
2. Trt 1 + 0.15% DL methionine	16	290^{b}	1.70^{d}
3. Sorghum RS671	20	305^{b}	1.63^{e}
4. Trt 3 + 0.15% DL methionine	20	406^{a}	1.44^{g}
5. Sorghum BR64	16	179^{d}	2.29^{a}
6. Trt 5 + 0.15% DL methionine	16	280^{b}	1.87^{bc}
7. Sorghum BR64	20	269^{b}	1.84^{c}
8. Trt 7 + 0.15% DL methionine	20	387^{a}	1.56^{f}

Means bearing the same superscripts are not significantly different ($P > 0.05$). Mean value for four replications of eight chicks each at 21 days of age.

BIOCHEMICAL AND NUTRITIONAL STUDIES IN SORGHUM

R. Jambunathan and E. T. Mertz

Effect of Polyvinylpyrrolidone on the Distribution of Sorghum Kernel Proteins.

It is a known fact that the presence of phenolic compounds in many plant tissues (loosely termed as tannins) makes it impossible to isolate active enzymes by conventional techniques. All phenols, unless sterically hindered, take part in hydrogen bonding and form complexes with proteins, nylon and polyvinylpyrrolidone (PVP). In addition, many phenols are readily oxidized to quinones, which are highly reactive compounds. Oxidation of phenols may occur non-enzymatically or it may be catalyzed by phenol oxidases or peroxidases, enzymes which are widely distributed in plants. Quinones are oxidizing agents and may oxidize essential groups of proteins. More important, they polymerize rapidly, and in the presence of protein, they also react rapidly to form covalent bonds with the protein. It was observed that the inhibition of plant enzymes by tannins could be prevented by the addition of insoluble proteins, such as collagen or hide powder. Since the use of foreign protein may contaminate the extract, a synthetic polymer was found to be equally effective and has been used for the extraction of plant enzymes relatively free of bound tannins. It is called insoluble polyvinylpyrrolidone (Insoluble PVP or Polyclar AT) (Loomis and Battaile, 1966).

We had observed earlier that the presence of tannins in sorghum alters its protein solubility pattern (Jambunathan and Mertz, 1973). Our preliminary data indicate that this altered distribution of proteins may be due

to the binding of proteins to tannins. Therefore, fractionation studies of high tannin sorghums were carried out in the presence of collagen, hide powder and insoluble PVP.

The results obtained are shown in Table 1. The presence of PVP alters the solubility of proteins of sorghum IS 2283, causing a substantial increase in the first fraction and reduction in the fifth fraction. The distribution pattern approached that of a normal sorghum (nitrogen of normal sorghum is higher in the first three fractions and lower in the last two fractions). A similar effect was also seen in the distribution of nitrogen in sorghum IS 6992 in the presence of PVP. Although the presence of collagen and hide powder also altered the solubility characteristics of sorghum proteins, the change in the distribution pattern was not as pronounced as that obtained in the presence of PVP. Also, there appears to be some solubility problem involved in the presence of hide powder and collagen at alkaline pH, as indicated by a higher percentage of nitrogen in the fourth and fifth fraction, thereby causing an error in the total percentage of extracted nitrogen.

Although the presence of PVP altered the solubility characteristics of the high tannin sorghums, it did not completely overcome the effect of tannins, since the distribution pattern is not identical to that of normal sorghum. Probably some of the tannins were already covalently bound to the sorghum proteins even before the addition of PVP, with the result that added PVP was not able to remove the bound tannins.

Effect of PVP on Rat Growth Response

A feeding experiment was carried out in order to find out the effect

Table 1. Effect on polyvinylpyrrolidone (PVP), collagen and hide powder on the distribution of sorghum kernel proteins.

	IS2283	IS2283	IS2283	IS2283	IS6992	IS6992
Weight of sample (g)	1.01	1.01	1.01	1.00	1.02	1.01
PVP (g)		1.01				1.00
Collagen (g)			0.20			
Hide Powder (g)				0.21		
Fraction* I	6.7	12.5	8.4	8.0	7.8	12.6
II	2.0	3.1	1.3	2.0	3.6	5.0
III	13.6	24.4	23.2	24.4	13.9	14.8
IV	12.8	10.3	13.7	19.3	15.1	12.1
V	63.4	50.3	78.1	62.3	49.0	47.1
% nitrogen extracted	98.5	100.6	124.7	116.0	89.4	91.6

* percent of soluble nitrogen

of PVP when fed along with a high tannin sorghum. Three sorghum samples were selected for this feeding experiment: 025274 containing 0.3% catechin equivalent, 925120 containing 2.08% catechin equivalent and Rx 025440 containing 3.08% catechin equivalent. They were mixed with 5% PVP in the diet along with minerals and vitamins. Starch was added where necessary to make all the diets isonitrogenous at an 8.6% protein level. Each diet was fed to six rats for a period of 28 days. The results are shown in Table 2.

There was a weight increase in rats fed the diet containing the highest level of tannin (Rx025440) with added PVP compared with rats fed a diet without PVP. The PER values showed the same trend. However, there was a weight loss in rats fed the PVP diets containing the two lower levels of

Table 2. Effect of PVP on rat growth response*

	Av. Total wt. gain	PER	Catechin Equivalent
025440	5.8	0.44	3.08
025440 + PVP	6.6	0.51	
025274	9.3	0.69	0.3
025274 + PVP	5.6	0.45	
925120	11.5	0.75	2.08
925120 + PVP	9.1	0.62	

* Six rats in each group, 28 days duration. Fed at 8.60% protein level. PVP was added to make 5% of the diet.

tannin. The addition of PVP seems to exert a beneficial effect on the sorghum that contains a very high amount of tannin. The level of PVP at 5% of the diet was arbitrarily chosen, and this level of PVP might have been excessive producing deleterious effect on rats fed the low tannin diet. Additional feeding experiments using different levels of PVP may give an idea of the true value of PVP when fed with sorghum diets.

Growth Response of Rats Fed on High and Low Tannin Sorghums Supplemented with Lysine

An experiment was carried out to observe the effects of feeding a high tannin sample supplemented with lysine on the growth response of rats. Although preliminary experiments had been carried out earlier, a systematic study using isonitrogenous protein levels was needed. Samples

which were fed previously with PVP were chosen for this study. The sample designation, protein and lysine values (based on protein) of the sorghum samples respectively were: RX 025440 - 9.64% - 2.05%; 025274 - 10.84% - 1.91%; 925120 - 9.90% - 2.08%. Vitamins and minerals were added to the diet and starch was added where necessary to make the diet isonitrogenous at the 9% protein level. The diets were divided into two portions and lysine in the form of L-lysine monohydrochloride was added to one-half of the diet to bring the total level of lysine to 1% in the diet, taking into consideration the level of lysine provided by the sorghum. The diets with and without added lysine were each fed to 10 weanling rats for a period of 28 days.

Results of this feeding experiment are shown in Table 3. Rats gained weight on the diet supplemented with lysine when compared to those fed the unsupplemented diet in all three sorghum diets. They also consumed more sorghum which was supplemented with lysine, however, the increase in food consumption was small as compared to the weight gain obtained in each group. This is more clearly reflected if we look at the protein efficiency ratio (weight gain/weight of protein consumed) values. Protein efficiency ratio of rats fed sorghum sample 025274, which had the lowest tannin level of the three samples, was 0.54 with the unsupplemented diet, (18.6% of the PER value of casein and 2.09 with the lysine supplemented diet, 72.1% of the PER of the casein). Similarly, the PER of rats on sorghum 925120 was 0.45 (15.5% of the PER of casein) and those on the lysine supplemented diet was 1.08 (37.2% of the PER of the casein). Rats which were fed sorghum sample RX 025440, which had the highest tannin level of the three, lost weight on this diet alone, while the PER of rats fed the lysine supplemented diet was

Table 3. Growth response of rats fed on high and low tannin sorghums supplemented with lysine[1]

Group	Av. Wt. Gain	Feed con-sumed	PER	% of casein	Cate-chin equiv.	Postmortem examination done by Dr. Kirkham (7/11/73) - 5 rats/group. General body condition	Liver condition	Ranking given from exam. of rats
I Rx025440	-0.5	121.2			3.08	Poor	1/5 Ave. 4/5 Below av.	5
II Rx025440 + lysine[2]	17.2	143.9	1.37	47.2		Fair	2/5 Good 1/5 Av. 2/5 Below av.	3
III 025274	6.6	137.1	0.54	18.6	0.3	Average	1/5 Av. 4/5 Below av.	4
IV 025274 + lysine[2]	31.4	167.7	2.09	72.1	2.08	Normal	5/5 Normal	1
V 925120	5.7	142.1	0.45	15.5		Poor	5/5 Below av.	5
VI 925120 + lysine[2]	14.4	148.1	1.08	37.2		Average+	5/5 Av. to above av.	2
Casein	62.4	240.9	2.90					

[1] Ten rats in each group, 28 days duration. Fed at 8.97% protein level.

[2] Lysine added to make 1% of the diet in total.

1.37 (47.2% of the PER of the casein). In conclusion, there was a weight gain in all cases where lysine was added regardless of the tannin levels. However, the increase in the weight gain (and in PER) was highest in the case of low tannin sorghums as compared to the high tannin sorghums.

Five rats in each group were taken for postmortem examinations to the Animal Disease Diagnostic Laboratory at Purdue University and were examined by Dr. W. W. Kirkham for liver abnormalities and general body changes. The ranking given by him after the examination of rats follow the expected pattern, with the rats fed the low tannin sorghums supplemented with lysine getting the first rank and those fed the high tannin sorghum alone getting the last two ranks. An independent examination of the liver also shows the expected pattern and indicates that, although rats gained weight with both of the diets of high tannin supplemented with lysine, the livers of the rats fed diet 925120 (2% catechin equivalent) with lysine were in a better condition than those fed Rx 025440 (3% catechin equivalent) with lysine.

In an earlier experiment it was observed that lysine supplementation of a high tannin sorghum (IS 2996, 4.6% catechin equivalent) did not improve the weight gain of rats substantially (Axtell and Oswalt, 1972). However, in the present experiment, lysine supplementation of the two high tannin lines improved the weight gain of rats in both cases. One explanation might be the interpretation of catechin equivalents in relation to the biological availability of proteins in sorghum. It appears from this experiment that in some of the sorghums the nutritional quality can be improved just by adding lysine alone even though they may have a high

tannin content. In other high tannin sorghums, the addition of lysine may not have any beneficial effect. It is not yet clear as to the compounds that do react in the catechin equivalent test and the nature of the reaction mechanism. Perhaps some of the compounds present in sorghum, which react in the catechin determination test thereby giving a high tannin value, are not deleterious per se for rats and these may respond to the addition of lysine. Thus, this experiment points out the importance of further research work that needs to be carried out in the area of tannin determinations in sorghum and their implications on the biological availability of proteins in sorghum.

Evaluation of the Performance of Different Small Animals on the Same Sorghum Diet

Sorghum was one of the diets that was included in a cooperative feeding trial conducted at different locations using the same supply of the diet. The results of this feeding experiment were presented at the International Symposium on Protein Quality in Maize in 1972 held at Mexico City, Mexico. The results obtained with the sorghum samples are presented in Table 4.

IS 2319 and 025042-1 are low tannin samples and IS 8165 is a high tannin sorghum sample. The mouse and chicken could not distinguish between the three sorghum samples. While the PER values of three sorghum samples were shown above the 70% when compared to casein in chick feeding trials, the PER of 025042 was 42% of casein, IS 2319 was 34% of casein and IS 8165 was 31% of casein in the case of mouse feeding trials. Protein efficiency ratios obtained with voles were very high, with IS 2319 and 025042 being 89% of the

Table 4. Summary of PER values obtained by feeding the cooperative sorghum and casein samples to different test animals.

Identification	Rat[1]	Vole[2]	Mouse[3]	Chicken[4]
IS 2319	1.28[d,e]	2.12	0.53[c,d]	2.02[f]
025042-1	0.97[e,f]	2.12	0.66[c,d]	2.09[e,f]
IS 8165	0.71[f]	1.19	0.49[d]	2.00[f]
ARNC casein	3.34[a]	2.37	1.58[a]	2.81[a,b]

[1] Ten male rats in each group, 28 days duration. Fed at 8.8% protein level. Casein was fed twice, once with maize samples and again with other cereals. Values with the same superscript are not significantly different from each other at the 1% level (Purdue University).

[2] Five male meadow voles in each groups, 5 days duration. Fed at 7% protein level. No significant differences could be demonstrated due to the variation between the animals within the group (CIMMYT).

[3] Five male mice in each group, 28 days duration. Fed at 8.8% protein level. Values with the same superscript are not significantly different from one another at the 10% level (University of Nebraska).

[4] Twenty chicks in each group, 14 days duration. Fed at 6% protein level on top of 8% protein provided by premix. (Total protein level 14%.) Values with the same superscript are not significantly different from each other at the 5% level (Washington State University).

casein control while IS 8165 was 50% of the casein control.

Of the four different test animals only the rat was able to differentiate between the high and low tannin sorghum samples. While the high tannin sample, IS 8165 showed a PER of o.71, which was 22% of the casein control, the low tannin samples 025042 and IS 2319 showed a PER of 0.97 (30% of casein control) and 1.28 (40% of casein control) respectively. Protein efficiency ratio values of sorghum obtained with the rat as the test animal

were as expected when compared to the PER obtained from other cereals, while this was not the case with other small animals. With the vole, the PER of sorghum was higher than that of opaque-2 maize and with the mouse the PER of sorghum could not be distinguished from that of opaque-2 maize. With the chicken the PER of sorghum could not be differentiated from that of wheat, triticale and floury-2 maize. Thus, the rat seems to be the ideal small animal for the biological evaluation of sorghum samples.

Protein Distribution Patterns in High Lysine Sorghum

The nitrogen distribution patterns obtained with the high lysine sorghum (hl hl hl) of Axtell and Singh (1973) (IS 11758 and IS 11167) and with the F_2 kernels obtained by crossing the high lysine sorghum, IS 11758, with Redlan is shown in Table 5. Normal (low lysine) seeds were plump with a vitreous endosperm while high lysine kernels were floury with a partially dented endosperm. For the fractionation studies of the F_2 kernel, normal and high lysine seeds were taken from the same head so that a valid comparison could be made of the influence of the high lysine gene on the distribution of protein patterns in both cases. Sorghum heads from five different locations were chosen for this study to take into consideration any location differences that might affect the distribution patterns.

The results in Table 5 clearly indicate that the percentage of soluble nitrogen in the first fraction has increased by about 7% in the high lysine sorghum as compared to the normal counterpart, while the alcohol soluble fraction has decreased in the high lysine sorghum as compared to the normal sorghum. Amino acid analyses of each of the protein fractions of other

Table 5. Nitrogen distribution in the whole kernels of normal (N) and high lysine (hl) sorghums.[a]

	Redlan x IS 11758 (F_2 Kernels)									
	55073		55074		55075		55076		55077	
Fraction	N	hl	N	hl	N	hl	N	hl	N	hl
I. Saline	16.5	22.3	16.6	22.4	15.3	23.3	13.2	22.2	14.7	21.8
II. Isopropanol	18.8	11.9	27.5	13.4	25.3	13.7	32.7	14.0	27.8	15.3
III. Isopropanol + 2-ME	28.7	19.1	23.5	20.8	28.5	20.3	26.1	21.5	25.5	19.5
IV. Borate buffer + 2-ME	5.2	4.5	4.8	4.6	4.1	4.0	3.7	4.5	3.9	3.9
V. Borate buffer + 2-ME + SDS	22.4	35.3	22.6	34.4	23.3	32.8	20.8	33.0	23.3	32.1
Total nitrogen extracted	91.6	93.1	95.0	95.6	96.5	94.1	96.5	95.2	95.2	92.6
% Protein	11.8	14.5	10.8	14.7	13.2	15.2	16.3	16.4	13.1	17.3

Fraction	IS11758	IS11167	Average 55073-55077	
			N	hl
I.	26.0	25.3	15.3	22.4
II.	10.3	15.2	26.4	13.7
III.	19.6	19.3	26.5	20.2
IV.	6.5	4.5	4.3	4.3
V.	27.2	29.5	22.5	33.5
Total nitrogen extracted	89.6	93.3	95.0	94.1
% protein	18.5	16.3	13.0	15.6

[a] Percent of soluble nitrogen.

sorghums had indicated that the first fraction is rich in lysine (as high as 5.3% based on protein), while the second and third fractions contain very low amounts of lysine (0 2 to 0.3%). Therefore, the changes in the distribution patterns with an increment in the percentage of nitrogen in the first fraction simultaneously raises the lysine level, thus causing an overall increase in the total lysine of the kernel. An increase of about 11% in the soluble nitrogen in the fifth fraction of the high lysine sorghum also increases the total level of lysine in the whole kernel. This indicates that the high lysine character can be inherited easily by simple crossing. If we compare the average values of normal and high lysine F_2 kernels of sorghum with the pattern obtained for the two high lysine lines, IS 11758 and IS 11167, the percentage of nitrogen in the first fraction in the two high lysine sorghums is slightly higher and the second and third fraction slightly lower as compared with the high lysine F_2 kernel data. Otherwise, the distribution pattern obtained with the F_2 kernels of the high lysine sorghum is very similar to that obtained with the two original high lysine lines. Since the protein distribution pattern in high lysine sorghums closely resembles that found previously in opaque-2 maize, it may be concluded that the hl gene in sorghum and the opaque-2 gene in maize have similar effect in controlling endosperm protein synthesis.

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RAPID NINHYDRIN COLOR TEST FOR SCREENING HIGH LYSINE MUTANTS OF MAIZE, SORGHUM, BARLEY, AND OTHER CEREAL GRAINS

E. T. Mertz, P. S. Misra, and R. Jambunathan

At a symposium in 1971 (1), we reported that the endosperm of opaque-2 maize had a higher level of water-soluble free amino acids than the normal counterpart. Sodek and Wilson (2) also reported increased levels of free amino acids in opaque-2 maize. In searching for a rapid color test to detect high lysine mutants, the idea occurred to us that the excess free amino acids in such mutants could be measured with ninhydrin.

The ninhydrin reagent rapidly detects the difference in total free amino acids between the normal and its mutant counterpart. Five kernels of maize, 15 kernels of sorghum, and 10 kernels of barley (from which the glumes had been removed) were split lengthwise with a sharp knife and placed in 0.75 x 6 in. Pyrex test tubes. The kernels were covered with 10 ml of water and 300 $\pm$ 50 mg of dry ninhydrin-buffer (pH 5) mixture (16% ninhydrin, 58% sodium citrate and 26% citric acid) was added. The contents were heated just to boiling and the tubes allowed to stand five minutes. The intensity of ninhydrin color is much greater in the tubes containing the high lysine mutants of maize, sorghum, and barley than in the tubes containing the normal grains. To identify single kernels, a section of the crown containing endosperm and pericarp can be removed and the cut surface stained with ninhydrin solution (3) and compared with the same fragment of a normal control kernel. The remainder of the seed can then be planted. The test should be performed only on freshly cut seeds. In normal sorghum, bisected seeds stored for one year gave a strong test for free amino acids

suggesting proteolysis on the cut surfaces.

In order to make a quantitative comparison, leucine equivalents per 100 mg of protein were calculated for the various cereal grains of economic importance and for the high lysine mutants of maize, sorghum and barley described in this paper. One hundred to 500 mg of defatted ground samples of whole kernels of the grains were suspended in 100 times their weight of distilled water and shaken at room temperature for 20 minutes. Samples were centrifuged and 0.15, 0.20 and 0.25 ml of supernate placed in separate 0.75 x 6 in. Pyrex test tubes, the volume made up to 0.5 ml, then 1.5 ml of ninhydrin reagent (3) was added. The tubes were heated in a boiling water bath for 20 minutes, cooled to room temperature, and 8 ml of 50% n-propanol was added. The tubes were briefly mixed on a Vortex mixer and were read in a Spectronic 20 colorimeter at 570 nm. Increments of standard L-leucine solution were treated in a similar fashion and the micromoles of leucine plotted against absorbance to obtain a standard curve. The color obtained with the samples was then expressed in terms of leucine equivalents.

The free amino acids as measured by the ninhydrin reaction, the total protein, and the lysine content of whole grain samples of seven cereal species are listed in Table 1. These data show that the high lysine mutants of maize, sorghum, and barley contain three to four times more water-soluble, ninhydrin-reacting molecules per 100 mg of protein than their normal counterparts. Rice, millet, and oat have leucine values in the range of those found for normal maize, sorghum, and barley. Wheat has slightly higher values. It is interesting to note that high protein rice has about one-half the leucine value of low protein rice.

Table 1. Free amino acids in cereal grains expressed as leucine equivalents.

Cereal Grain	Total free amino acids μM leucine/ 100 mg protein	Protein %	Lysine g/100 g protein
Maize 4854[a]	14.3	11.0	2.6
Maize 4858[a]	17.6	10.5	2.7
Maize 4855[a]	46.0	10.9	4.5
Maize 4856[a]	50.8	10.7	4.4
Maize 4857[a]	69.3	10.1	4.7
Sorghum, normal[b]	10.7	13.1	1.8
Sorghum, high lysine[b]	49.5	18.5	3.3
Barley, Bomi[c]	19.5	19.8	3.3
Barley, high lysine[c]	68.0	19.4	5.6
Rice BP1761[d]	8.9	15.4	3.4
Rice BB[d]	16.8	6.9	3.7
Wheat, Purdue 4930[e]	20.2	17.3	2.9
Wheat, Arthur[e]	23.1	15.4	2.7
Wheat, Genesee[e]	27.7	12.1	3.0
Millet[f]	9.8	14.5	1.9
Oat, Noble[g]	15.9	18.9	3.8
Oat, Dal[g]	16.1	20.3	3.9

[a] 4854 and 4858: CIMMYT normal, yellow synthetics; 4855: CIMMYT yellow, hard endosperm, opaque-2 synthetic; 4856 and 4857: CIMMYT yellow, soft endosperm, opaque-2 synthetics. CIMMYT: International Maize and Wheat Improvement Center, Mexico City.

[b] Normal sorghum kernels: high lysine (hl hl hl) kernels from F_2 segregating heads derived from crosses between "normal" (low lysine) plants and the high lysine sorghum lines IS 11758 (4).

[c] Bomi: parent barley variety (5); high lysine mutant: mutant 1508 from Risø, Denmark (5).

[d] BP1761: high protein rice, IRRI (6); BB: Blue Bonnet rice (6).

[e] Purdue 4930: A6-28-2-1 Indiana high protein; Arthur: variety of Indiana soft wheat; Genesee: variety of New York soft wheat.

[f] Finger millet: variety from Uganda supplied by S. A. Eberhart, Iowa State University, Ames, Iowa.

[g] Noble: Wisconsin variety; Dal: Purdue University variety.

These determinations were made on mature seeds. Data which will be published elsewhere show that the free amino acid levels of both normal and opaque-2 maize are higher in immature grain; thus, the differences observed might not be found if an immature normal grain was compared with a mature high lysine mutant. Also, some variation in leucine values has been observed among normal maize genotypes. It is therefore recommended that any screening for high lysine mutants be done using normal controls with comparable genetic backgrounds.

Hard vitreous endosperm types of opaque-2 maize indistinguishable from normal maize have been developed recently (maize 4855, Table 1 is an example). The simple test described here should quickly identify such kernels in the field or in the marketplace (7).

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3. Ninhydrin reagent: dissolve 400 mg of reagent grade $SnCl_2 \cdot 2\ H_2O$ in 250 ml of sodium citrate buffer, 0.2M, pH 5. Mix with 250 ml methyl cellosolve containing 10 g of ninhydrin (practical).

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NUTRITIONAL QUALITY OF SORGHUM BICOLOR (L.) MOENCH AS ESTIMATED BY POLYPHENOLS, CRUDE PROTEIN, AMINO ACID COMPOSITION AND RAT PERFORMANCE

D. L. Oswalt

Grain sorghum, Sorghum bicolor (L.) Moench, is one of the world's four leading sources of food nutrients from cereal crops. It ranks third as a source of food in the arid or semi-arid tropics where several million people live at marginal levels of nutritional requirements. Improving levels of education and methods of communication, increases in the world population, improvements in health care and standards, and higher economic standards of living, all demand more food and improved food quality. The ability to meet the predictable food requirements of the world necessitates the most concerted efforts of all scientists concerned with efficient manipulation of mechanisms of plant inheritance, conservation of crop variabilities and adaptations, and the production of nutritional food sources which maintain acceptability.

Chemical procedures have been developed which can be used to identify and predict components related to nutritional qualities of cereals, but the chemical analyses have not consistently predicted the biological quality or nutrient availability in grain sorghum selections. Variations in the performance of monogastric and ruminant animals utilizing grain sorghum rations having similar levels of crude protein, and carbohydrates, and having similar amino acid profiles suggested that nutritional quality was being affected by factors not measured by any single chemical procedure normally considered. A rapid and

efficient identification of genotypic selections from established genotypes and from segregating populations which have improved nutritional and production factors is paramount to meet future food requirements demanded from grain sorghum producers.

It was hypothesized that: (a) rat weight gains from grain sorghum samples could be correlated with chemical tests indicating nutritional quality of protein which would eliminate the necessity for biological testing of all selections in a screening program, (b) at a given level of protein, grain sorghums differ significantly in animal performance due to factors related to pigmentation and bird resistance, and (c) supplementation of selected sorghum grain samples with identified deficient amino acids would result in rat weight gains similar to complete and balanced rations.

MATERIALS AND METHODS

Grain samples for all chemical and biological tests were obtained from replicated yield trials or seed increase plots grown on the Purdue Agronomy Farm, Lafayette, Indiana. The commercial hybrid grain samples were grown in 1969 and the other lines and hybrids in 1970 or 1971. Year of production, soil fertility level, size of plots, crude protein and amino acid analyses were utilized in making original sample selections and groupings of the samples subsequently selected for feeding trials. Seed maturity was verified by field data. Low density seed and foreign materials were removed by use of screens and by air separation.

Rat Feeding

Weanling male rats were obtained from the Wistaur Inbred Colony maintained by the Biochemistry Department at Purdue University, West Lafayette, Indiana. Six individually caged rats comprised a replication. The six rats were matched initially by weight and assigned randomly to cages and replications so that each replication had a similar average starting weight. The individually caged rats were placed on feeding shelves in a temperature, humidity, light and noise controlled room at Shuman Laboratories, Battle Ground, Indiana. The rats were provided water and feed ad libitum. Spilled feed was returned to the feeders daily. Individual weight gains were

calculated at the end of 14 days. No respiratory or other illnesses were detected at any time.

To obtain heavier rats for comparison of their growth response to the level of protein available in grain sorghum, weanling rats in excess of the required experimental number were fed a complete commercial ration until they reached the desired weight. The rats were then sorted by weight and randomly assigned to treatments and placement as was done for the smaller weanling rats.

Feed consumed per rat was calculated by subtracting the feed residue from the initial supply and dividing by the number of rats fed in that replication. A feed efficiency ratio was then calculated by dividing the amount of feed consumed by the weight gain and expressed as grams of feed consumed per gram of gain.

Chemical Analyses

The micro-Kjedahl procedure (A.O.A.C. 1960) was used to determine the nitrogen content of selected samples and mixed rations. Percent nitrogen was multiplied by 6.25 to obtain the percent crude protein.

All samples were defatted, hydrolysed and analyzed for amino acid content using an automatic Beckman Model-120 C ion-exchange resin chromatography amino acid analyzer.

Percent oil in whole grain samples was determined at the University of Illinois, Urbana, Illinois, by use of the nuclear magnetic resonance spectroscopy (NMR) technique.

Catechin equivalents were determined as percent of sample by using the vanillin-HCl procedure described by Burns (1971). One gram of grain ground to pass through a 20 mesh screen was soaked in 50 ml

methanol for 24 hours. Standards were prepared by mixing one part of 8% HCl, one part 4% vanillin and 40 mg catechin in 100 ml methanol. New standard curves were prepared daily. One ml of grain-free sample extract was mixed with 6 ml of the vanillin solution and read after 20 minutes at 500 mu on a colorimeter. The readings were then calculated as catechin equivalents from the standard curve established daily from known samples.

The *in vitro* dry matter disappearance (IVDMD) percentages were determined by use of a modified Tilley and Terry (1963) procedure. One gram of grain was digested at 39 C in 20 ml of nutrient buffer solution and 5 ml of strained rumen fluid. The vials were flushed with carbon dioxide, stoppered and periodically shaken in the stoppered vials which were equipped with gas releases. After 48 hours, 2 ml of 6 N HCl and 0.1 g of pepsin was added and mixed for continued solubilization at 39 C for 24 hours. The samples were then filtered, washed with distilled water and the dry weight determined after drying the samples at 100 C overnight. The loss in weight of a sample was calculated, corrected for rumen fluid solids and reported as the percent of the sample that disappeared during the *in vitro* digestion procedure (IVDMD).

Whole grain samples were tested for anthocyanogens by heating approximately 1 g of grain in 12N HCl. A magenta color formation indicates the presence of anthocyanogens (Blessin et al. 1963). The colors produced were rated as follows: 1 clear, 2 yellow, 3 magenta, 4 darker red, and 5 dark red and cloudy.

Other Measurements and Statistical Analysis Procedures

Pericarp color was evaluated according to the following scale: 1 white, 2 straw, 3 yellow, 4 orange, 5 light brown, 6 brown, 7 red brown, 8 gray, and 9 purple.

The grain was sectioned by cutting in half and the endosperm classified visually as follows: 3 floury, 5 normal, and 7 corneous. The endosperm was further identified as being waxy or non-waxy (blue-black color produced with iodine solution indicated non-waxy type endosperm).

Standard analysis of variance, correlations, and regressions were calculated according to methods suggested by Steel and Torrie (1960).

Field Data Collection

The number of days from planting until half the plants in the central portion of a plot were flowering half way down the panicles were recorded as days to half bloom.

At the time of seed maturity, height to the top of the panicle of the average plants in the central section of a plot was measured and recorded in centimeters.

Plots utilized for grain yield evaluations and grain sample production were a minimum of 455 cm long with 75 cm between rows. Where heights were known and uniform in the commercial yield trials, 2-row plots were utilized with 305 cm sections harvested from both rows. In other yield trials either the two center rows of 6-row plots or the center row of 3-row plots were used to calculate yields and for obtaining chemical and feeding supplies of grain. Threshed grain from two or more replications in a given experiment were pooled and

completely mixed to insure sufficient grain for feeding trials. Starting at a randomly selected spot within each harvested area of a plot, 10 consecutive panicles were cut, weighed, and subpacketed for obtaining estimates of moisture content at harvest, dry weight, and threshing percentage after being dried and threshed. All panicles in the harvested area of a plot were cut just below the bottom branches and the number of panicles per plot was recorded. Fresh weight of each plot was recorded at harvest time. Plant density for each plot was obtained by use of number of panicles harvested in each plot.

Grain samples were dried at 40 C to less than 12 percent moisture and allowed to equilibrate at room temperature prior to threshing. Samples were threshed, weighed and a Brown-Duvel grain moisture tester was used to determine grain sample moisture content. All yield data were adjusted to 15 percent moisture levels. All grain samples were stored under controlled conditions where the temperature ranged from 15-20 C and the relative humidity ranged from 30 to 40 percent.

Five plants were selected at random from the harvested plants and divided into leaf, stem and panicle components for determining plot yields. Plot dry weights were determined after the components were dried to constant weight (9% moisture) at 45 C. These plant samples were utilized to calculate yields and for chemical analysis.

Feeding Sample Preparation

All samples were uniformly prepared for feeding by cleaning, blowing, grinding, and mixing of 97 g of grain with 4 g mineral mix and 1 g vitamin mix or 96 g of grain with 4 g mineral mix and 2 g vitamin mix unless specified differently in a given experiment.

Grain samples were freshly ground just prior to feeding trials and chemical analyses. The Hawk-Oser salt mixture number three was obtained from Nutritional Biochemical, Inc., Cleveland, Ohio and the Vitamin Supplement was produced by Biochemical Research Products, Chagrin Falls, Ohio. Both contained above minimum levels required for normal weanling rat growth.

A large sample of the line identified as IS (India Sorghum) 2319 was obtained, subdivided and stored unground. These subsamples were used as the check sample for comparisons among samples fed to different groups of rats at different times. Rat weight gains were calculated as actual grams of weight gained and expressed as percent of the check gain obtained with a given set of rats at a given time.

Partial removal of the pericarp and testa was accomplished by steeping the grain for 5 minutes in 60 C water, followed by heating it for 5 minutes in 20 percent sodium hydroxide solution at 60 C. The grain was rinsed six times in water at 60 C, neutralized with 5% acetic acid at 60 C for 5 minutes, washed five times with 60 C water, and dried at 60 C as described by Blessin (1971). This procedure was used to reduce the amount of tannins and/or pigmented complexes measured as catechin equivalents from selected samples of grain sorghum.

Iso-nitrogenous samples were prepared by utilization of crude protein and amino acid analyses of selected representative grain samples. The concentration of crude protein in the grain was determined and the amount of a selected grain sample that would supply a required amount was calculated. The grain sample was then diluted to a selected % protein by the addition of commercial corn starch.

When supplementation of a given amino acid was desired, the amount provided by the grain was calculated and the commercially prepared amino acid was added to bring its level to 125% of the rat requirement (Mertz 1969), replacing equal amounts of the corn starch filler. Thus the major source of protein supplied to the rats was from the grain sorghum samples with a very small amount added via the corn starch and amino acid supplementation. Subsamples of each iso-nitrogenous ration were analyzed after mixing to verify the actual crude protein and amino acid content being fed.

RESULTS AND DISCUSSION

Differences found in IVDMD rates of selected commercial hybrids.

Table 1 shows yield, crude protein concentration, and % of IVDMD for leaves, stems, and grain from selected commercial hybrids. The crude protein concentrations ranged from 8.3 to 13.3% in the grain selections. For some grain samples the IVDMD percentages were lower than expected for grain as compared to IVDMD percentages for stems and leaves. The IVDMD percentages for grain were significantly different among the varieties in this trial and ranged from 3150 to 7070 kg/ha. The range of total solubilized dry matter in the stems, leaves, and grain among hybrids was from 6,800 kg/ha to 11,460 kg/ha. These differences among commercial hybrids led to the subsequently described studies designed to identify the factor(s) in the grain of a specific variety which reduced the solubility rate of the *in vitro* procedure.

In vivo differences of the magnitude demonstrated among these selected hybrids by the *in vitro* results, if and when translated into animal performance, could be of considerable economic importance. For human nutrition, understanding and controlling the factor(s) related to availability of dry matter differences of such magnitudes could be critical for making steps toward improvement or maintenance of health. This would be of primary importance in isolated geographic areas where people are dependent upon grain sorghum as a major food source.

Table 1. Comparisons of yield, protein content, and *in vitro* dry matter disappearance (IVDMD) percentages of selected commercial hybrids (planted June 17 and harvested October 21, 1969 from replicated yield trials on the Purdue Agronomy Farm) (1).

Variety		Plant Part	Dry Matter					
			Yield		Protein		IVDMD(2)	
			%	kg/ha	%	kg/ha	%	kg/ha
DeKalb	A-25	Grain	43	7350	13.3	970	50	3680
		Leaves	15	2530	12.0	300	55	1380
		Stems	28	4820	4.4	210	55	2640
	C-42a	Grain	44	8580	9.7	830	70	6000
		Leaves	18	3490	10.9	380	55	1930
		Stems	22	4440	3.4	160	40	1780
	BR 44	Grain	40	6350	10.0	630	50	3150
		Leaves	16	2480	12.4	300	57	1410
		Stems	30	4720	4.1	190	47	2240
Pioneer	866	Grain	42	9910	9.8	970	71	7070
		Leaves	13	3070	12.0	370	55	1680
		Stems	28	6700	3.7	250	40	2710
	846	Grain	41	8160	9.7	800	67	5500
		Leaves	18	3540	12.7	450	54	1930
		Stems	24	4840	3.9	190	40	1930
Taylor Evans		Grain	40	9960	8.3	820	67	6740
Exp.	11105	Leaves	17	4380	11.1	480	53	2340
		Stems	26	6480	3.1	720	35	2260
RS	610	Grain	41	7360	9.7	720	67	4940
		Leaves	14	2600	12.1	310	60	1580
		Stems	30	5470	3.7	200	41	2260
Averages		Grain	41	8230	10.1	820	63	5300
		Leaves	16	3160	11.9	370	56	1750
		Stems	27	5350	3.7	200	43	2250
C.V. (%)		Grain	4.9	12.1	5.8	11.1	5.9	13.8
		Leaves	6.5	9.3	4.0	11.2	5.0	8.7
		Stems	5.1	12.1	9.6	18.5	4.7	11.4
L.S.D. (.05)		Grain	NSD	NSD	1.4	NSD	5.6	1530
		Leaves	2.5	720	NSD	100	4.2	320
		Stems	3.3	NSD	NSD	NSD	2.9	380

(1) Yields were calculated from 7 replications, two replications were used in the IVDMD calculations, and all plots were fertilized with 309-64-360 kg/ha.

(2) *In vitro* dry matter disappearance percentages as determined by the modified method of Tilley and Terry (1963).

When supplementation of a given amino acid was desired, the amount provided by the grain was calculated and the commercially prepared amino acid was added to bring its level to 125% of the rat requirement (Mertz 1969), replacing equal amounts of the corn starch filler. Thus the major source of protein supplied to the rats was from the grain sorghum samples with a very small amount added via the corn starch and amino acid supplementation. Subsamples of each iso-nitrogenous ration were analyzed after mixing to verify the actual crude protein and amino acid content being fed.

Weanling (50g) rat compared to 80g rat weight gains when fed grain sorghum rations.

Weanling rats have been used extensively for nutritional studies. It was hypothesized that dilution of grain sorghum selections to an iso-nitrogenous level would lower the crude protein content of the rations to below 8% in order to feed the desired samples. The alternatives were to supplement all rations with a fixed amount of crude protein or feed the samples at their natural concentration of crude protein. To avoid confounding protein quality by the addition of some other source of crude protein in the rations, grain sorghum selections mixed with only vitamins and minerals were fed. Considerable variability was found among rats on a selected ration.

Starting weanling (50g) rats on an all cereal diet was thought to cause much of this noted variability among a set of rats. Weanling rats were fed on complete rations until they reached 80g and then compared with 50g weanling rats on rations containing varying concentrations of crude protein and CE. It was hypothesized that older or heavier rats would have a lower protein requirement and thus show less variability than the 50g weanling rats in 14-day weight gains on grain sorghum rations.

It was found that 50g rats showed a lower coefficient of variability than 80g rats (Table 2) and were thus as useful for test animals in samples fed with varying concentrations of CE and crude protein as older rats. These data indicated that the younger rats gained more than the older rats when fed rations made up of the high CE samples and gained less than the heavier rats when fed rations made up of the low CE samples. When considered over both rat sizes and

without regard to CE differences of the grains, weight gains were very similar and the range in weight gains was not related to crude protein concentrations of the rations.

The effect of CE concentrations was evident by the comparison of rations made from RS 610 and BR 64. These selections had similar crude protein concentrations, but differed greatly in rat weight gain. BR 64, which contained a higher CE concentration than RS 610, resulted in significantly less weight gain than RS 610. Further, the 80g rats showed a tendency to gain less than the 50g rats when fed these two grain selections. These data indicated that 50g rats could be used as effectively as 80g rats to compare rat weight gains from grain sorghum samples fed at various concentrations of CE and crude protein.

Table 2. A comparison of 14-day rat weight gains on variable concentrations of crude protein and CE in grain sorghum samples starting with rats having initial weights of 50 and 80 grams (1).

Sample Identification	Catechin Equivalent	Crude Protein	Rat Weight Gain	
			50 g	80 g
		%	g	g
RS 610	.48	9.9	5.32	3.59
IS 2319	.51	11.8	14.24	15.98
IS 0062	.52	12.3	12.32	17.56
IS 3982	.63	12.6	8.21	10.29
IS 0075	.97	12.5	2.12	2.25
Low CE Average	.62	11.8	8.44	9.93
IS 6992	4.61	9.3	3.19	2.20
IS 2942	5.70	10.0	2.21	-2.91
BR 64	8.10	9.3	1.54	-2.28
High CE Average	6.14	9.5	2.31	-.99
Average of all		11.0	6.15	5.84
C.V. (%)			.36	.59
LSD (.05)			2.98	7.35

(1) 6 rats were fed per replication. Means of 2 replications are reported.

Use of weanling rats for screening grain sorghums for nutritional quality.

Grain sorghums vary greatly in crude protein, amino acid, pigment, and oil concentrations as well as endosperm types. To correlate chemical data with biological estimates of nutritional quality, weanling rats were fed the grain sorghum samples supplemented only with minerals and vitamins. This reduced the dilution of pigments, carbohydrates, and other factors, but confounded the normal consideration of feeding rations for nutritional quality at iso-nitrogenous levels to avoid differences due to variable concentrations of crude protein. To overcome this consideration, it was hypothesized that crude protein differences could be minimized by observing weight gains within groupings having similar concentrations of protein in the grain. The data shown in Figure 1 indicated that at any selected crude protein concentration, there was a wide range of rat weight gains, expressed as % of the IS 2319 sample fed as a control (% of check). Selections with a high CE concentration tended to result in lower average rat weight gains. These high CE concentrations were found to be associated with a dark colored testa. A dark colored testa was found also on the commercial varieties showing lower rates of IVDMD (Table 1). It was further noted that visual ratings, according to testa and pericarp characteristics, did not consistently predict CE concentrations and subsequent rat response.

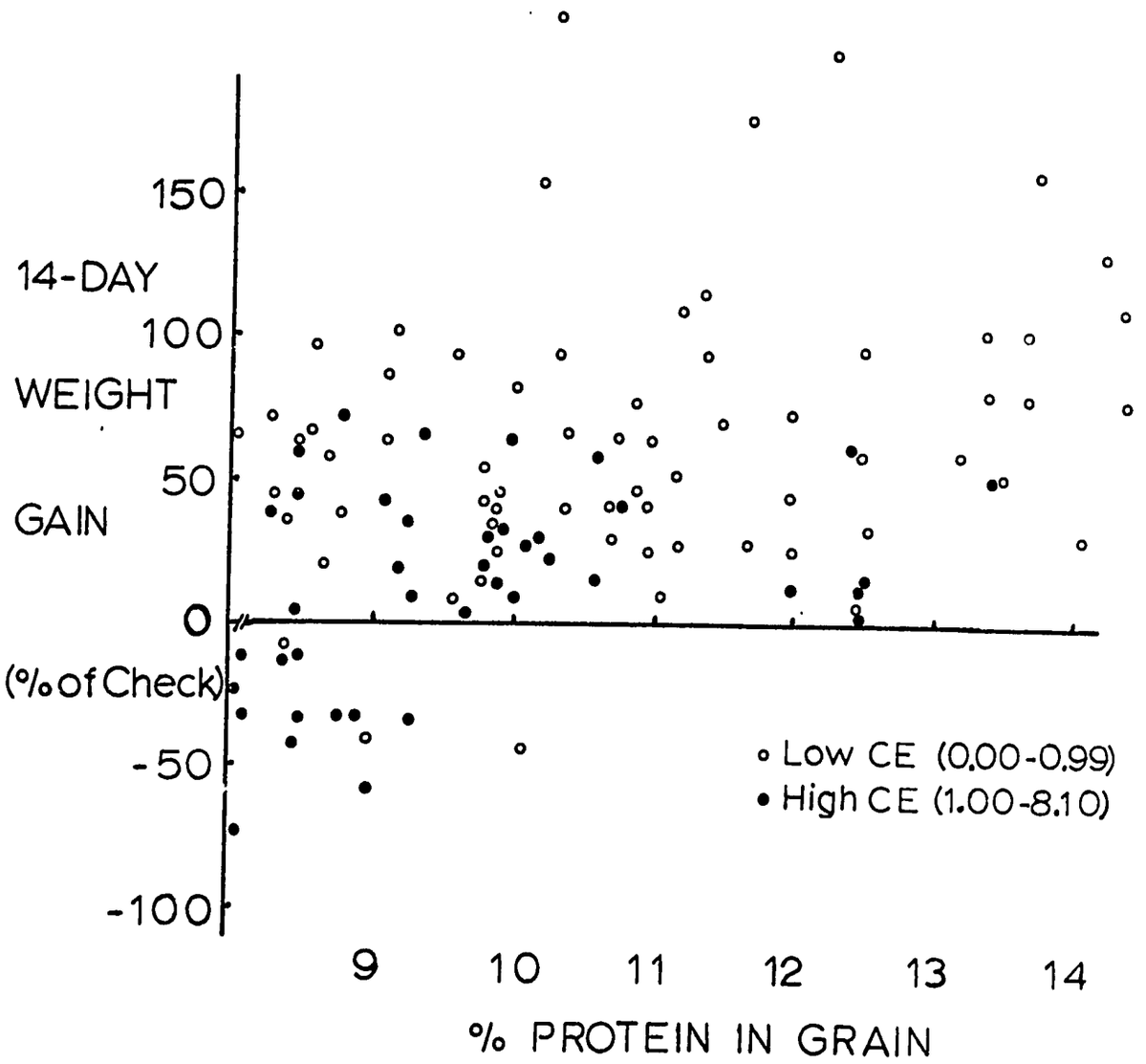

Figure 1. Rat weight gain from 110 grain sorghum genotypes of differing crude protein and catechin equivalent (CE) concentrations.

Relationship of polyphenols (tannins), as measured by catechin equivalents (CE), to the evaluation of grain sorghum nutritional quality.

Data for 172 grain sorghum samples fed to weanling rats over 14-day feeding periods were obtained. To study the relationships of rat weight gain to chemical analyses, 110 of the 172 samples were chosen to represent a random selection of the range of crude protein and CE concentrations within the genotypes fed. CE concentrations were used as a measure of the amount of polyphenols (tannins) in the grain (Maxon et al. 1972).

Within the 110 genotypes, a natural break seemed evident by the spread in the sample frequency, means for CE concentration and amount of weight gain between the group of samples just below and just above the CE concentrations of 1.00 (Table 3). The five groups based on CE concentrations, as shown in Table 3, were then grouped into 66 samples below a CE concentration of 1.00 (low CE group) and 44 samples containing a CE concentration of 1.00 or above (high CE group). Data relative to means and correlations of weight gains with selected means are shown for the high and low groups in Table 4.

Interpretation of the data for all 110 of the selected genotypes (Table 3), when divided into low and high CE concentration groups, was difficult, because correlation of weight gain, as % of check, with grams of lysine per 100 grams of protein (lysine % of protein) was found to be negative rather than positive at low CE concentrations. Amino acid analyses indicated that all genotypes contained less lysine than required for normal growth of weanling rats. Therefore, a positive correlation of weight gain with lysine as % protein was anticipated. However, since these grain sorghum samples were not fed

Table 3. Rat weight gain as related to CE concentrations, IVDMD and correlations with lysine content of 110 selected sorghum genotypes (1).

No. of Samples	CE Range	CE Mean	Protein Mean %	Weight Gain Mean g	Weight Gain Mean % Check	IVDMD Mean %	Correlation (r) of % Check with: Protein %	Lysine % Protein	Lysine % Sample	IVDMD %
42	.00 - .49	.25	10.60	5.75	63	78	.268	.047	.221	.309*
24	.50 - .99	.61	10.79	5.68	62	74	.306	.218	.537**	.656**
11	1.00 - 1.49	1.43	10.38	3.37	27	66	.084	.445	.475	.285
12	1.50 - 3.99	3.13	9.09	3.21	28	58	.552	.035	.416	.281
21	4.00 - 8.10	5.96	9.44	.92	4	55	.751**	-.503*	.046	.295
				Low CE Group						
66	.00 - .99	.38	10.70	5.72	63	77	.280*	.104	.293*	.459**
				High CE Group						
44	1.00 - 8.10	4.13	9.69	2.15	17	59	.392**	-.331*	.055	.301*
				All Samples						
110	.00 - 8.10	1.90	10.28	4.29	44	69	.400**	-.090	.292**	.548**

(1) All rat rations were composed of 94% grain sorghum, 2% vitamin mixture, and 4% mineral mixture.

* Significant at .05 level
** Significant at .01 level

Table 4. Correlations of 14-day weanling rat weight gain, expressed as percent of check, with percent protein, amino acids as percent protein and selected factors.

Selected Factors	Low CE Samples 66 Means	High CE Samples 44 Means	Low CE Samples 66 r	High CE Samples 44 r
Weight gain (% of check)	63	17	1.000	1.000
% Protein	10.63	9.69	.292*	.392**
CE	.38	4.13	.049	-.472**
Weight (g)	5.75	2.28	.788**	.928**
Days Flower	80	82	-.074	-.098
Height (cm)	183	187	.408**	-.015
HCl test	2.91	3.94	.234	-.176
% Oil	3.13	3.21	.184	.174
IVDMD	77	59	.444**	.301*
100 seed (g)	2.46	2.36	.051	.247
mg P/seed	2.78	2.43	.214	.364*
mg L/seed	.062	.055	.276	.244
Pericarp	4.08	5.74	-.087	-.360
Endosperm	5.36	4.47	-.217	-.171
Lysine % of sample	.254	.233	.278*	.055
Meth+Cyst	2.64	2.80	.001	.305*
Iso/Leuc	.289	.292	-.071	-.331*
Lysine	2.29	2.33	.088	-.373**
Histidine	2.22	2.56	-.346*	.217
Arginine	4.05	4.17	.112	-.066
Asp. acid	7.31	7.17	-.006	-.190
Threonine	3.23	3.19	-.120	.012
Serine	4.42	4.37	-.064	.200
Glu. acid	22.98	22.44	-.075	.316*
Proline	8.05	7.72	-.103	.605**
Cystine	1.25	1.32	-.352**	.273
Glycine	3.21	3.25	.013	-.238
Alanine	9.75	9.67	-.196	.233
Valine	5.21	5.11	-.093	-.013
Methionine	1.41	1.53	.157	.239
Isoluecine	4.11	4.05	-.268*	.148
Leucine	14.14	13.91	-.237	.278
Tyrosine	4.88	4.60	-.081	.397**
Phenylalanine	5.35	5.35	-.280*	.114

* Significant at .05 level
** Significant at .01 level

at iso-nitrogenous levels, the varying levels of protein confounded the direct use of lysine expressed as percent of protein as a measure to predict protein quality and thus be a predictor of rat weight gains. Had the selected samples all been fed on an iso-nitrogenous basis, then lysine as percent of protein would have been equivalent to lysine content or lysine as % of sample (grams of lysine per 100 grams of sample). As fed in this study, correlation of weight gain with lysine expressed as percent of sample, for the low CE concentration group of samples, showed the expected dependency of rat growth on lysine % of sample (r=.293*). However, the high CE group did not show a significant correlation of weight gain with lysine % of sample (r=.055) which is indicative of an overshadowing influence of the factors associated with high concentrations of CE on nutritive quality of sorghum grain.

Weight gains (g) for 48 low CE samples and 35 high CE samples were plotted against their respective lysine percent of sample (Figure 2 a & b) and protein percent of sample (Figure 3). The regression of rat weight gain on lysine % of sample for the low CE samples was positive (r=.290*), but for the high CE samples the regression of weight gain on lysine % of sample was not significantly different from zero (r=.055). These differences indicated that the low and high CE samples should be studied separately due to factors associated with the high CE concentrations which reduced rat weight gains more than the lysine content which normally limits rat growth in cereal crops. The lysine content was lower in these samples than the amount required for normal rat growth. (See discussion later and Figures 5 and 6).

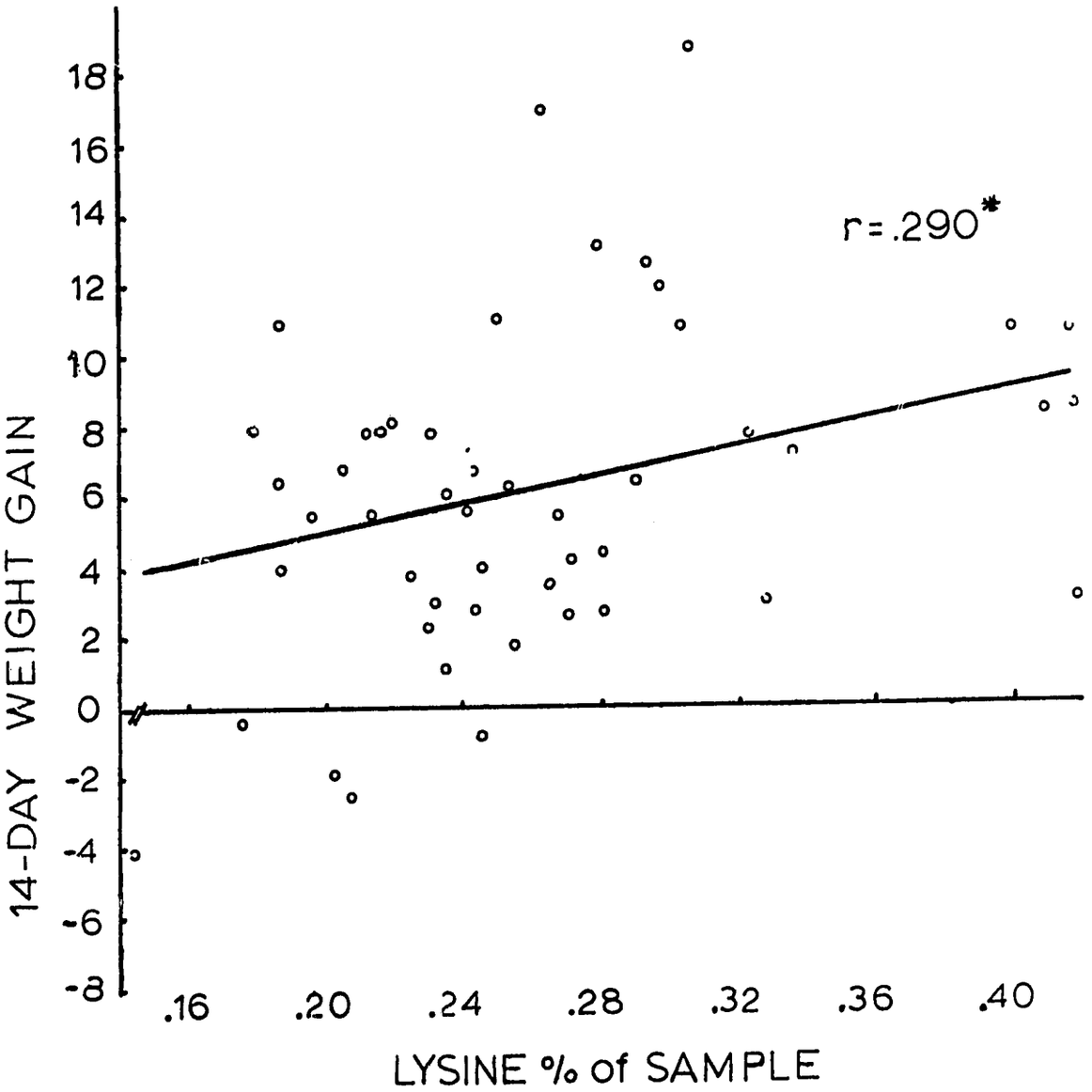

Figure 2a. Regression of rat weight gain (g) on grams of lysine per 100 grams of sample for 48 low CE concentration grain sorghum samples.

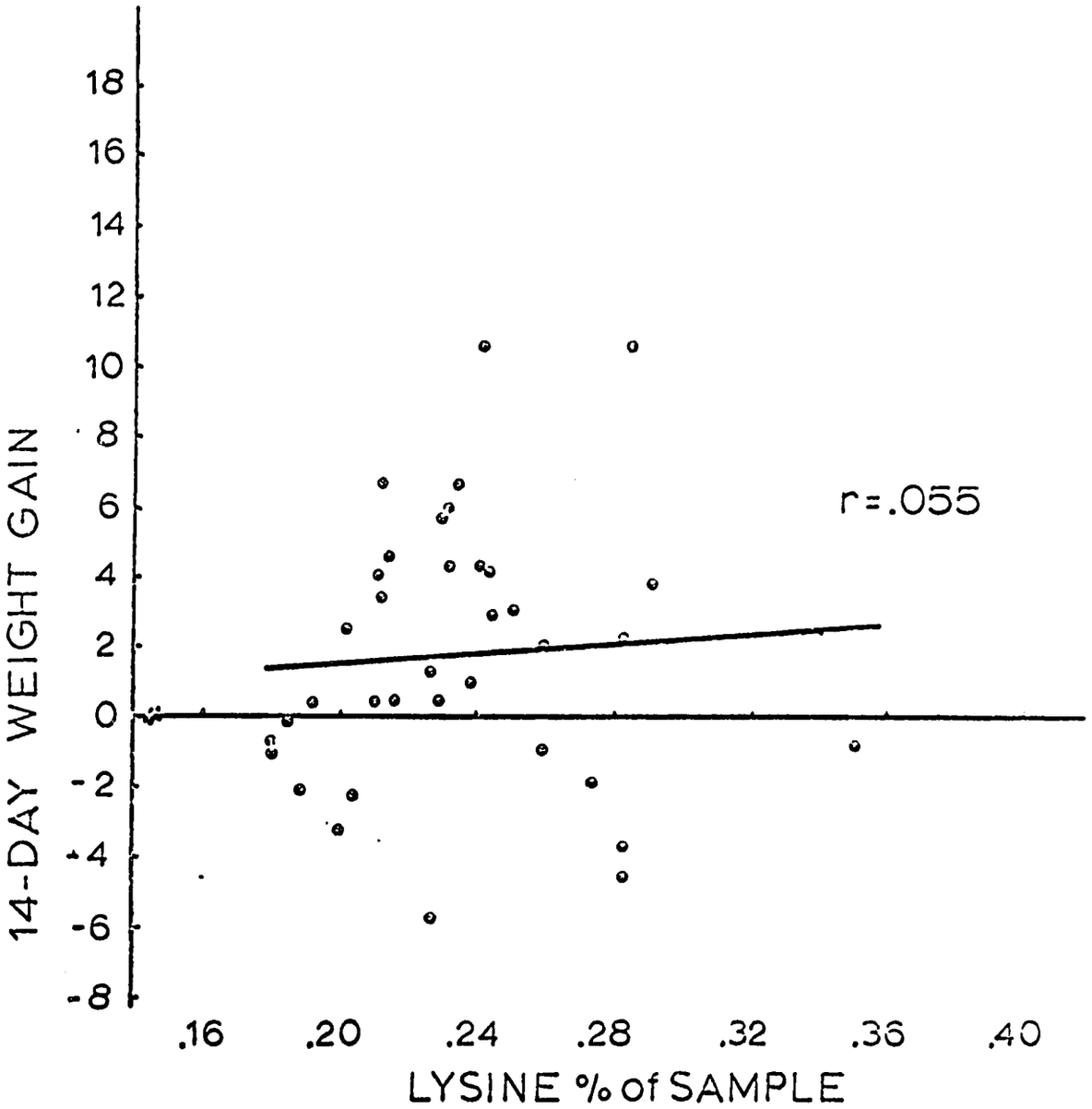

Figure 2b. Regression of rat weight gain (g) on grams of lysine per 100 grams of sample for 36 high CE concentration grain sorghum samples.

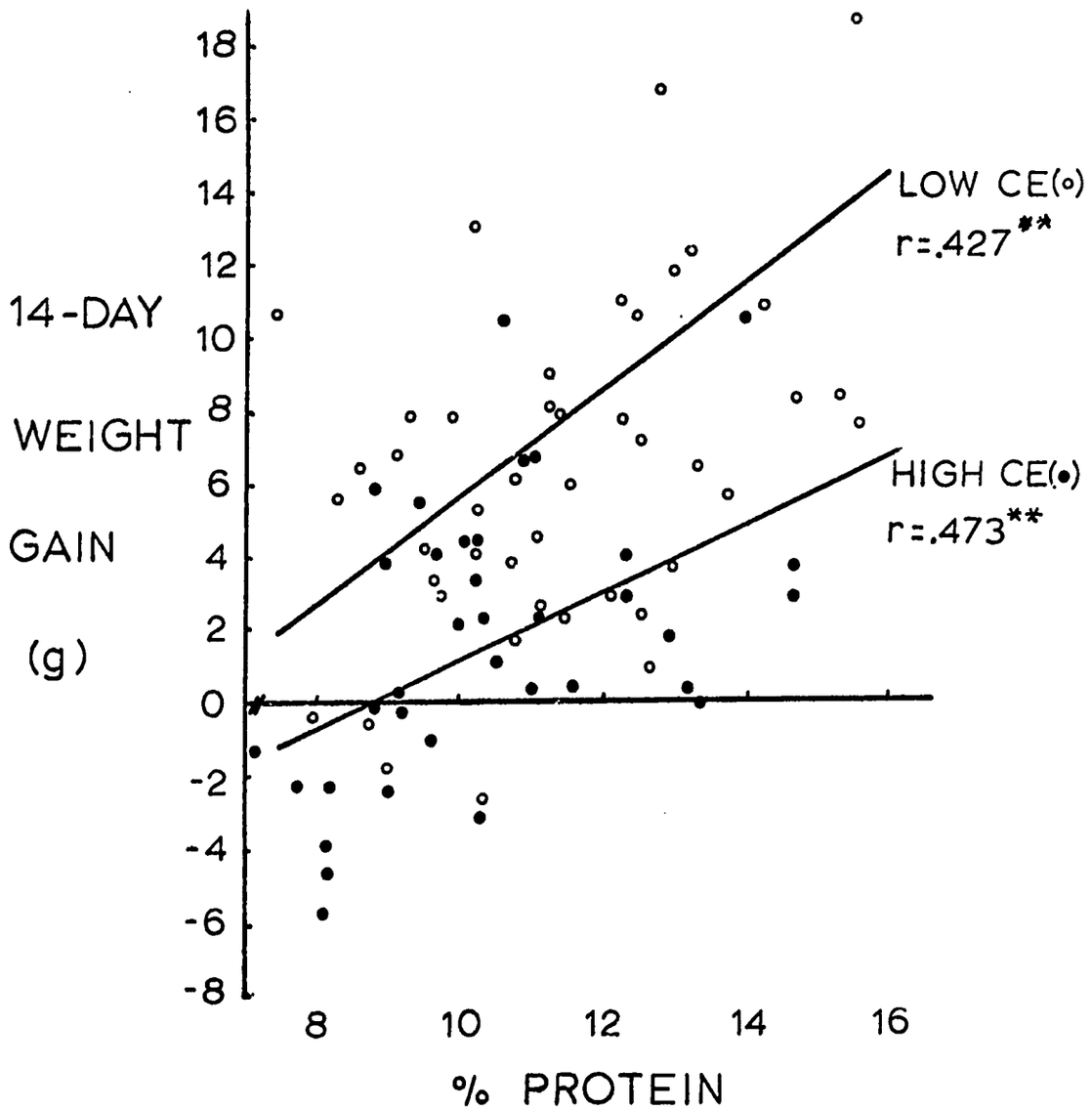

Figure 3. Regression of rat weight gain (g) on percent crude protein in 48 low and 36 high CE concentration grain samples.

Rat weight gains from low and high CE genotypes showed a positive response to increased protein content as anticipated (Table 3 and Figure 3). Since the low CE sample weight gains were correlated with both % crude protein and lysine % of sample and the high CE weight gains were correlated only with % crude protein, this would suggest that the limiting factor in the high CE sample was associated with % crude protein and its availability rather than lysine % of sample. This is supported by the lower weight gains obtained at a given % crude protein and the difference in the regression lines shown in Figure 3 which is lower for the high CE samples than for the low CE samples (Schaffert 1972). However, the regressions for low and high CE group weight gains on % crude protein were not statistically different.

The high level of error associated with these data, representing the means of six rats fed in unreplicated rat trials at inherent crude protein content, limits their use to general implications. In the screening of large numbers, identification of extremes was possible and useful to select genotypes within the high and low CE concentrations for more detailed iso-nitrogenous studies of factors associated with the CE concentrations and the nutritional quality of grain sorghums.

Use of the <u>in vitro</u> dry matter disappearance (IVDMD) procedure (modified Tilley and Terry 1963) for estimating rat weight gain and thus estimating the nutritional quality of grain sorghum was shown by the data in Table 3. The mean IVDMD for each CE group was consistently lower as the CE mean increased. The correlation of weight gain as % check was positively correlated with IVDMD for low CE samples (r=.459**), high CE samples (r=.301*) and all samples together (r=.548**).

Correlations of IVDMD with CE concentrations (not shown) were r= -.185 for the low CE samples, r= -.457** for the high CE samples and when considered all together r= -.623**. These data indicate that IVDMD percentages were reliable estimates of nutritional quality in groups of grain sorghum where high and low CE samples were studied together or when high CE samples would be studied in a separate group. The IVDMD percentage was a good estimator of the combined influences of crude protein and CE concentrations as shown by the IVDMD means and their correlations with weight gain.

Comparison of rat weight gains in high and low CE groups of grain sorghum genotypes at near iso-nitrogenous levels.

Each of the near iso-nitrogenous groups had similar CE values (Table 5). The average crude protein and lysine as % protein concentrations of the low and high CE groups at a given level of crude protein were similar. However, the lysine content (g of lysine per 100g sample) increased as the % crude protein levels increased. This indicated that the % lysine in the crude protein remained constant or decreased only slightly as the % crude protein increased. Thus, in the low CE samples as the % crude protein increased the lysine as % sample increased and this resulted in an increase in rat weight gain.

Increased weight gain was also indicated in the low CE group by the general increase of the IVDMD percentages. High CE group means were consistently lower in rat weight gains (except at the 10% crude protein level), rat weight gains expressed as % of check and IVDMD % for all levels of crude protein as compared to the low CE sample means. Two of the eight genotypes in the high CE group at the 10%

Table 5. Comparisons of means of grain sorghum genotypes grouped by crude protein levels and high and low concentrations of catechin equivalents (CE).

No. of Samples	C.E.	Protein %	Rat Weight Gain g	Rat Weight Gain % Check	IVDMD %	Lysine* % Sample	Lysine* % Protein
			Below 8.9% crude protein				
12	.40	8.37	4.10	47	70	.20	3.00
17	4.00	8.24	.13	1	55	.23	2.82
			From 9.0 to 9.9% crude protein				
15	.26	9.63	4.87	46	74	.24	2.59
13	4.96	9.48	2.39	17	52	.22	2.64
			From 10.0 to 10.9% crude protein				
11	.51	10.47	5.19	71	73	.25	2.75
8	4.09	10.34	5.20	39	68	.24	2.96
			From 11.0 to 11.9% crude protein				
13	.34	11.26	6.86	66	84	.28	2.45
0							
			From 12.0 to 14% crude protein				
15	.42	13.19	6.94	80	80	.30	2.50
6	2.21	12.58	3.33	30	71	.26	2.75

* Lysine rat requirement is 1% of sample (Mertz 1969).

crude protein level gave unusually high rat weight gains and thus caused the mean weight gains for this protein level to be higher than would be indicated for the other high CE samples in the protein level group.

Weight gains increased in the low CE genotype as the % crude protein and the lysine content of the sample increased. Likewise weight gain expressed as % of check for the high CE samples showed a general increase as % crude protein increased but at much lower levels (1-39) than for the low CE samples (46-80). This showed that CE concentration was a dependable indicator of the presence of a factor(s) in the high CE genotypes which resulted in reduced rat weight gain at a given crude protein and lysine content.

Data in Table 5 showed, as was indicated in Table 3, that the IVDMD procedure was an effective method of predicting rat weight gain when CE, crude protein and lysine concentrations of a given sample were considered simultaneously. This was especially true when samples were studied at various levels of crude protein in order to screen large numbers of samples having relatively limited amounts of grain for feeding purposes. These data indicate that the IVDMD procedure would be useful to identify genotypes having exceptional nutritional quality for detailed isonitrogenous or other studies that could lead to the identification of the specific factors in a genotype related to its inherent nutritional qualities and inheritance of these qualities.

Supplementation of high and low CE commercial grain sorghum rations for weanling rats with choline and methionine.

Methionine and choline are potential methyl donors (Mitjavila et al. 1970) that could be expected to furnish methyl groups for the breakdown of tannins in the intestinal tract of a rat. Since it is thought that the test for CE concentrations measures tannins, weanling rats were fed a low CE selection (RS 610) and a high CE selection (BR 64) ration supplemented and unsupplemented with methionine and choline alone and in combination with each other.

No significant differences in weight gains, feed consumed or feed efficiency ratios were found for any of the rations containing RS 610 (Table 6). However, BR 64 fed unsupplemented or supplemented with methionine or choline alone showed a significantly lower rate of rat gain than the rations made with RS 610 and low in CE. The feeding of choline with methionine as a supplement in the BR 64 ration showed weight gains and a feed efficiency ratio similar to that of rations from RS 610. In addition, the amounts of feed consumed on each of the 4 BR 64 rations were similar to the RS 610 containing rations.

These data indicate that some factors limiting rat growth in the BR 64 rations were corrected for rat growth when both methionine and choline were added simultaneously to the grain sorghum ration containing the high CE concentration. The data showed, in addition, that feed consumption was not significantly reduced for the 3 BR 64 rations which produced the lowest amount of rat weight gain. Thus, palatability or acceptability in this trial was not the factor causing the high CE concentration sample (BR 64) weight gains to be significantly lower

Table 6. Supplementation of low (RS 610) and high (BR 64) CE commercial grain sorghum rations for weanling rats with methionine and choline *.

Sample Identification	14-day Weight Gains g	Feed Consumed g/rat	g feed/ g gain
RS 610 (low CE group: 0.48 CE)	5.27 a	112 a	21 a
RS 610 + methionine (1)	5.95 a	107 a	18 a
RS 610 + choline (2)	4.05 a	117 a	28 a
RS 610 + methionine + choline (3)	6.50 a	105 a	16 a
BR 64 (high CE group: 8.10 CE)	1.31 b	116 a	88 b
BR 64 + methionine (1)	.91 b	97 a	106 b
BR 64 + choline (2)	.76 b	136 a	179 b
BR 64 + methionine + choline (3)	4.39 a	129 a	29 a
Average	3.64	115	
C.V. (%)	45	9	

* Numbers followed by the same letters are not significantly different at the 5 percent level based on Duncans multiple range test. All values are the average of 2 replications containing 6 rats each fed for a period of 14 days. All rations contained 2% vitamin mixture and 4% mineral mixture.

1) Methionine was supplemented at the rate of .15 g per 100 g of ration.

2) Choline was supplemented at the rate of .20 g per 100 g of ration.

3) Methionine and choline were supplemented at .15 g and .20 g respectively per 100 g of ration.

than the RS 610 ration gains. The apparent benefit from combining methionine with choline is not understood and merits further investigation.

The effect of dehulling and lysine supplementation of high CE grain sorghums on weanling rat weight gain.

Removal of the outer layers of the caryopsis with hot sodium hydroxide lowered the CE (tannin) level of the remaining grain without significantly altering the crude protein content of the grain as Blessin (1971) had reported was possible (Table 7). Amounts of feed consumed by rats fed rations based on dehulled grain was not significantly different from whole grain rations. However, the feed efficiency ratio was higher for the whole grain sample of BR 64 which contained a higher CE concentration than the low CE selection fed (RS 610) and the dehulled samples. This indicated that the factor(s) associated with the CE concentration test were removed by the dehulling procedure and that weanling rats could then gain weight on the dehulled-sample ration at a rate similar to weight gains obtained from low CE selections. The data indicated that dehulling did not alter the rate of weight gain, feed consumed or the feed efficiency ratio for the low CE selection, but did remove the factor(s) measured by the procedure to determine high CE concentrations that were causing reduced rat weight gains.

Supplementation with lysine of two high CE samples did not significantly increase 14-day weight gains for weanling rats (Figure 4). However, dehulling grain of these genotypes did result in increased weight gain and an additional increment of weight gain resulted when the dehulled samples were also supplemented with lysine. Dehulled

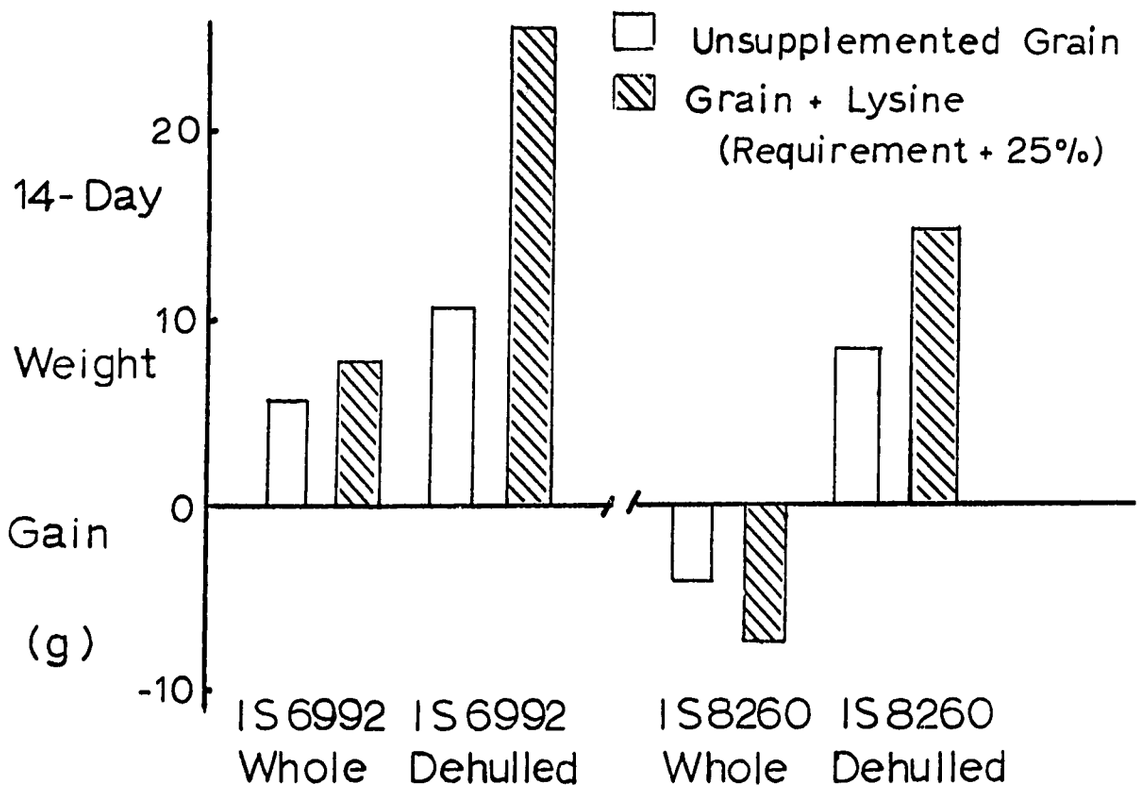

Figure 4. Rat weight gain on rations with and without lysine supplemented whole and dehulled high CE concentration grain sorghum genotypes.

IS 6992 supplemented with lysine produced rat weight gains similar to those of low CE grain sorghum genotype rations for a 14-day period. Apparently, factor(s) related tc poor rat growth present in high CE sorghum lines were removed by the dehulling process since the weight gains following dehulling were similar to those of low CE lines with and without lysine supplementation.

Table 7. Effect of alkali dehulling of grain on rat weight gain of a high and a low catechin equivalent (CE) grain sorghum ration.

Identification	C.E.	Protein %	14-day Weight g	Feed Consumed g	g feed/ g gain
RS 610 (Whole)	0.48	9.90	5.27	112	21.3
RS 610 (Dehulled)	0.10	9.95	6.73	132	19.6
BR 64 (Whole)	8.10	9.30	-.99	122	--
BR 64 (Dehulled)	.35	9.38	8.42	118	14.1
Mean			4.86	121	
C.V. (%)			42	01	
L.S.D. (.05)			6.47	28	

Essential amino acid content of high and low CE grain sorghums compared to weanling rat amino acid requirements and rat gain responses to amino acid supplementation.

Concentrations of nine amino acids considered essential for normal weanling rat growth are shown as grams of amino acid per 100 grams of protein of grain sorghum in Figure 5. The means for 36 low and 34 high CE concentration grain sorghum genotypes are compared to the weanling rat requirements. The mean protein concentration for the low CE samples was 11.61% while the mean for the high CE samples was 10.33%.

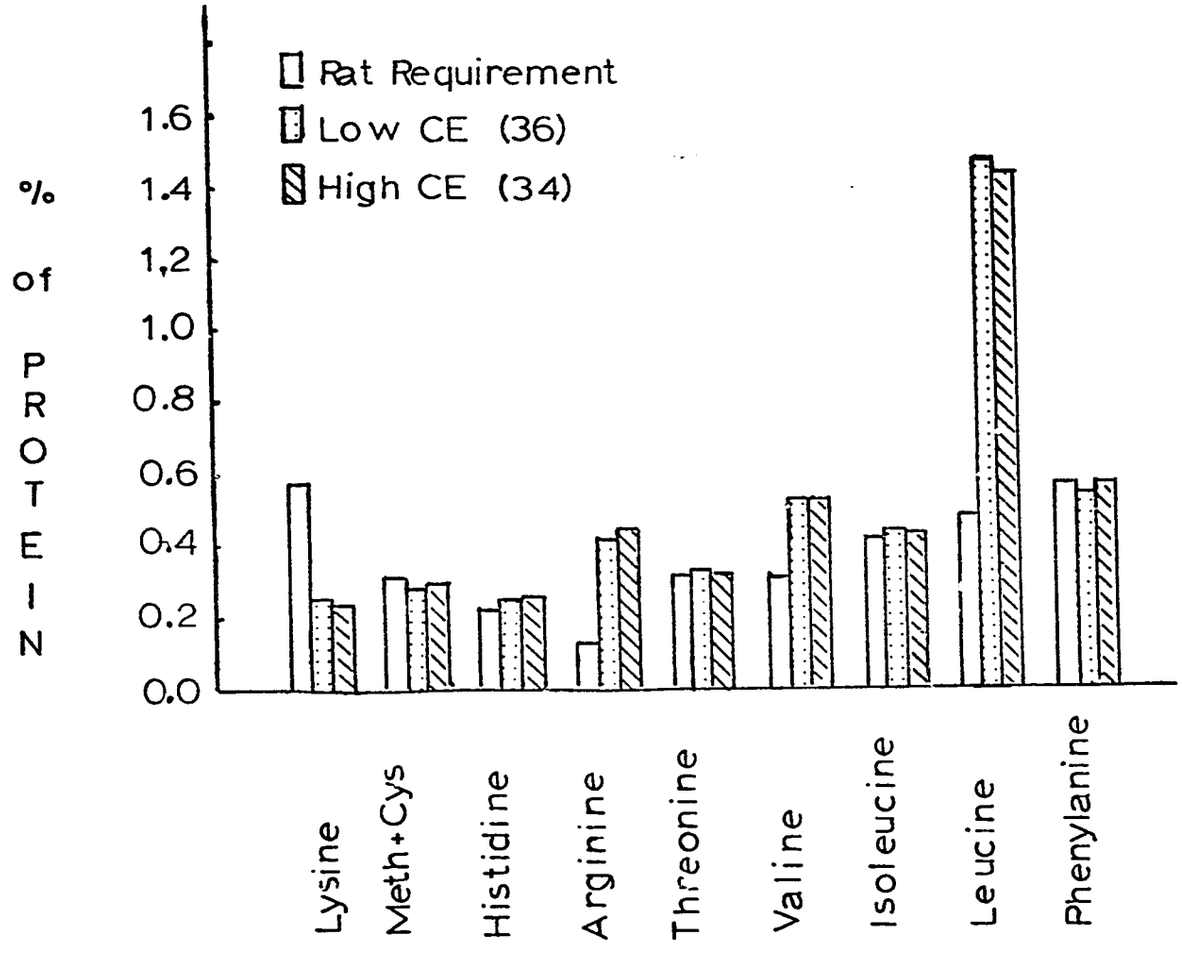

Figure 5. Comparison of weanling rat essential amino acid requirements expressed as percent of protein with high and low polyphenol (CE) grain sorghum selections.

In Figure 6, similar comparisons are made except the amino acid values are expressed as grams of amino acid per 100 grams of sample. Essentially no differences in amino acid concentrations were apparent between the high and low CE selections. The slight difference was consistent and due to the lower crude protein concentration found in the high CE selection means as compared to the low CE selection means.

Expressing the amino acids as % of protein (Figure 5) or as % of sample (Figure 6) showed concentrations of lysine in the grain samples to be very low compared to the weanling rat requirement for normal growth. In addition, the amounts of leucine and arginine were shown to be present in concentrations of more than double the weanling rat requirement. These excessive amounts could be expected to be potentially toxic to normal weanling rat growth (Mertz 1972). Grain sorghums would appear to be also deficient in methionine, threonine and possibly in isoleucine and phenylalanine when compared to concentrations of these amino acids required by weanling rats expressed as % of sample (Figure 6).

To test the validity of supplementing grain sorghums with lysine alone, as indicated, being the only amino acid needed when expressing amino acids as % of protein (Figure 5), compared to the need for additional amino acids when they were expressed as % of sample (Figure 6), two low CE grain sorghum samples which possess a complete or partial testa, but which were both low in CE concentration and one sample containing a high concentration of CE were fed at iso-nitrogenous levels in replicated trials. Rations composed of the selected grain sorghum (94%), mineral mixture (4%), and vitamin mixture (2%) were fed 14 days unsupplemented or supplemented with sufficient lysine to bring

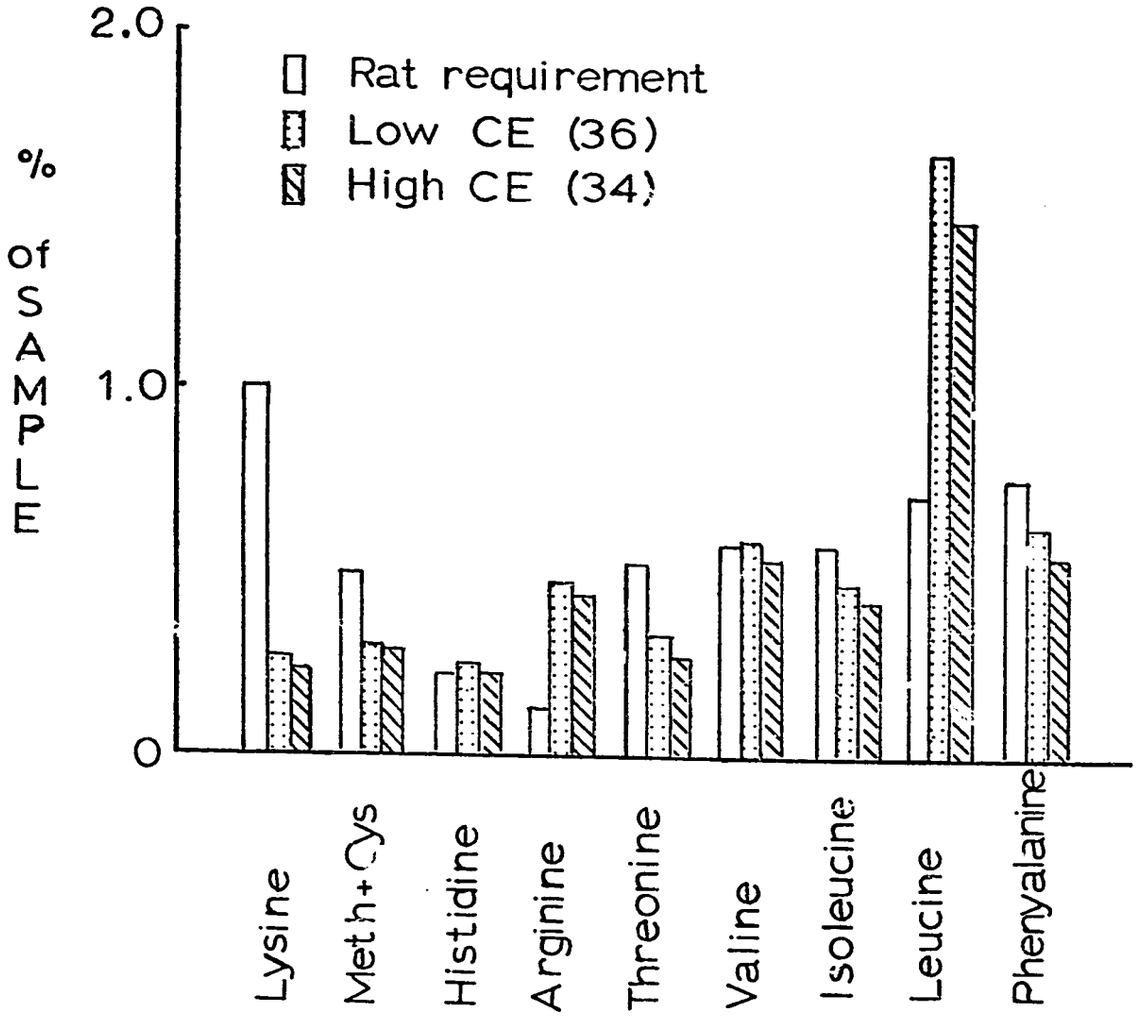

Figure 6. Comparison of essential amino acid requirements of weanling rats with high and low polyphenol (CE) content of grain sorghum.

the content up to 125% the rat requirement for normal growth. Figure 7 shows that IS 2319 responded well to lysine supplementation and that IS 0129 also responded significantly to lysine supplementation by increased rat gain. However, lysine supplementation of the ration based on the high CE selection (IS 6992) did not result in normal rat gains. Data in Figure 4, discussed earlier, showed that IS 6992, if dehulled, did respond favorably to lysine supplementation.

To test the influence of toxicity due to excessive amounts of arginine and leucine indicated in Figure 6, and to test the influence of supplementation with additional amino acids, the same grain sorghum samples discussed in the previous section and shown in Figure 7 were supplemented with lysine alone and with lysine in combination with methionine, threonine and phenylalanine. Each of the amino acids were supplemented to 125% of the rat requirement based on grams of amino acid per 100 grams of the mixed ration. Data presented in Figure 8 indicated that the addition of all four amino acids, significantly increased rat gains when fed iso-nitrogenous rations containing grain from the low CE genotypes. The slight increase in weight gain noted for the high CE genotype was not significant at the .05 level of probability. The lack of weight gain by weanling rats when fed IS 6992 (high CE) supplemented with lysine and when supplemented with all four amino acids again points out the difference between selections that contain high and low CE concentrations. In order to effectively identify the nutritional potentials of the sorghum genotypes containing high concentrations of polyphenols (tannins or tannin-like compounds) measured as CE concentrations (catechin equivalents), the samples must

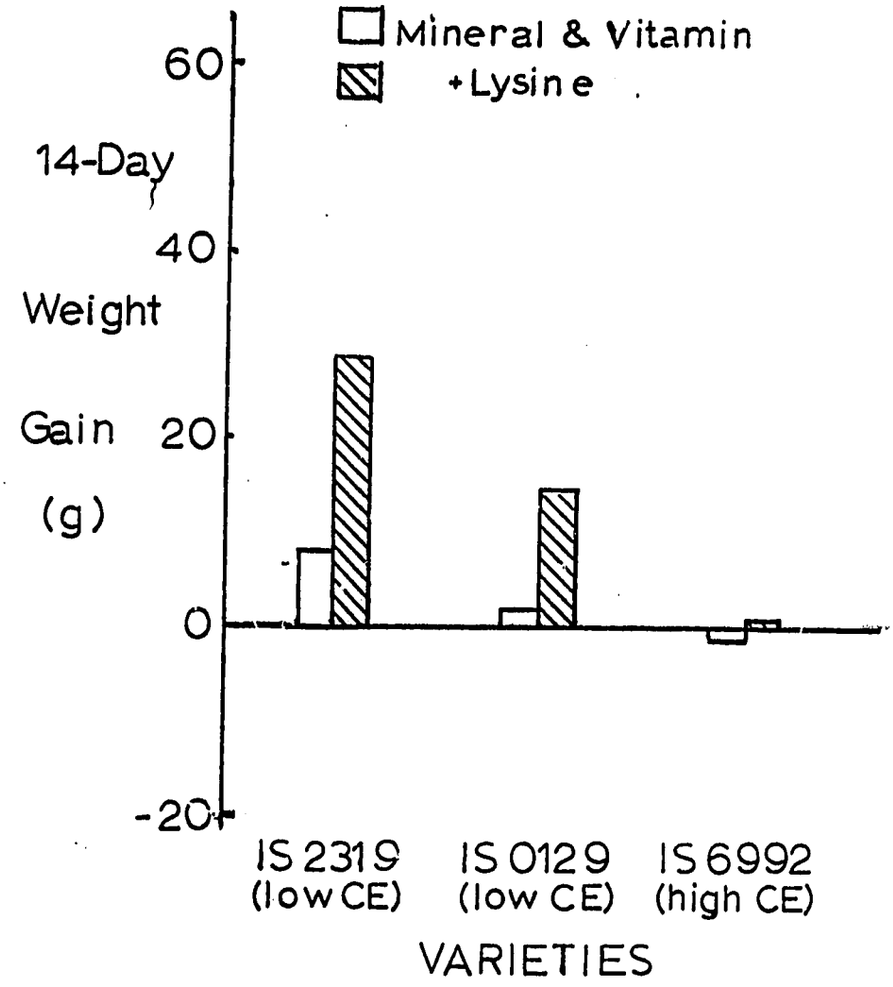

Figure 7. Influence of lysine supplemented compared to non-supplemented iso-nitrogenous low and high CE concentration grain sorghum rations on 14-day weanling rat weight gain.

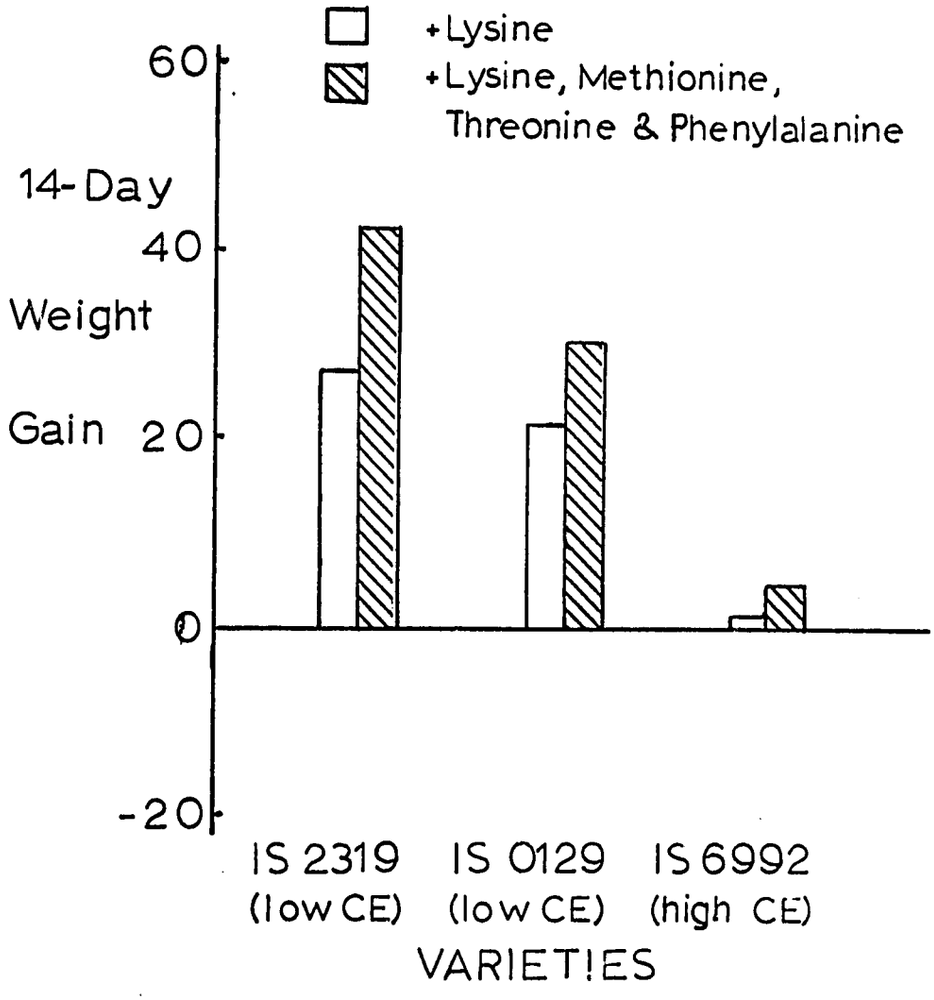

Figure 8. Influence of lysine and lysine, methionine, threonine and phenylalanine supplemented iso-nitrogenous low and high CE concentration grain sorghum rations on 14-day weanling rat weight gain.

be dehulled or have these pigment-associated substances de-activated before normal rat growth at the inherent level of crude protein can be obtained.

Work reported by Schaffert (1972) indicated that supplementing high CE genotypes with soybean meal (and likely other forms of protein) can result in normal rates of rat growth if sufficient protein was supplied by the soybean meal. Work reported by Rostagno (1972) indicated that such supplementation would result in increased fecal losses of protein compounds, and the 4 amino acids used to supplement the high CE sorghum did not improve significantly rat weight gain. Therefore, total protein is indicated as being the primary limiting factor for rat growth in rations containing high concentrations of CE.

The amount of weight gained on the rations of low CE sorghums supplemented with all four amino acids indicated that in the 14-day period measured in this experiment, the excessive amounts of arginine and leucine did not significantly retard rat weight gains since gains in excess of 3 grams per rat per day were observed.

SUMMARY AND CONCLUSIONS

Wide variations in commercial hybrid grain sorghum *in vitro* dry matter disappearance percentages (IVDMD) were found. Part of these differences in IVDMD percentages were found to be associated with the presence or absence of polyphenolic compounds associated with the pigmented testa and pericarp of the mature grain sorghum caryopsis.

Phenotypic differences in polyphenol concentration were expressed in catechin equivalents (CE). Sorghum lines with CE concentrations below 1.00 did not affect IVDMD percentage. Genotypes containing CE concentrations above 1.00 had significantly reduced IVDMD percentages. IVDMD percentages were negatively correlated with CE concentrations (r=-.623**).

Fourteen-day weanling rat weight gains from 172 sorghum selections were positively correlated with IVDMD percentages (r=.484**) and negatively correlated with CE concentrations (r=-.493**). However, when all grain sorghum selections were considered as a single group, at inherent crude protein concentrations and with varying CE concentrations, identification of genotypes having high nutritional quality by amino acid analyses was masked by the influence of factors associated with high CE concentrations in some of the pigmented sorghum selections. Thus, to relate the amino acid data to rat weight gains it is necessary to consider the low and high CE concentration groups independently.

Positive rat weight gain responses to lysine supplementation were obtained when rations made from low CE concentration selections were supplemented with lysine. Additional increases in weight gains were obtained by supplementing the rations made from low CE selections with three other essential amino acids (methionine, threonine and phenylalanine) when compared to lysine supplementation alone. Weight gains by rats fed rations supplemented with all four amino acids were in excess of 3g per day, indicating that the high leucine and arginine content in grain sorghum grains did not induce toxic effects for these short (14-day) feeding trials.

Rations from grain sorghum genotypes containing similar concentrations of crude protein and amino acid profiles, but containing CE concentrations greater than 1.00 produced significantly lower weanling rat weight gains than those obtained from the rations containing a low CE concentration. Supplementation with choline plus methionine of the ration from a sample containing a high CE concentration apparently corrected the adverse effects of high CE concentration since rat weight gains were similar to those of the ration having a low CE concentration. However, supplementation of rations from the genotypes having a high CE concentration with lysine alone or with lysine, methionine, threonine and phenylalanine combined, resulted in no significant increase in rat weight gain.

Partial removal of the pericarp and testa of the genotypes containing high CE concentrations lowered the measurable level of CE concentration. When rations containing dehulled grain were fed to weanling rats, weight gains were comparable to gains from rations with

low CE selections of similar crude protein and lysine content. Rations from dehulled high CE genotypes when supplemented with lysine resulted in improved feed efficiency ratios and weight gains increased to rates comparable with rations from genotypes with low CE concentrations.

Lysine was shown to be a limiting factor for weanling rat growth in grain sorghum by amino acid analyses. However, it was shown that lysine was not the most limiting factor in genotypes containing high CE concentrations. Therefore, genotypes containing high CE concentrations must be studied separately from genotypes containing low CE concentrations to identify amino acid deficiencies relative to nutritional quality. The chemical nature and activity of the factors associated with the pericarp and testa of genotypes containing high CE concentrations merits further investigation.

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AGRONOMICALLY ACCEPTABLE LINE SELECTIONS FROM THE WORLD COLLECTION OF GRAIN SORGHUM FOR BREEDING AND DEVELOPMENT PROGRAMS.

D. L. Oswalt and G. Srinivasan

The principal objective for the selection of this group of lines was to identify agronomically acceptable lines with a known protein production from the diversity of the present world collection of germplasm for use in protein quality improvement breeding programs.

Selection procedures:

Temperate flowering lines were phenotypically selected from the Puerto Rico and Lafayette, Indiana production of the world collection in 1969 and 1970 by R. E. Schaffert and B. Gelaw. The Indiana evaluation of lines in 1971 and 1972 was followed by replicated three-row yield trials at three diverse Indiana locations in 1973 (Dubois, Lafayette and Wanatah). All yield and other data was obtained by evaluating the center row of a three-row plot. The following data (table 1) was collected:

a) Experiment 367 entry number.

b) Identification: IS (India Sorghum) number, Purdue base (six-digit) number or commercial hybrid number (included for checks).

c) Days to flower: days from planting until one-half the plants of an entry had flowered 50% of the way down a panicle.

d) Height: cm to top of average panicle of mature plant.

e) 100 seed weight: grams per 100 randomly selected whole kernels.

f) Color: the color of the pericarp is coded as 1=white, 2=straw, 3=yellow, 4=orange, 5=light brown, 6=brown, 7=red brown, 8=gray, 9=purple.

g) Catechin equivalents (CE): the estimate of tannins in 1 g of ground whole grain samples utilizing 50 ml extracted 24 hours in methanol compared to catechin standards. (Ref: Burns, R. E. 1971. Method of estimation of tannins in grain sorghum. Agron. J. 63:511-512.)

h) Dye binding capacity (DBC): determined by use of the Udy dye binding method and used as a screen for lysine in consideration with estimates of crude protein.

i) Protein percent: micro-Kjeldahl nitrogen x 6.25 adjusted to 10% moisture.

j) Lysine percent: determined by ion-exchange resin chromatography.

k) Lysine percent of sample: protein percent x lysine percent of protein.

l) First replication yield: kg/ha of 10% moisture grain for center row of three-row plots of replication 1 at Lafayette, Indiana and used for chemical analysis.

m) Average yield: kg/ha of 10% moisture grain from center row of three-row plots, the mean of three Indiana locations (* mean of one location, ** mean of two locations).

Discussion:

This group of temperate (non light-sensitive) lines and selected commercial hybrids (Table 1) represents a diversity of phenotypes with a high incidence of white pericarp (with complete, partial or no testa) and yellow endosperm selections (color rating of 3). Selections from this group are being screened for disease and insect resistance and adaptation to adverse tropical environments in additional international yield trials.

The average yield of the nine commercial hybrids included as checks was 7250 kg/ha, with a range of 6010 to 8090 kg/ha. The average grain yield of the 204 lines was 7050 kg/ha with a range of 4580 to 9860 kg/ha.

The protein average of 8.86% is low compared to the average of similar trials in previous years at a similar yield level, but protein yields per hectare of these lines are above average due to the total grain yield obtained.

Entry no. 202 (Pioneer 931) shows a very high lysine percent of sample value. This is a typical chemical analysis of an immature grain sample which had a low yield. Entries 5, 164, 168 and 215 show above average protein quality, as indicated by lysine percent of sample, but still should be considered normal sorghums as compared to a high lysine sorghum.

These lines are being incorporated into random mating populations and individually selected for crossing with a genetic source of a hl gene to improve quality in a diversity of phenotypic characteristics which are agronomically acceptable.

Table 1. Agronomic and chemical data of selected temperate lines and commercial hybrids grown in three Indiana locations in 1973.

Entry No.	Ident.	Fl days	Ht cm	100 seed wt	Color	C.E.	DBC	% P	L % of P	L % of sample	Yield kg/ha 1st rep	Ave. yield kg/ha
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)
001	131044	76	180	2.47	2	.20	26.25	7.5	1.77	.1327	6970	7330**
002	131048	76	160	2.29	2	.45	30.25	8.7	2.16	.1879	7860	6710
003	IS0114	71	100	2.28	2	.27	30.25	9.5	2.10	.1995	6300	6340
004	IS16365	92	360	4.64	5	3.19	30.25	8.6	2.12	.1823	13080	8410
005	IS15391	102	330	3.41	3	.05	34.75	11.4	2.62	.2987	6060	6170*
006	IS16468	91	210	3.27	3	3.38	25.25	6.6	1.79	.1181	7080	7620
007	IS15106	91	360	4.12	7	4.76	31.75	8.7	2.15	.1870	11580	8580**
008	IS15455	94	250	3.43	5	3.50	29.50	8.8	2.09	.1839	6600	8100**
009	IS15661	96	300	3.06	5	3.26	33.25	8.5	2.01	.1708	6830	7430**
010	131160	92	160	2.97	5	2.48	28.00	8.5	1.97	.1674	3470	5600
011	131161	71	130	2.99	5	.57	32.25	10.7	2.25	.2407	8330	6250
012	131162	80	220	2.00	5	7.31	28.50	7.1	2.01	.1427	6100	7160**
013	IS15526	95	280	3.07	5	3.79	27.75	7.9	2.09	.1651	7560	6180**
015	IS15612	90	240	3.26	3	2.75	38.00	9.7	2.68	.2600	10980	8330**
016	IS15991	79	240	4.12	5	4.47	30.00	8.5	2.18	.1853	10560	8670**
017	IS15346	90	290	3.15	5	2.34	32.75	9.3	2.54	.2362	1100	5760
018	131305	71	110	2.27	5	1.19	30.50	8.3	1.99	.1652	6760	6800
020	IS1522	77	250	1.95	2	.28	33.25	11.5	2.40	.2760	5640	5260*
021	IS8164	81	160	1.52	5	3.29	28.75	7.7	1.90	.1463	8930	6160
022	IS1461	90	300	2.33	2	.25	32.75	9.6	2.52	.2419	7490	7560
023	IS9528	81	240	2.89	5	.44	32.00	8.4	2.32	.1949	7370	8090
025	IS8377	71	200	2.82	2	.41	32.75	11.3	2.26	.2554	3990	5750
026	IS2057	71	120	2.10	2	.67	31.25	10.0	2.25	.2250	5120	5580
027	IS0466	82	150	1.82	3	.17	29.25	8.0	2.02	.1616	4920	5600**
028	IS0339	79	210	2.70	5	1.01	31.00	10.0	2.10	.2100	8630	6470

Table 1. (Contined)

Entry No. (a)	Ident. (b)	Fl days (c)	Ht cm (d)	100 seed wt (e)	Color (f)	C.E. (g)	DBC (h)	% P (i)	L % of P (j)	L % of sample (k)	Yield kg/ha 1st rep (l)	Ave. yield kg/ha (m)
029	IS6451	76	150	4.52	3	.18	34.25	8.7	2.28	.1984	9410	7420
030	048005	74	210	3.65	2	1.33	35.00	11.8	2.24	.2643	6080	5540
031	158120	77	190	2.60	2	1.14	30.25	9.6	2.20	.2112	4760	5320**
032	IS0135	81	210	1.51	2	.56	35.00	9.3	2.53	.2353	6090	5110**
033	121112	78	170	2.76	3	.44	33.25	9.8	2.35	.2303	4640	5260**
034	121142	76	120	2.76	5	2.79	30.75	8.7	2.05	.1783	7030	6590
035	121166	80	150	2.06	5	.82	28.00	7.4	1.96	.1450	6770	7430
036	121168	75	120	2.76	5	.92	34.00	10.0	2.56	.2560	5450	6350
037	121169	102	230	1.89	2	6.97	32.75	9.4	2.17	.2040	4600	6530**
038	IS10493	72	100	1.92	3	1.47	31.00	9.1	2.03	.1847	7500	8180*
039	121179	80	330	2.75	5	.66	36.25	11.0	2.43	.2673	3520	4820*
040	121180	71	200	2.58	2	.44	30.25	9.2	2.29	.2107	6030	6580**
041	121183	81	170	3.40	3	.86	28.00	8.1	1.99	.1612	5020	5320**
042	121186	73	200	3.20	3	.55	30.50	8.8	2.19	.1927	6720	6400**
043	IS15414	91	360	4.22	2	.58	33.00	8.7	2.22	.1931	7840	7460**
044	IS15799	90	330	3.57	2	1.63	37.25	9.9	2.54	.2515	8600	6520
045	IS15720	100	350	3.00	2	2.08	33.50	8.6	2.46	.2116	8400	6720*
046	IS9180	71	100	2.20	5	1.01	31.25	9.3	2.14	.1990	4370	5610
047	IS8687	73	120	2.15	5	1.36	32.00	9.4	2.32	.2181	6460	5550
048	IS10594	72	100	2.11	3	.35	30.00	8.4	2.24	.1882	5420	6290
049	IS9750	71	110	2.23	5	.60	29.00	7.2	1.92	.1382	6190	6450
050	IS9926	71	150	1.89	5	.95	34.00	10.7	2.45	.2621	6900	6680**
051	IS9954	76	120	2.14	5	.80	32.00	8.7	2.15	.1870	4290	6280
052	IS2042	71	130	2.62	2	.39	30.25	9.3	2.33	.2167	7660	7460
053	IS2279	94	120	2.51	5	1.88	31.50	8.7	2.16	.1879	5390	4850

Table 1. (Continued)

Entry No. (a)	Ident. (b)	Fl days (c)	Ht cm (d)	100 seed wt (e)	Color (f)	C.E. (g)	DBC (h)	% P (i)	L % of P (j)	L % of sample (k)	Yield kg/ha 1st rep (l)	Ave. yield kg/ha (m)
054	IS1232	79	130	2.21	5	1.64	33.75	10.0	2.52	.2520	6170	6030
055	IS0835	76	110	2.19	5	.86	31.75	9.4	2.24	.2106	5670	5720
056	IS2701	72	150	2.32	5	.41	28.50	8.7	2.10	.1827	5880	6080**
057	IS3161	72	100	2.01	2	.42	28.25	6.9	1.75	.1207	6800	7160
058	IS0222	72	160	2.78	2.	.65	30.75	9.2	2.17	.1996	6080	6580
059	IS10929	71	100	3.34	3	.66	33.00	10.1	2.32	.2343	5240	5830
060	IS8356	79	100	1.92	5	.96	28.50	8.8	2.18	.1918	5190	6850
061	IS8352	71	140	2.58	5	.50	32.25	9.6	2.20	.2112	6800	5630
062	IS5275	72	150	2.72	2	.38	28.75	8.8	2.17	.1910	6670	7440
063	IS8708	79	150	2.06	3	.31	30.00	9.0	2.01	.1809	5810	5630
064	IS8583	99	120	2.16	2	3.99	27.50	7.2	2.08	.1498	6270	6380**
065	IS10486	79	110	1.92	5	1.08	30.00	8.5	2.08	.1768	7590	6900
066	IS10484	85	120	1.63	5	1.01	30.00	8.7	2.16	.1879	8220	5550
067	IS10477	71	110	2.40	5	1.25	32.25	10.1	2.27	.2293	6620	7000
068	IS10584	74	140	2.18	3	.68	27.25	7.7	1.99	.1532	6690	7050
069	IS10596	71	120	2.05	5	.51	33.25	10.3	2.36	.2431	7960	6310
070	IS10262	74	110	2.62	2	.56	29.00	9.0	2.23	.2007	8200	6880
071	IS10254	79	130	2.46	2	.34	30.75	10.9	2.22	.2420	6160	6430
072	IS10246	71	130	1.97	2	.13	34.75	8.9	2.59	.2305	7420	6380
073	IS10524	72	160	2.47	2	.57	33.00	9.7	2.26	.2192	6680	6420
074	IS10523	74	130	3.20	2	.35	32.00	9.9	2.30	.2277	5810	6410
075	IS10562	73	160	2.65	2	.44	30.25	9.2	2.36	.2171	7510	7190
076	IS10680	76	150	2.49	2	.44	32.50	9.9	2.33	.2307	6290	5920
077	IS10642	92	130	1.72	2	.88	30.00	10.6	2.18	.2311	4430	6200
078	IS10644	74	120	2.26	3	.11	29.75	9.4	2.18	.2049	6610	6540

Table 1. (Continued)

Entry No.	Ident.	FL days	Ht cm	100 seed wt	Color	C.E.	DBC	% P	L % of P	L % of sample	Yield kg/ha 1st rep	Ave. yield kg/ha
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)
079	IS12278	79	130	2.63	5	.53	32.25	10.2	2.40	.2448	8510	8480
080	IS2091	76	190	2.60	2	.49	27.00	7.4	1.88	.1391	6990	7570**
081	IS2217	78	160	1.87	3	.07	28.00	7.9	1.69	.1335	5470	6080
082	IS0893	71	130	1.86	5	7.64	35.00	9.3	2.16	.2009	6580	6260
083	IS0416	78	140	2.31	5	.56	31.00	10.1	2.23	.2252	6890	6890
084	IS0475	76	150	2.18	3	.25	29.00	8.5	1.97	.1674	6170	7050**
085	IS8544	74	100	1.80	2	4.77	32.25	9.8	2.36	.2313	7410	7440
086	IS8768	88	150	1.77	2	.44	28.50	6.7	1.81	.1213	5820	5570
087	IS2812	95	160	2.27	2	1.12	31.00	8.7	1.97	.1714	6760	6400
088	IS0158	76	150	2.00	2	.82	32.00	8.1	2.18	.1766	5710	6710
089	IS0102	79	150	2.43	2	.36	30.75	8.9	1.90	.1691	7150	7290
090	IS8563	82	170	2.56	1	.42	32.00	8.1	2.12	.1717	5080	6230
091	IS8330	71	170	2.31	2	.59	27.25	7.1	1.91	.1356	6630	7030
093	IS8584	88	150	1.85	5	4.12	27.50	7.0	1.79	.1253	5860	6230
094	IS10252	74	150	2.30	2	.34	31.50	9.9	2.31	.2287	7920	6620
095	IS9288	79	170	2.77	2	.38	33.50	9.3	2.28	.2120	8710	7790
096	IS10354	75	140	2.29	2	.42	28.00	8.7	2.05	.1783	6120	6745
097	IS10648	71	160	1.75	2	.35	29.25	9.2	2.22	.2042	8540	7470
098	IS0508	81	140	2.36	5	1.34	30.75	8.4	2.13	.1789	8510	7380
100	IS12282	79	130	2.79	2	.46	32.00	8.4	2.12	.1781	7810	8090
101	IS12279	71	180	3.02	5	3.04	32.00	9.1	2.20	.2002	8990	7460**
102	IS12281	74	130	2.67	2	.81	31.75	8.8	2.16	.1901	8150	6670
103	IS0223	88	150	2.24	2	.64	31.00	8.6	2.15	.1849	6620	7260
104	IS0862	79	170	2.43	2	.60	29.25	7.0	1.80	.1260	8750	7360
105	IS0874	71	160	2.19	5	.54	29.50	7.7	2.12	.1632	8820	7870

Table 1. (Continued)

Entry No. (a)	Ident. (b)	Fl days (c)	Ht cm (d)	100 seed wt (e)	Color (f)	C.E. (g)	DBC (h)	% P (i)	L % of P (j)	L % of sample (k)	Yield kg/ha 1st rep (l)	Ave. yield kg/ha (m)
106	IS0919	90	180	2.66	2	.54	29.00	7.8	1.73	.1349	7590	6680
107	IS1031	77	190	2.50	4	.54	34.25	8.8	2.50	.2200	10880	9230*
108	IS8070	90	150	3.13	2	3.54	28.00	7.9	1.71	.1351	6580	7530
109	IS7034	90	160	2.15	2	.76	30.25	7.8	1.99	.1552	7290	7020
110	IS2830	74	110	1.93	5	3.38	32.50	8.8	2.15	.1892	5650	6420
111	IS8248	73	150	1.53	2	1.28	30.25	7.7	1.96	.1509	5140	5440
112	IS9285	79	190	2.98	2	.48	32.25	8.7	2.26	.1966	8520	6830
113	IS8545	79	220	2.45	4	3.23	32.75	9.5	2.05	.1947	7170	6930
114	IS8193	89	190	2.17	5	4.87	31.75	7.7	2.14	.1648	8280	6570
115	IS9746	81	130	2.37	5	1.27	32.75	9.6	2.60	.2496	6930	6830
116	IS10266	76	160	2.44	2	.54	23.50	6.7	1.80	.1206	5860	7380**
117	IS8614	80	190	3.04	4	4.31	32.00	9.0	2.21	.1989	7440	7600
118	IS9347	81	210	2.53	2	.60	33.00	9.2	2.43	.2236	5670	6160
119	IS9333	79	180	2.26	2	.64	30.00	8.4	2.16	.1814	9300	8180
120	IS9183	79	170	1.88	6	4.40	28.00	6.4	2.00	.1280	6800	6910
121	IS10644	71	120	2.35	2	.11	30.50	10.0	2.05	.2050	6770	6520**
122	IS8742	71	190	3.28	2	.54	30.25	9.8	2.26	.2215	7870	6570
123	IS9844	80	180	2.71	5	4.26	27.00	7.3	2.00	.1460	7040	7440
124	IS12317	71	200	2.43	5	2.48	29.25	7.8	2.04	.1591	10670	8000
125	IS0630	75	200	3.06	1	.22	31.00	10.2	2.35	.2397	9130	7450
126	IS0185	87	220	2.39	1	.56	29.00	8.8	2.11	.1857	7240	6120
127	IS0170	71	130	2.32	2	.18	30.00	9.0	2.10	.1890	4550	6110
128	IS0865	79	170	2.81	2	.55	29.25	7.6	1.78	.1353	6540	6420
129	IS8574	90	240	2.25	3	.14	33.00	9.5	2.32	.2204	6280	6580*
130	IS2740	92	220	3.19	2	.43	34.25	10.1	2.20	.2222	6450	7630**

Table 1. (Continued)

Entry No. (a)	Ident. (b)	Fl days (c)	Ht cm (d)	100 seed wt (e)	Color (f)	C.E. (g)	DBC (h)	% P (i)	L % of P (j)	L % of sample (k)	Yield kg/ha 1st rep (l)	Ave. yield kg/ha (m)
131	IS2287	80	210	2.88	3	.54	30.00	8.5	2.09	.1776	7920	6920
132	IS3798	92	200	2.88	3	.47	32.00	8.9	2.26	.2011	6430	6840*
133	IS0452	81	230	2.12	3	.07	35.00	10.3	2.35	.2420	6700	6680*
134	IS9285	89	180	3.09	2	.29	30.00	7.7	2.02	.1555	5070	7830
135	IS8612	88	220	2.99	4	.79	30.50	8.9	2.22	.1976	7000	6490
136	IS10256	71	210	2.78	2	.28	30.00	9.3	2.38	.2213	8320	7170**
137	IS9337	100	170	2.66	5	.86	28.25	7.9	1.87	.1477	4490	6970
138	IS0829	81	220	2.43	5	8.82	34.00	9.3	.22	.2065	7950	7540**
139	IS2692	80	180	2.86	5	.92	29.25	8.3	2.05	.1701	8380	7210**
140	IS3163	89	200	3.19	4	.81	28.25	6.9	2.05	.1414	5920	7400*
141	IS9706	88	240	2.81	2	.20	31.75	8.3	2.19	.1818	7430	6710
143	IS8753	79	170	2.53	2	.49	29.00	7.8	2.15	.1677	7930	7620*
145	IS9958	71	160	2.19	5	.72	29.00	9.1	2.16	.1966	9200	7120
146	IS1291	78	210	1.01	5	5.88	32.75	9.5	2.34	.2223	5880	6000
147	IS9459	79	210	2.41	2	.51	30.00	9.5	2.34	.2223	6160	7120
148	IS9454	93	250	3.21	5	.69	32.25	9.2	2.33	.2144	6370	4760**
149	IS2692	88	210	2.68	5	.82	27.50	7.1	2.03	.1441	7820	7270
150	IS2239	71	130	2.07	2	.08	31.50	9.3	2.54	.2362	6950	6550
151	IS0469	82	170	1.90	3	.07	33.75	9.4	2.30	.2162	4160	5570*
152	IS3974	74	160	2.33	2	.45	28.25	7.6	1.87	.1421	7480	6640
153	IS3952	81	330	2.17	3	.22	32.00	10.4	2.20	.2288	6850	6400*
154	IS2057	80	150	2.24	2	.55	28.75	8.3	2.02	.1677	4280	6220
155	IS2863	71	160	2.40	2	.51	30.25	8.6	2.15	.1849	7070	7390**
156	IS8671	96	180	1.82	2	4.89	32.00	8.6	2.06	.1772	7240	7130**
157	IS9358	77	170	2.84	2	.52	30.25	8.1	2.11	.1709	6810	7480

Table 1. (Continued)

Entry No.	Ident.	Fl days	Ht cm	100 seed wt	Color	C.E.	DBC	% P	L % of P	L % of sample	Yield kg/ha 1st rep	Ave. yield kg/ha
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)
158	IS9323	90	230	2.85	4	.42	28.75	7.7	2.05	.1578	6440	6570*
159	145106	77	180	2.54	5	.53	31.00	8.3	2.32	.1926	8690	7480
160	145107	72	100	2.10	5	.66	34.00	9.8	2.46	.2411	4650	5160
161	145109	81	160	2.27	5	4.37	31.00	8.1	2.16	.1750	5690	6060
162	IS6921	79	210	2.87	5	1.88	30.00	8.7	2.14	.1862	8670	7230
163	IS9370	80	210	3.48	2	.53	29.25	8.9	2.23	.1985	7990	6830*
164	IS0071	88	200	2.38	1	.50	36.25	11.1	2.86	.3175	7460	6080
165	145122	81	170	2.48	2	.22	30.75	7.6	1.97	.1497	6820	6060
166	IS12303	90	230	2.50	7	4.57	33.00	9.3	2.29	.2130	9950	8030**
167	IS9719	79	140	2.75	2	2.91	29.25	8.8	2.15	.1892	8060	7050
168	IS12102	71	330	3.18	2	.55	38.00	10.6	2.83	.3000	7380	7180**
169	IS9708	76	220	2.83	2	.60	28.50	8.1	2.10	.1701	7990	6830**
170	IS12156	102	290	3.13	2	.17	31.25	8.7	2.19	.1905	5740	7480*
171	IS12202	100	240	2.57	2	.17	31.00	9.9	2.31	.2287	4900	4580**
173	IS2670	79	150	1.90	5	3.57	29.50	7.9	2.03	.1604	6360	6590
174	IS2840	76	170	2.90	2	.29	30.00	9.5	2.04	.1938	7900	6980**
175	IS9282	79	140	2.28	5	2.40	30.25	9.2	2.11	.1941	7990	6780
176	IS9576	77	110	2.29	2	.27	28.00	7.0	1.79	.1253	8690	7420
177	IS10219	75	200	2.53	2	.34	29.75	7.1	2.17	.1541	9030	7300
178	IS9563	71	150	2.87	5	2.48	29.75	8.0	1.93	.1544	6430	6990
180	132005	78	230	2.53	2	.82	28.25	9.1	2.00	.1820	8740	6820*
181	IS0811	70	130	2.73	5	1.34	33.50	11.5	2.28	.2622	6260	5440
182	IS7031	79	160	2.18	2	.56	31.00	7.7	1.93	.1486	6100	6380
183	IS12316	74	200	2.38	5	2.08	29.00	7.9	1.98	.1564	8030	8010
184	IS8129	74	150	1.78	5	3.05	28.25	7.7	1.89	.1455	3870	5710

Table 1. (Continued)

Entry No. (a)	Ident. (b)	Fl days (c)	Ht cm (d)	100 seed wt (e)	Color (f)	C.E. (g)	DBC (h)	% P (i)	L % of P (j)	L % of sample (k)	Yield kg/ha 1st rep (l)	Ave. yield kg/ha (m)
185	IS9119	81	200	2.70	5	.57	29.00	7.7	2.08	.1602	5320	4970*
186	IS4806	78	130	2.49	2	.34	29.75	6.7	2.08	.1394	9810	7920**
193	RS610	74	140	2.49	5	.38	30.00	8.6	2.03	.1746	8360	8050
194	NK300	80	220	2.04	5	4.30	28.00	6.9	1.88	.1297	6610	8000
195	FS24	92	260	2.30	5	.76	31.25	8.7	1.86	.1618	6360	5850
196	FS26	100	330	2.85	5	5.63	33.00	9.2	2.46	.2263	4680	8090*
197	RS671	71	100	2.37	5	.55	30.75	9.5	2.17	.2061	8400	7790**
198	IS7579	79	370	2.43	5	.38	35.00	8.9	3.09	.2750	3780	6060*
199	IS4225	77	300	2.95	5	3.68	34.00	9.0	2.43	.2187	8740	7180**
201	BR64	72	160	2.23	5	7.31	29.50	6.8	1.93	.1312	9980	7860
202	Pion931	98	410	2.37	5	7.31	46.50	13.1	3.05	.3995	4970	6010**
203	RS626	71	130	2.43	5	.70	29.25	8.5	2.11	.1793	7070	6950
204	FS4	78	320	2.30	5	6.89	34.75	10.1	2.37	.2394	8050	6640**
205	IS2319	79	260	3.50	2	.40	39.00	9.9	2.79	.2762	7170	6280
206	232006	91	290	3.47	3	.09	32.50	9.9	2.35	.2326	10550	9200**
207	IS10670	74	100	1.87	5	.54	31.25	10.0	2.27	.2270	7150	6780
208	232058	89	160	2.01	4	1.23	27.00	7.4	2.01	.1487	7890	7440
209	IS15676	79	250	4.81	4	3.95	32.00	8.1	2.24	.1814	13300	9860*
210	232047	79	230	3.02	2	.67	30.25	8.9	2.26	.2011	9760	8200**
211	IS16046	76	280	4.09	7	2.56	32.25	9.7	2.28	.2212	5820	7800**
212	IS15070	98	280	3.50	5	5.12	29.25	7.6	2.04	.1550	6740	8020*
213	IS16327	92	380	3.03	5	2.86	32.50	9.6	2.47	.2371	9120	6990**
214	IS15414	91	370	4.40	2	.69	34.00	9.2	2.47	.2272	7620	6730**
215	IS16117	92	290	3.13	2	.58	39.00	10.7	3.09	.3306	7810	6800**
216	232067	72	100	2.33	2	.37	32.00	8.5	2.12	.1802	5450	6040

Table 1. (Continued)

Entry No.	Ident.	FL days	Ht cm	100 seed wt	Color	C.E.	DBC	% P	L % of P	L % of sample	Yield kg/ha 1st rep	Ave. yield kg/ha
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)
217	IS2778	77	160	2.88	2	1.43	32.00	8.8	2.16	.1901	7480	6240
218	232043	79	140	2.43	5	.48	30.75	8.4	2.21	.1856	8460	7550
219	232052	79	170	3.53	2	.70	31.25	10.0	2.23	.2230	7440	7970
220	232021	79	170	2.72	3	.61	30.00	8.3	2.09	.1735	5580	5370*
221	IS16100	81	250	2.62	4	4.03	29.75	7.6	2.11	.1604	8620	9620*
222	IS16145	89	320	3.98	5	3.32	27.00	7.4	1.83	.1354	12640	7700
223	IS15871	91	310	2.98	2	.20	28.00	6.7	1.97	.1320	7830	5900
224	IS16419	92	370	3.71	7	7.06	37.50	10.4	2.56	.2662	9090	6220
225	IS7521	94	280	2.44	2	.70	28.75	8.7	2.17	.1888	8740	5970**
226	IS8902	78	230	2.49	5	.56	29.75	8.3	2.28	.1892	8100	8430*
227	IS9747	100	360	2.77	1	.06	35.00	9.5	2.46	.2337	7410	4660**
228	IS4904	91	230	2.68	5	3.82	36.00	8.1	2.70	.2187	5360	4620**
229	232008	94	275	3.20	3	.11	31.75	9.9	2.31	.2287	6930	8080**

* mean of one location.

** mean of two locations.

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