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COMPONENTS OF NUTRITIONAL QUALITY IN GRAIN SORGHUM

J. D. Axtell, R. C. Pickett, D. L. Oswalt, R. Jambunathan and G. Srinivasan

There are several factors which effect nutritional quality in cereal crops. The nutritional problems in sorghum parallel those in other major cereals in many respects, but also differ in some important respects. The major components of nutritional quality in sorghum are as follows:

I. Protein Quality
   - A. Lysine Content
     1. Prolamine Content
     2. Embryo Size

II. Protein Availability
   - A. Tannin Content

Protein quality is limited by the low lysine content of sorghum grain, which reflects the high prolamine content of the endosperm and the relatively small embryo size as a proportion of the mature grain. Protein availability is apparently limited in some sorghum genotypes by the presence of unidentified polyphenolic compounds located primarily in the testa layer of the grain. These pigmented compounds have not been well characterized chemically and are referred to generically as "tannins". We do not fully understand how these tannin compounds influence the nutritional quality of sorghum. Our hypothesis at present is that the seed proteins become complexed or bound with the tannin compounds of the whole grain, and that the complexed

proteins are substantially less available for utilization by monogastric animals.

This report will present some of the data and experimental evidence which supports these general observations, and briefly describe the status of our efforts toward improving the nutritional quality of sorghum.

Amino Acid Composition - The average protein and amino acid composition of 522 lines from the world sorghum collection is presented in Table 1.

Table 1. Protein and Amino Acid Composition of Sorghum Grain*

Composition	Mean	Standard Deviation
Protein	12.61	1.89
Lysine	2.14	0.35
Histidine	2.01	0.20
Arginine	3.59	0.44
Aspartic Acid	7.83	0.77
Threonine	3.26	0.21
Serine	4.52	0.32
Glutamic Acid	23.22	1.99
Proline	8.16	0.89
Glycine	3.07	0.27
Alanine	9.89	0.71
Cystine**	1.50	-
Valine	5.35	0.31
Methionine**	1.80	-
Isoleucine	4.08	0.25
Leucine	14.27	1.21
Tyrosine	4.50	0.32
Phenylalanine	5.19	0.37
Tryptophan***	1.31	0.14

* Weighted average for 522 lines.
** Weighted average for 3 lines.
*** Weighted average for 9 lines.

The average protein content of these lines is 12.6 percent and the average lysine content (expressed as a percent of protein) is 2.1 percent. A more meaningful assessment of amino acid content from a nutritional point of of view is a comparison of essential amino acid content in sorghum with the rat requirement, as shown in Figure 1. This illustrates the deficiency in lysine, and the excessive leucine content present in sorghum. The methionine content of sorghum is low, but if one considers the cystine content of 1.5 percent the overall sulfur amino acid content approaches that required by the rat. The tryptophan content of sorghum based on our evidence at this time seems to be adequate which is in contrast to the low tryptophan content in normal corn.

Fractionation of Whole Grain and Endosperm Proteins - Dr. Jambunathan and Dr. Mertz (1973) at Purdue University have recently completed the fractionation of sorghum proteins using the procedure of Landry and Moreaux (1970). This procedure solubilizes most of the nitrogen of sorghum and yields five different solubility fractions. Results of the fractionation of whole grain from two low tannin and two high tannin sorghum lines are shown in Table 2. The average percent nitrogen in each fraction for the low and high tannin lines is shown in each column, with the average lysine content expressed as percent of protein for each fraction in parenthesis. Two conclusions can be drawn from this data. First, the distribution of protein in various fractions is distinctly different between the high tannin and low tannin lines. This suggests that the presence of tannins has altered

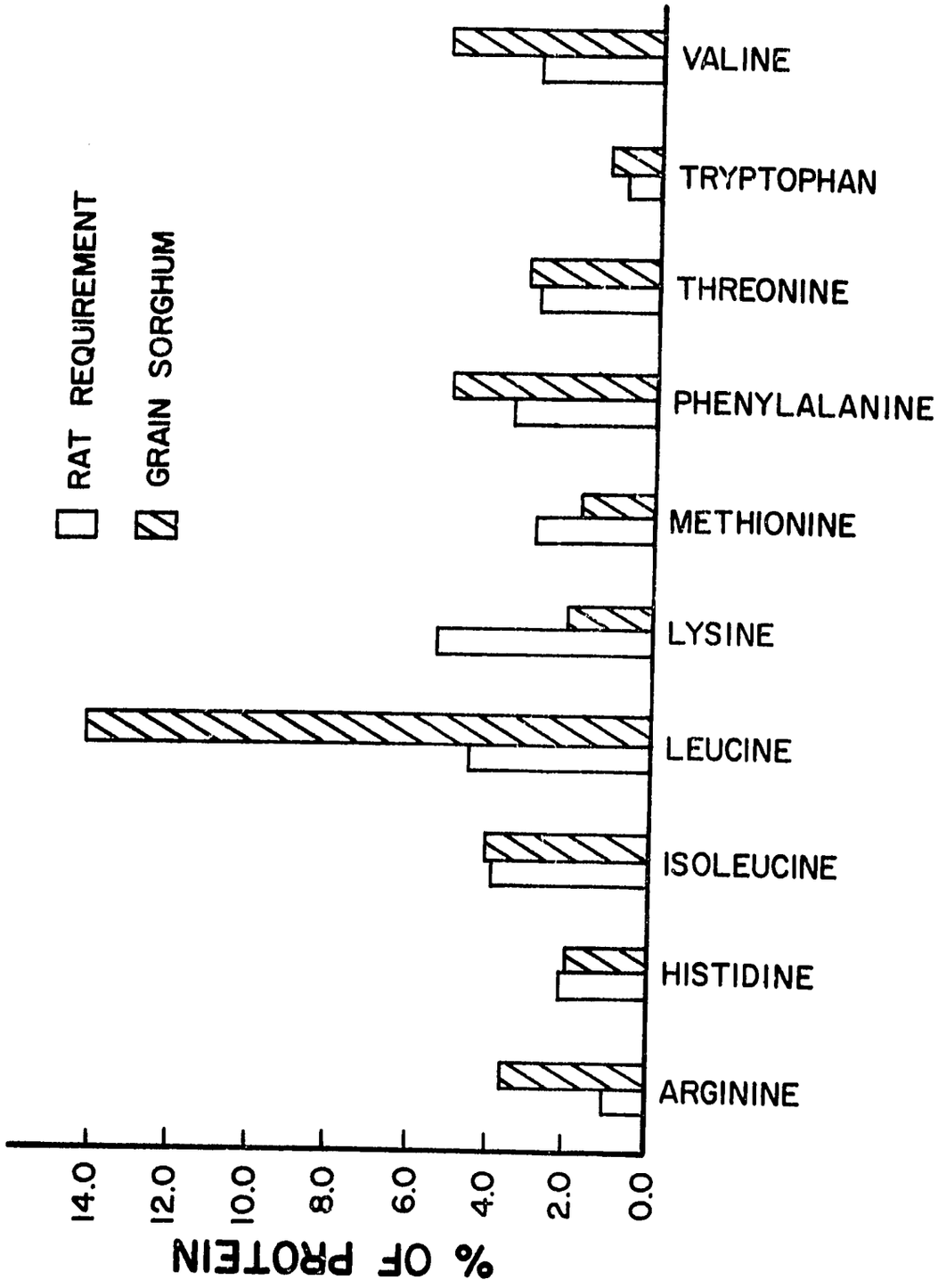

Figure 1. Essential Amino Acids.

Table 2. Nitrogen Distribution in Whole Grain* (Lysine as Percent of Protein in Parenthesis).

Fraction	Low Tannin	High Tannin
I (Saline)	16.1 (5.8)	4.9 (3.1)
II (Isopropanol)	14.5 (0.3)	4.2 (0.4)
III (Isopropanol + 2-Mercaptoethanol)	18.6 (0.1)	17.7 (0.2)
IV (Borate buffer + 2-Mercaptoethanol)	6.3 (2.3)	16.2 (2.9)
V (Borate buffer + 2-Mercaptoethanol + Sodium Dodecyl Sulfate)	32.1 (2.8)	52.6 (1.3)
Total Nitrogen Extracted	87.5	89.9

* Percent of Soluble Nitrogen

the solubility of sorghum proteins, perhaps by binding or forming complexes with the protein. This idea is supported by the observation that the first fraction in the high tannin samples is very low in protein content, although one would expect it to be reasonably high since fraction I represents the albumins and globulins which are indispensable for the germination and sustenance of the plant. Second, within the low tannin sorghums, the protein content of fractions II and III is high, but the lysine content of these proteins is very low. In the case of endosperm fractions which are presented in Table 3, the differences in protein content between high and low sorghum samples are less pronounced, possibly because a part of the tannin containing testa layer was removed with the pericarp during separation of the endosperm from the whole grain. In the endosperm the proportion of protein with low lysine content in fractions II and III is higher than in whole grain fractions as expected, since these are primarily storage proteins.

Table 3. Nitrogen Distribution in Sorghum Endosperm* (Lysine as Percent of Protein in Parenthesis).

Fraction	Low Tannin	High Tannin
I (Saline)	7.3 (5.1)	2.9 (3.0)
II (Isopropanol)	16.8 (0.3)	11.3 (0.3)
III (Isopropanol + 2-Mercaptoethanol)	32.4 (0.2)	27.6 (0.3)
IV (Borate buffer + 2-Mercaptoethanol)	7.8 (1.2)	10.9 (2.4)
V (Borate buffer + 2-Mercaptoethanol + Sodium Dodecyl Sulfate)	25.3 (3.6)	40.4 (3.0)
Total Nitrogen Extracted	89.5	92.9

---

* Percent of Soluble Nitrogen

Growth Response of Rats Relative to Lysine and Tannin Content - Since lysine is the first limiting amino acid in sorghum an experiment was designed to verify the growth response of rats when fed sorghum rations supplemented with adequate lysine hydrochloride to fulfill the rat requirement for lysine plus a 25 percent excess. The results of this experiment are shown in Figure 2. Two low tannin lines (IS2319 and IS0129) and one high tannin line (IS6992) were included in this trial. The rat growth on low tannin sorghum rations supplemented with lysine, vitamins and minerals was substantially greater than growth on the ration supplemented only with vitamins and minerals. In contrast, rats fed the high tannin sorghum (IS6992) lost weight on the unsupplemented ration and gained only slightly on the ration supplemented with lysine. These results suggest that lysine is the first limiting amino acid in low tannin sorghum lines, but other factors are

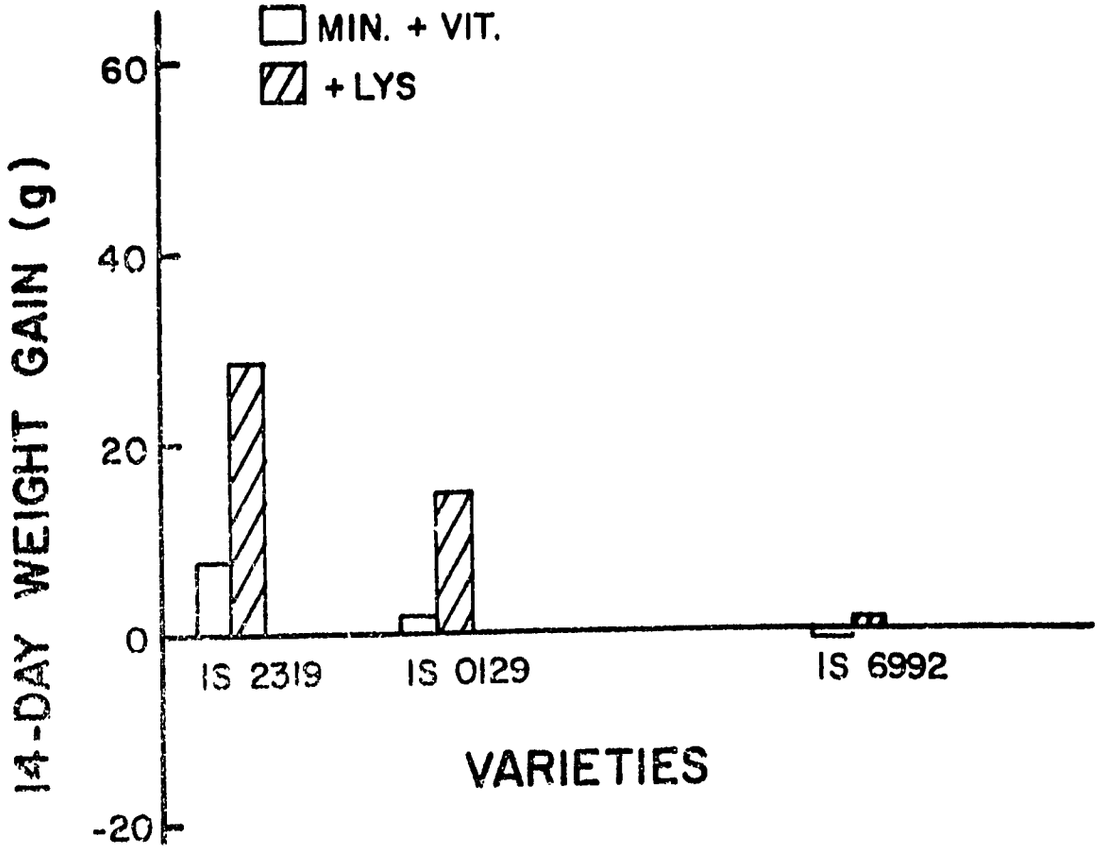

Figure 2. Rat Weight Gains on Iso-Nitrogenous Samples of Supplemented Grain Sorghum Lines.

first limiting in high tannin lines.

The results obtained from lysine supplementation experiments are substantiated by data from rat growth trials conducted at Purdue. In this experiment we used 17 low tannin sorghum lines and 10 high tannin lines which represented the range in variation for protein and lysine content of sorghum lines available from our screening program of the world sorghum collection. The results of this experiment, illustrated in Figure 3 demonstrate that rat weight gain is dependent on lysine content in the low tannin sorghum groups, but not in the high tannin sorghum group. The regression of rat weight gain on lysine content for the high tannin group is nearly zero whereas the regression coefficient for the low tannin group is 64.79. We have used the acidified vanillin method of tannin analysis described by Burns (1971) which estimates tannin content as catechin equivalents. Maxson and Rooney (1972) have reported that catechin equivalent values for grain sorghum are correlated with tannin values obtained with other methods. In our experience the range of catechin equivalents for sorghum is from 0 to 10. We have classified sorghum lines as low tannin if the catechin equivalent value is below 1.00.

Frequency of High Tannin Sorghum Genotypes in the World Collection - We have only limited evidence at this time on the frequency of high tannin sorghum genotypes in the world sorghum collection. Among 472 lines which we have analyzed for amino acid content, shown in Table 4, 300 were classified as low tannin and 172 were high tannin lines. The average catechin equivalent values for the low and high tannin lines were 0.38 and 3.40 respectively.

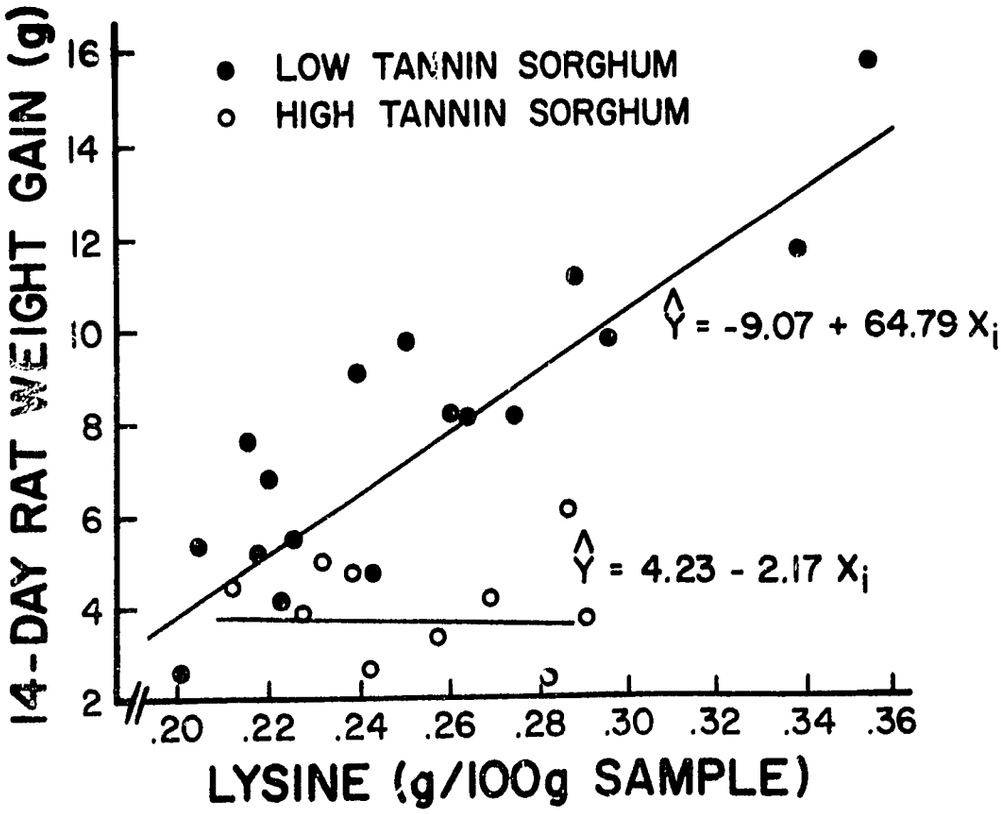

Figure 3. Relationships of Rat Weight Gain with Lysine Content of High and Low Tannin Sorghum Lines.

Table 4. Protein and Amino Acid Composition of High and Low Tannin Grain Sorghum. (Amino Acid Content Expressed as Percent of Protein)

Essential Amino Acids	300 Low Tannin ± S.D.	172 High Tannin ± S.D.
Catechin Equivalents	0.38 ± 0.24	3.40 ± 2.35
Protein	12.71 ± 1.86	12.42 ± 1.95
Lysine	2.11 ± 0.33	2.15 ± 0.35
Histidine	2.02 ± 0.19	1.99 ± 0.21
Arginine	3.56 ± 0.42	3.60 ± 0.43
Threonine	3.26 ± 0.20	3.24 ± 0.18
Cystine	0.94 ± 0.22	0.89 ± 0.22
Valine	5.33 ± 0.29	5.37 ± 0.31
Methionine	1.02 ± 0.53	1.01 ± 0.36
Isoleucine	4.08 ± 0.25	4.07 ± 0.22
Leucine	14.33 ± 1.20	14.17 ± 1.10
Phenylalanine	5.21 ± 0.36	5.12 ± 0.34

There were no significant differences in protein or essential amino acid content between the average of the two groups. We have just completed evaluating 288 lines collected recently in Cameroon, Africa. The data on average tannin and protein content of these lines is reported in Table 5.

Table 5. Frequency of High and Low Tannin Sorghum Genotypes Among 288 Lines Collected in Cameroon, Africa.

	High Tannin	Low Tannin
Number of lines	229	59
Average Catechin Equivalent Value	4.07	0.49
Average Percent Crude Protein	10.61	10.93

Approximately 80 percent of these lines were classified as high tannin genotypes with an average catechin equivalent value of 4.07. There was no

difference in average protein content between high and low tannin groups, although both were lower than the overall average of 12.6 percent reported in Table 1. The lower average protein content can probably be accounted for by the observation that the Cameroon lines represent an unusually large seeded collection. In general, it can be concluded that high tannin sorghum genotypes comprise a significant proportion of the sorghum germplasm in some areas of the world.

Alkali Dehulling of High and Low Tannin Sorghum Grain - Since the tannin in sorghum grain is contained primarily in the testa layer beneath the pericarp we have tried with some success to remove the tannin with an alkali dehulling technique described by Blessin *et al*, (1971). The data in Table 6 shows the effect of dehulling on tannin content, protein content, rat gain and feed consumption for one low tannin Texas hybrid (RS610) and two high tannin lines (IS6992 and IS8260) from the world sorghum collection.

Table 6. Alkali Dehulling of High and Low Tannin Grain Sorghum.

Entry	Catechin Equiv.	Percent Protein	14-Day Rat Weight Gain (g)	Feed Consumed (g)
RS610	.48	9.9	5.26	112
RS610 (Dehulled)	.10	9.9	6.72	132
IS6992	1.74	9.4	5.73	99
IS6992 (Dehulled)	.07	9.8	10.20	101
IS8260	3.71	8.1	-4.12	95
IS8260 (Dehulled)	.10	8.4	7.98	97

Catechin equivalent values were reduced without substantial change in protein content. Rat weight gain was not significantly improved by dehulling the low tannin RS610 hybrid, but was substantially increased by dehulling of both high tannin lines. There was no large difference in the amount of feed consumed between hulled and dehulled samples which indicates that the poor weight gain for whole grain samples of IS6992 and IS8260 was not caused by reduced intake.

All of our previous tests on the difference in response to lysine supplementation between high and low tannin sorghum lines have been made by comparing lines from the world sorghum collection which differ in tannin content but are not isogenic for other characteristics. A more direct test can be made by utilizing the alkali dehulling technique to obtain "low tannin" control lines which can then be compared with whole grain with the same genetic background but with a high tannin content. The results of an experiment comparing the biological value of whole grain samples from two high tannin lines, and dehulled counterparts of each line, with and without lysine supplementation are shown in Figure 4. In both cases the rats gained little or no weight on the whole grain (high tannin) rations supplemented with lysine, but responded to lysine supplementation of the dehulled (low tannin) counterpart rations. The evidence from this and other studies discussed demonstrates that low lysine content is the major factor limiting the biological value of low tannin sorghum grain, but that lysine is not first limiting for high tannin sorghum grain.

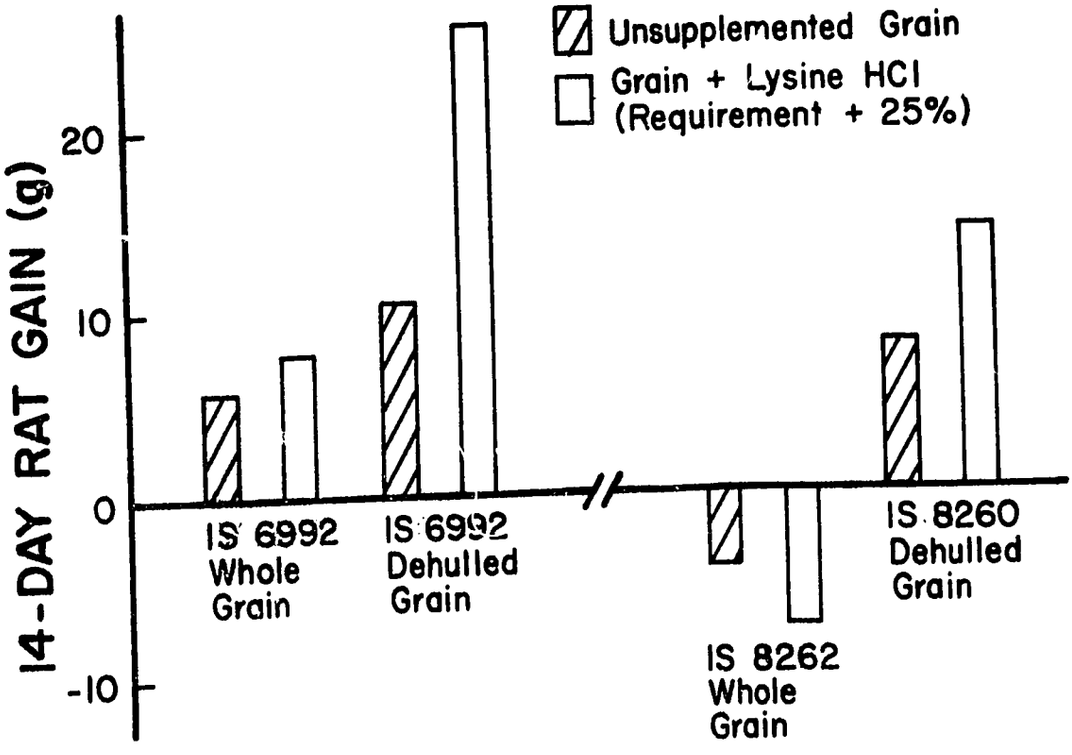

Figure 4. Lysine Supplementation of Whole Grain and Dehulled High Tannin Sorghum Lines.

Supplementation with Soybean Meal - One possible explanation for lack of rat growth response in lysine supplemented high tannin sorghum rations is that the tannin compounds may produce a toxic effect which counteracts the expected growth response from lysine supplementation. The data in Figure 5 (Schaffert, 1972) suggest that this is not the case since the addition of only five percent soybean meal to high tannin rations produced a very significant increase in rat growth. In this experiment rations containing two low tannin and two high tannin lines were supplemented with 0, 5, 10, or 15 percent soybean meal, and rat growth response measured in a 13-day trial. It seems unlikely that the addition of small quantities of soybean meal would dilute any toxic effect to the extent required to account for the substantial rat gain illustrated in Figure 5. We would expect the low tannin lines to respond to soybean supplementation because of its relatively high lysine content. It is quite apparent that something else is added with the soybean meal which can correct a nutritional deficiency or imbalance in the high tannin rations. The effect of soybean meal supplementation on feed efficiency ratios is illustrated in Figure 6. This again shows the striking improvement in amount of feed consumed per gram of gain when high tannin rations are supplemented with 5 percent or more soybean meal. The most viable possibility at this time is that soybean meal supplies available protein which is deficient in high tannin lines because the sorghum protein is complexed or by some mechanism made unavailable for monogastric animals. The evidence obtained by Drs. Jambunathan and Mertz on protein fractionation supports this explanation,

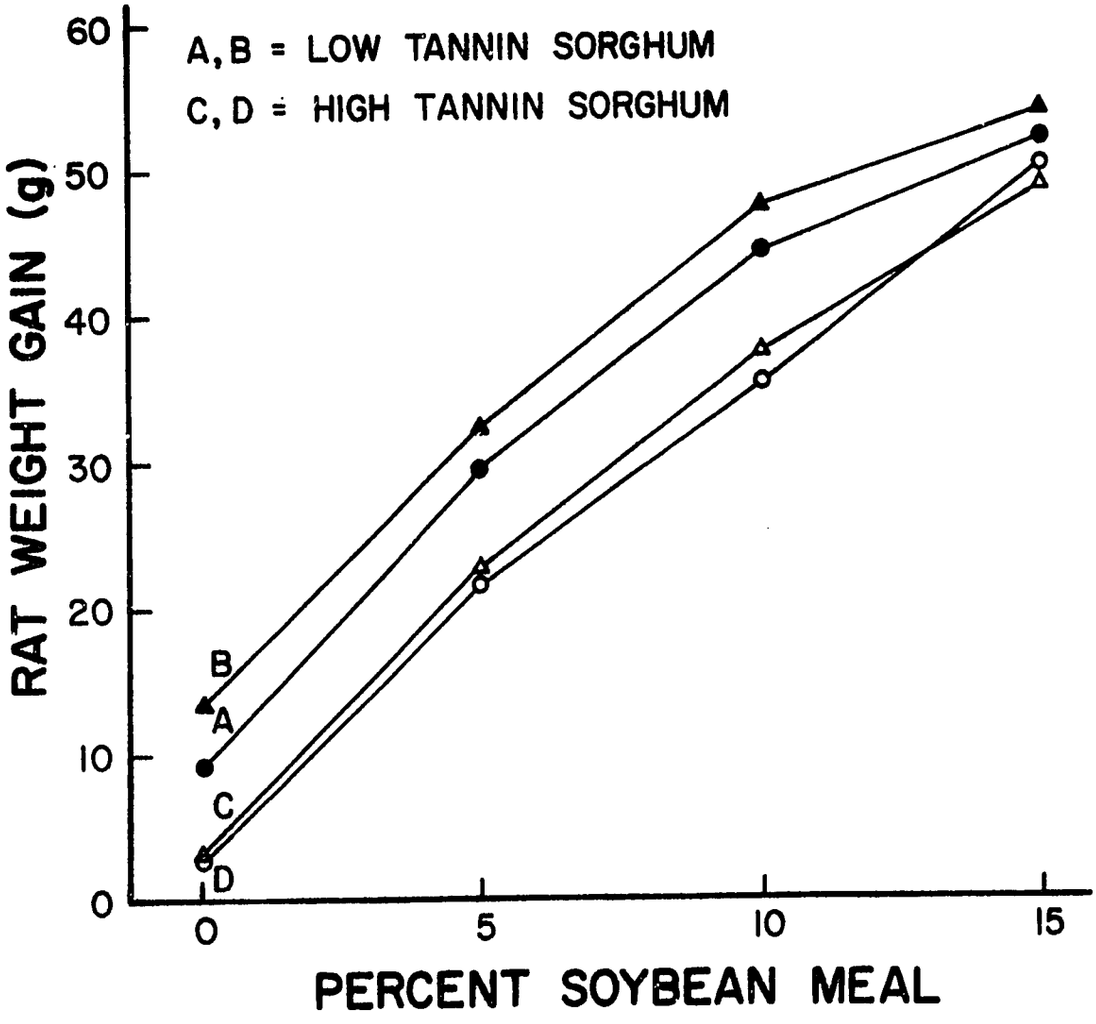

Figure 5. Average Weight Gain Per Rat for 13 Days.

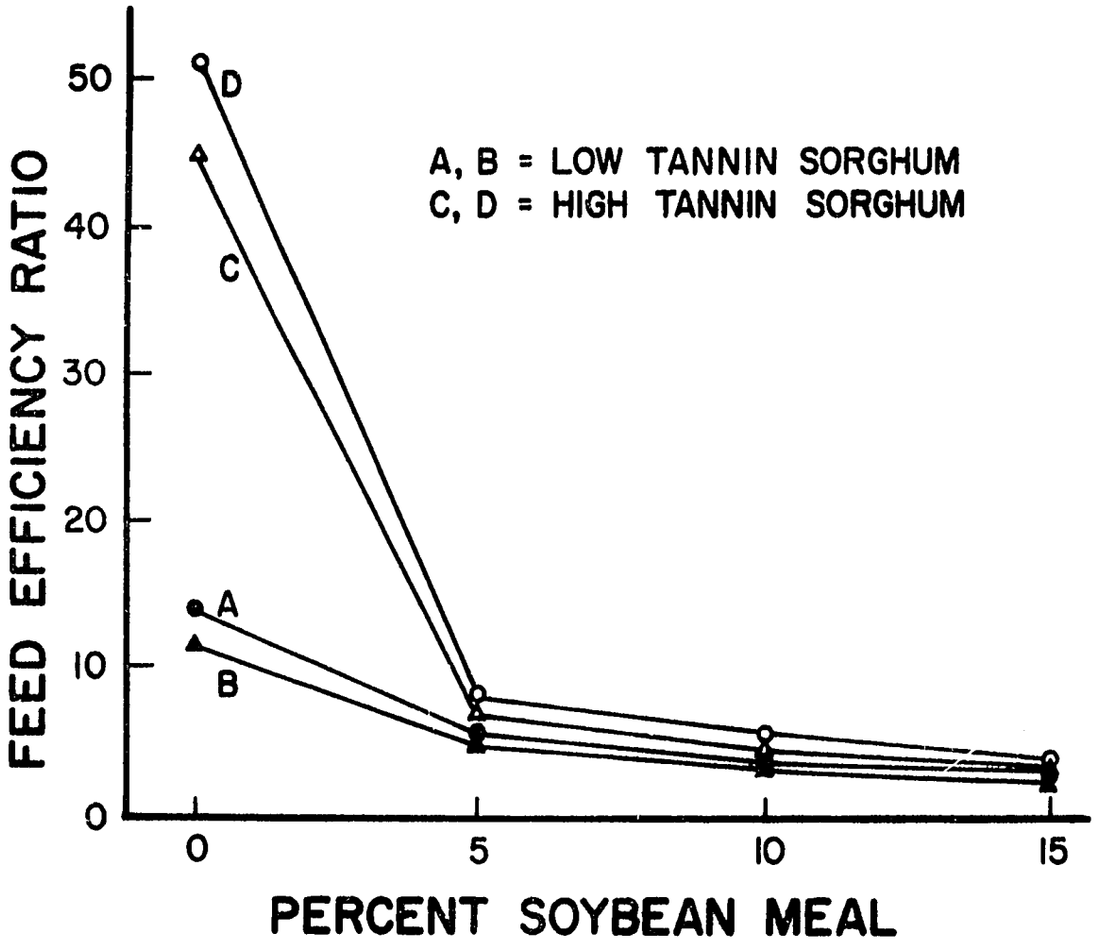

Figure 6. Feed Efficiency Ratios for Rats Fed 13 Days.

since the presence of tannin compounds apparently alters the solubility of major protein components in high tannin sorghum lines. There are other possible explanations which need to be tested, including the following:

a) Soybean meal may provide some other essential amino acid which is selectively unavailable in high tannin rations.

b) There may be a deficiency of available inorganic nitrogen, needed for biosynthesis of non-essential amino acids and/or other nitrogenous compounds vital for normal growth and development in high tannin sorghum rations.

c) There may be compounds in soybean meal which can bind more competitively with tannins, and thereby replace the protein bound to tannin making it more available.

What is the status of our efforts in improving the nutritional quality in sorghum? If we are correct in our assumption that tannins complex with seed proteins and that these proteins are then less available for monogastric animals there are several alternatives:

a) Use only low tannin sorghums for consumption by human and other monogastric animals and find other methods for controlling birds, which is one major reason for using high tannin sorghum germplasm. Since tannin content is simply inherited and controlled by only one or two major genes, it is an easy task for the breeder to remove the tannin factors.

b) Use high tannin sorghum lines for their desirable agronomic characteristics and plan to supplement with additional protein to meet the nutritional requirement. If this approach is adopted one should bear in mind the possibility that you are essentially wasting some of the protein in the sorghum grain. However, this may not be economically feasible in most areas of the world where sorghum is a major component of the human diet.

c) There may be dehulling procedures or methods of dry milling that could be used to remove the pericarp and testa cells which contain the tannin compounds.

d) There may be additives that will preferentially bind with the tannins and allow the protein to be utilized.

With regard to improvement of lysine content there are excellent opportunities for increasing germ size. Grain sorghum germ size ranges from 7.8 to 12 percent of the whole grain. This is smaller than the germ size in corn. This difference explains part of the difference in lysine content between normal corn and sorghum since normal corn protein contains approximately 2.5 to 2.8 percent lysine whereas normal sorghum protein contains approximately 1.9 to 2.2 percent lysine. The difference in average oil content between corn (4 to 5 percent) and sorghum (2 to 3 percent) also suggests differences in germ size between these species since most of the oil is contained in the germ. We have identified several sorghum lines with protein containing 2.5 to 2.6 percent lysine which have a relatively large germ size. We should be able to make quite rapid progress in

selections from random mating populations derived from selected large germ lines without reduction in yield potential. This should lead to improved nutritional quality.

The prolamine content of sorghum endosperm is high, as we have seen, and the lysine content of the prolamine fraction is very low in sorghum as it is in corn. The situation in sorghum parallels that in normal corn in many respects. There is every reason to believe that mutants which reduce or block prolamine synthesis also occur in sorghum. We have now completed examining sectioned kernels of most lines in the world collection and identified possible opaque kernel phenotypes. Some have been grown in Lafayette in 1972 and others (light-sensitive lines) will be grown in Puerto Rico. Protein and lysine analysis of these putative opaque lines will reveal whether any low prolamine lines have been identified.

In addition we have initiated chemical mutagen treatments of two colorless pericarp, low tannin, vitreous endosperm sorghum lines for identification of low prolamine mutants. M_2 rows will be grown in Puerto Rico for evaluation in 1973.

In summary, it will be necessary to consider each of these parameters (prolamine content, germ size and tannin content) in the improvement of nutritional quality in sorghum. It is unlikely that any line in the world sorghum collection will have all three necessary characteristics, therefore it appears that it will be necessary to identify lines with each component of nutritional quality and then recombine them to obtain genotypes with the desired combination of characteristics for high quality.

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GENOTYPE-ENVIRONMENT INTERACTIONS FOR YIELD, PROTEIN, LYSINE, OIL, AND SEED WEIGHT IN SORGHUM BICOLOR (L.) MOENCH

R. E. Schaffert, R. C. Pickett, D. L. Oswalt and J. D. Axtell

Since the discovery of high lysine protein in the opaque-2 mutant of maize, a tremendous amount of research effort has been devoted to finding equivalent mutants in the other cereals. Much effort has been expended searching the World Collections for varieties with superior protein and lysine properties. Due to the large number of entries in the collections and also because of the high cost of amino acid determintions, genotypes grown in only one environment have generally been used in the initial screening procedure. The existence, size, and nature of genotype-environment interactions could reduce the effectiveness of such a screening procedure. The purpose of this investigation was to measure the genotype-environment interactions for protein, lysine, and other agronomic traits thought to be correlated with protein and lysine content in grain sorghum.

Materials and Methods

Experimental. Thirty-five genotypes were grown in six locations in 1970 and 1971. The genotypes consisted of: Martin B, Redlan B, India Sorghum number (IS)0062, IS0075, IS0187, IS0418, IS0616, IS2288, IS5437, IS7125, IS8165, IS8361, the hybrid of Martin and each IS entry, the hybrid of Redlan and each IS entry, two commercial hybrids, RS610 and NK300, and

one commercial variety, Atlas. These 10 IS genotypes represented an array of variability for maturity, plant height, protein content, and lysine content that was considered typical of the World Collection of Sorghum, except poor yielding IS selections were not included in this investigation. In both years these 35 genotypes were planted in a split-plot modified randomized complete block design with two replications at the DeKalb Agresearch Station in Lubbock, Texas, the University of Nebraska Research Center at Mead, Nebraska, the Southern Indiana Purdue Agriculture Center (SIPAC) in Dubois County, Indiana, the Pinney-Purdue Agriculture Center (PPAC) in Porter and LaPorte Counties, Indiana, and normal and high fertility fields at the Purdue Agronomy Farm (PAF) in Tippecanoe County, Indiana. Years were considered whole plots and the modification of the randomized complete block design consisted of separating the genotypes into three height groups within each block. The experiments in Texas and Nebraska were irrigated. The experiments in Indiana were not irrigated since there is usually adequate rainfall for normal production.

The experiments at PPAC were planted on a Tracy sandy loam which, like SIPAC, usually has some moisture stress during the growing season. The high fertility field at the PAF has been fertilized at the beginning of each season to have 560 kg/ha of N, 245 kg/ha of available P, and 466 kg/ha of available K based on soil tests. All the other experiments were fertilized according to soil tests for optimal production.

Each plot consisted of two rows 76 cm apart and 5.2 m long except at SIPAC where the distance between rows was 51 cm. Two border rows of each height group (RS610, NK300, or Atlas) were planted between height

groups to minimize competition effects. Each plot was over-planted and thinned to approximately 240,000 plants per hectare. Weeds were controlled with herbicides and cultivation. Insecticides were applied on the experiments in Texas and Nebraska. No insect problems occurred in Indiana. All of the environments were relatively disease free.

Measurements were taken on competitive plants from three meters in the center of each row. Data on the following agronomic traits were recorded: days from planting to median flower, plant height, percent protein, protein yield, percent lysine of protein, percent lysine of sample, lysine yield, percent oil, 100 seed weight, and protein content per seed. Plant height was determined by measuring the distance from the base of the plant to the top of the panicle of an average plant. Days to flower were recorded as the number of days from planting to when 50% of the plants were in 50% anthesis. Grain yield was determined by harvesting all the panicles from the center three meters of both rows and adjusted to 14% moisture. Ten consectuvie heads from the center of the plot were dried and threshed to determine moisture and threshing percentage and were used for chemical analysis. Percent protein[1] (crude protein) was calculated by multiplying 6.25 times the percent nitrogen determined by the micro-Kjeldahl procedure on a fat free sample. Percent lysine[1] in the protein was determined by ion-exchange resin chromatography. Percent oil was determined by nuclear magnetic resonance (NMR)[2]. Seed weight was determined by weighing 100 random grains. The other variables

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1 Shuman Chemical Laboratory, Battle Ground, Indiana.

2 University of Illinois, Champaign, Illinois.

were determined by making the appropriate calculations.

Statistical. A split plot design was used in this experiment. The mathematical model for an observation on the lth genotype at the jth location in the ith year (replication in time) and the kth block is:

$$Y_{ijkl} = \mu + \tau_i + \delta_{(i)} + \lambda_j + (\tau\lambda)_{ij} + \beta_{(ij)k} + {}_{(ijk)} + \gamma_l + (\tau\gamma)_{il} + (\lambda\gamma)_{jl} + (\tau\lambda\gamma)_{ijl} + \varepsilon_{ijkl}$$

where μ is the mean, τ_i is the year (replication in time) effect, $\delta_{(i)}$ is the first restriction error (Anderson, 1970), λ_j is the location effect, $(\tau\lambda)_{ij}$ is the interaction effect of years with locations, $\beta_{(ij)k}$ is the effect of blocks within years and locations, ${}_{(ijk)}$ is the second restriction error, γ_l is the genotype effect, $(\tau\gamma)_{il}$ is the interaction effect of years with genotypes, $(\lambda\gamma)_{jl}$ is the interaction effect of locations with genotypes, $(\tau\lambda\gamma)_{ijl}$ is the effect of years, locations, and genotypes and ε_{ijkl} is the interaction effect of blocks within years and locations with genotypes or within error. All the effects were assumed to be random and uncorrelated with variances σ_y^2, σ^2, σ_l^2, σ_{yl}^2, σ_b^2, σ^2, σ_g^2, σ_{gy}^2, σ_{gl}^2, σ_{gyl}^2, and σ^2, respectively. Since the blocks were adjacent to each other within each year-location combination, the σ^2 may not be large enough to test for σ_{gyl}^2 in the analysis of variance, if the inference is to be made over the population of years and locations from which the ones in the experiment were "randomly" drawn.

The analysis of variance and expected mean squares of interest are presented in Table 1.1. Estimates of the variance components were calculated using the method described by Comstock and Moll (1963). The standard errors of the variance components were calculated according to the method outlined by Anderson and Bandcroft (1952). The variance of a genotype mean is:

$$\sigma_y^2 = \frac{\sigma^2}{ysr} + \frac{\sigma_{gyl}^2}{ys} + \frac{\sigma_{gy}^2}{y} + \frac{\sigma_{gl}^2}{s}$$

Table 1.1. Analysis of variance and expected mean squares for a completely random model.

Source of Variance	df	Expected Mean Squares
Years (Y)	(y-1)	
First Restriction Error	0	
Locations (L)	(s-1)	
Y × L	(y-1)(s-1)	
Blocks within Y and L (R)	ys(r-1)	
Second Restriction Error	0	
Genotypes (G)	(g-1)	$\sigma^2 + r\sigma_{gyl}^2 + rs\sigma_{gy}^2 + ry\sigma_{gl}^2 + rys\sigma_g^2$
Y × G	(y-1)(g-1)	$\sigma^2 + r\sigma_{gyl}^2 + rs\sigma_{gy}^2$
L × G	(s-1)(g-1)	$\sigma^2 + r\sigma_{gyl}^2 + ry\sigma_{gs}^2$
Y × L × G	(y-1)(s-1)(g-1)	$\sigma^2 + r\sigma_{gyl}^2$
Experimental Error	ys(n-1)(r-1)	σ^2

where y = number of years, s = number of locations, r = number of blocks, and g = number of genotypes.

where y equals the number of years, s equals the number of locations, and r equals the number of blocks per environment. The optimum combination of years and locations and least significant differences (LSD) were calculated following the method used by Shutz and Bernard (1967). The homogeneity of variances was tested using the procedure recommended by Burr and Foster (1972).

Results and Discussion

The average and range of genotype values across the six locations and two years are presented in Table 1.2. The results from 4 genotypes were omitted from the data due to premature seed shattering and poor yield estimates. Data for yield and height were not obtained at the Nebraska location in either year due to adverse weather conditions prior to harvest. However, valid chemical samples were obtained in both years. These data indicate a range of values that might be encountered in a screening or breeding program. Significant differences between genotypes were observed for all variables. The average value for locations and years for the traits studied are presented in Table 1.3. Neither locations nor years (Ignoring restriction errors, a conservative test for non-significance) were significantly different when they were considered random. However, if a breeder was interested in specific years and locations and considered them fixed they both were highly significant. (Assuming the restriction error variance component to be zero.) This indicates that the breeder must be cautious about combining data for comparison of genotypes from more than one environment. The above means were presented to give the reader a feeling of the inference

Table 1.2. Average value and range of the genotypes for the agronomic traits studied.

Trait	Statistic			
	Average	Minimum Value	Maximum Value	Range
Grain yield (kg/are)*	69.1	55.4	91.7	36.3
Protein (percent)	12.7	10.2	15.0	4.8
Protein yield (kg/are)*	8.7	6.0	11.3	5.3
Lysine (percent of protein)	2.05	1.83	2.34	0.51
Lysine (percent of sample × 100)	25.8	23.7	32.4	8.7
Lysine yield (kg/ha)*	17.0	13.8	21.4	7.6
Oil (percent)	3.32	2.62	4.07	1.45
Seed weight (gm/100 seeds)	2.75	1.75	3.78	2.03
Protein/seed (mg/seed)	3.53	1.92	5.41	3.49
Height (cm)	200	99	323	224
Days to Flower	79.4	67.6	93.3	25.7

* Atlas a low yielding genotype was omitted from these values.

Table 1.3. Average values of locations and years for all the genotypes of the agronomic traits studied.

Trait	Location						Year	
	Texas	Nebraska	PAF*	PAF+	SIPAC	PPAC	1970	1971
Grain yield (kg/are)	72.9	--	72.7	71.7	61.3	66.9	70.4	67.8
Protein (%)	13.7	13.2	12.4	13.2	11.2	12.6	12.4	13.1
Protein yield (kg/are)	10.0	--	9.0	9.3	6.8	8.4	8.6	8.8
Lysine (percent of protein)	1.94	2.40	2.00	1.88	2.10	1.98	2.01	2.09
Lysine (percent of sample × 100)	26.6	31.6	24.8	24.7	22.8	24.8	24.5	27.2
Lysine Yield (kg/ha)	19.2	--	17.9	17.4	14.0	16.5	16.8	17.1
Oil (percent)	3.33	3.07	3.39	3.44	3.29	3.44	3.43	3.22
Seed weight (gm/100 seeds)	2.83	2.45	2.90	2.84	2.81	2.66	2.80	2.69
Protein/seed (mg/seed)	3.91	3.27	3.65	3.79	3.21	3.39	3.36	3.72
Height (cm)	180.7	--	222.3	200.2	203.8	193.1	185.9	214.1
Days to flower	77.8	--	76.8	79.0	80.9	82.4	78.7	80.1

* Normal fertility.

\+ High fertility.

space for genotypes, locations and years.

The estimates of the variance components and their standard errors are presented in Table 1.4. The negative estimates of variance components are within the limits of their standard errors for a value of zero and should be interpreted as zeros. The variances were all homogeneous when tested with the Burr and Foster (1972) procedure. Estimates of the genotypic variance component were significantly greater than zero for all the traits analyzed. All the genotype-year-location components were significantly greater than zero except lysine as a percent of protein. The sum of the interaction components was greater than the error component for grain yield, protein yield, percent oil, seed weight, protein per seed, height and days to flower.

The non-significant genotype-location component of variance estimates for grain yield, protein yield, and lysine yield could have been due in part to the relative high yields across all environments. Data presented by Liang and Walter (1966) indicated that the genotype-location effect for yield of sorghum grown in seven locations in Kansas was non-significant. However, due to an inaccurate F-test (F = 1.25 for 120,120 df is not significant) they did not reach this conclusion. Collins (1969) also reported that the genotype-location effect for sorghum yield was non-significant. Matzinger (1963) presented data that showed non-significant genotype-location effects for yield in cotton, tobacco and soybeans. However, these yield data should be interpreted cautiously and may be an indication that the locations are not a random sample of locations but a more uniform subset than a random set.

Table 1.4. Estimate of variance components and their standard error (in parenthesis) for yield, protein, lysine, oil, seed weight, height and days to flower.

Trait	Component of Variance									
	σ_g^2		σ_{gy}^2		σ_{gl}^2		σ_{gyl}^2		σ^2	
Grain yield (kg/are)	116**	(40)	50**	(20)	5	(14)	94**	(20)	113	(9)
Protein (percent)	1.22**	(0.33)	0.04	(0.04)	0.19**	(0.07)	0.30**	(0.08)	0.78	(0.06)
Protein yield (kg/are)	3.45**	(0.60)	1.40**	(0.30)	-0.24	(0.21)	1.45**	(0.32)	1.99	(0.16
Lysine (% protein)	0.017**	(0.006)	0.005**	(.002)	.004*	(.002)	0.005	(0.003)	0.034	(0.002)
Lysine (% sample x 100)	2.59**	(0.81)	0.25	(0.27)	0.61	(0.42)	2.51**	(0.54)	4.08	(0.30)
Lysine yield (kg/ha)	5.09**	(1.90)	2.58**	(1.10)	-0.02	(0.78)	4.87**	(1.15)	7.56	(0.61)
Oil (percent)	0.139**	(0.036)	0.008**	(0.003)	-0.002	(0.002)	0.023**	(0.004)	0.019	(0.001)
Seed weight (gm/100 seeds)	0.238**	(0.062)	0.010**	(0.005)	0.012*	(0.005)	0.034**	(0.006)	0.036	(0.003)
Protein/seed (mg/seed)	0.672**	(0.180)	0.050**	(0.020)	0.044**	(0.017)	0.096**	(0.020)	0.138	(0.010)
Height (cm)	4280**	(1078)	9	(14)	38*	(22)	131**	(26)	140	(11)
Days to flower	52.4**	(14.5)	5.4**	(1.0)	32.9**	(2.6)	5.0**	(0.9)	4.2	(0.3)

* Significantly different from zero at the 0.05 probability level, determined by the F-test in the analysis of variance.

** Significantly different from zero at the 0.01 probability level, determined by the F-test in the analysis of variance.

The non-significant genotype-location and genotype-year interaction for lysine as a percent of the sample was an encouraging sign for developing a high lysine genotype in one set of locations and years and having it hold up in other locations and years. The sum of the interaction components of variance for lysine as a percent of the protein was less than either the error or genotype component of variance. Lysine expressed as a percent of the sample is more important in selection for improved lysine than is lysine expressed as a percent of protein.

The estimates of the components of variance for the first order interaction were smaller than the component of variance for the second order interaction for all variables except for days to flower. The component of variance for the second order interaction was smaller than the experimental error component of variance for all variables except percent oil and days to flower. The relative size of these components play an important roll in determining the optimum number of years, locations, and replications to minimize the variance of a genotype mean. Weighing the cost factor of an additional replication versus an additional year or location, the most economical combination of years, locations and replications can be utilized to minimize the variance of a genotype mean.

Least significant different (LSD) values, calculated by multiplying the appropriate t-value by the standard error of the difference between two genotype means for various combinations of replications, years and locations are presented in Table 1.5. Ostle (1963) cautions against the use of the LSD technique, for if enough treatments are compared, the probability is high that significant differences will be observed due to chance alone. The LSD is presented here only to show the

Table 1.5. LSD values for different combinations of replications, years, and locations for the agronomic traits studied.

Trait	Numbers of							
Blocks	1	2	2	2	2	2	2	2
Years	1	1	2	4	1	2	4	8
Location	1	4	2	1	8	4	2	1
Yield (kg/are)	45.8	26.6	22.8	21.1	23.5	19.0	16.5	15.6
Protein (percent)	3.24	1.45	1.53	1.74	1.11	1.12	1.25	1.51
Protein yield (kg/are)	6.22	4.02	3.24	2.78	3.70	2.84	2.29	1.96
Lysine (percent protein)	0.61	0.30	0.28	0.30	0.25	0.22	0.22	0.25
Lysine (percent of sample x 100)	7.72	3.51	3.54	3.80	2.74	2.60	2.73	3.11
Lysine yield (kg/ha)	10.95	6.16	5.25	4.74	5.41	4.33	3.70	3.34
Oil (percent)	0.63	0.35	0.31	0.28	0.30	0.25	0.21	0.20
Seed weight (gm/100 seeds)	0.85	0.45	0.43	0.46	0.37	0.33	0.34	0.39
Protein/seed (mg/seed)	1.62	0.90	0.84	0.88	0.78	0.67	0.66	0.75
Height (cm)	50.6	23.5	24.3	27.0	17.8	17.8	19.3	22.8
Days to flower	19.5	11.1	12.9	17.0	9.1	9.7	12.2	16.6

reduction in the variance of a genotype mean by the addition of various numbers of replications, locations, and years, and it is not recommended as the best statistical tool for detecting differences between genotypes. The LSD values for a single observation such as might be encountered in a screening program were generally quite large compared to the range of means in Table 1.2. However the LSD for percent protein, protein per seed, percent oil, seed weight, and lysine as a percent of the sample were less than the range of the observations in this experiment indicating that some of the entries could effectively be eliminated in the first round of screening. The LSD values were generally reduced by 50% or more by increasing replications, years and locations from one to two each. The addition of more years and locations also reduced the LSD values but by a smaller magnitude. The substitution of years for locations did not appreciably change the LSD value expect for the yield measurements. This fact allows the plant breeder to substitute locations for years when selecting in a screening or a breeding program. However, the contribution of σ_{gyl}^2 to the variance of a genotype mean and hence the LSD is kept at a minimum when the product of the number of years and the number of locations is kept at a maximum value.

The large error-variances and LSD values for grain yield, protein yield, and lysine yield may have been due to border and competition effects when adjacent plots within a height group varied up to 50 cm or more in height. This variance could be reduced by either employing larger plots or subdividing each block into more height groups. The latter would probably be less efficient than larger plots due to a large and significant genotype-year-location interaction effect for height.

Also, in screening and breeding programs the plant breeder often does not have a reliable height estimate. Variations in maturity may have also caused competition effects and increased the experimental error.

The simple correlation coefficients of the genotype means across environments between the 11 agronomic traits investigated are presented in Table 1.6. Since the genotypes represent a random sample of genotypes from the World Collection of Sorghum, subject to the restriction of excluding extremely low yielding genotypes, and were averaged across all environments the phenotype mean approximates the genotype mean and the correlations computed are in reality the "genetic correlations" in a broad sense. All the yield variables were highly correlated as was expected. The negative correlation between grain yield and percent oil, an estimate of embryo size, and a positive correlation between grain yield and seed weight indicated that as yield increased a smaller percent of the seed was embryo. This was also supported by the fact that percent protein was positively correlated with percent oil. Lysine as a percent of protein and percent protein were highly negatively correlated as has generally been recorded (Collins and Pickett, 1972). However, the more useful expression lysine as a percent of the sample, was highly significantly correlated with percent protein and protein per seed.

The fact that protein per seed was positively correlated with percent protein and lysine as a percent of the sample and not significantly correlated with grain yield indicates that increased protein per seed may be selected without a reduction in grain yield. The fact that both percent oil and seed weight were positively correlated with percent protein means they both might be useful tools in selection for increased protein. However, percent oil was negatively correlated with yield and

Table 1.6. Correlation coefficients between agronomic traits based on genotype means across two years and five locations.

	Grain yield	Percent Protein	Protein Yield	Lysine (Percent of Protein)	Lysine (Percent of Sample)	Lysine Yield	Percent Oil	Seed Weight	Protein per Seed	Height	Days to Flower
Grain yield	1.00	-0.28	0.87**	-0.07	-0.42*	0.92**	-0.44*	0.28	-0.02	-0.06	0.14
Percent Protein		1.00	0.22	-0.66**	0.68**	-0.02	0.44*	0.51**	0.70**	0.05	-0.56**
Protein yield			1.00	-0.42*	-0.09	0.93**	-0.21	0.55**	0.33	-0.06	-0.14
Lysine (% of protein)				1.00	0.09	0.05	-0.35*	-0.52**	-0.53**	0.16	0.61**
Lysine (% of sample)					1.00	-0.05	0.27	0.21	0.42*	0.24	-0.13
Lysine yield						1.00	-0.36*	0.40*	0.15	0.01	0.09
Percent oil							1.00	0.17	0.28	0.24	-0.43*
Seed weight								1.00	0.73**	0.25	-0.41*
Protein/seed									1.00	0.18	-0.38*
Height										1.00	0.29
Days to flower											1.00

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

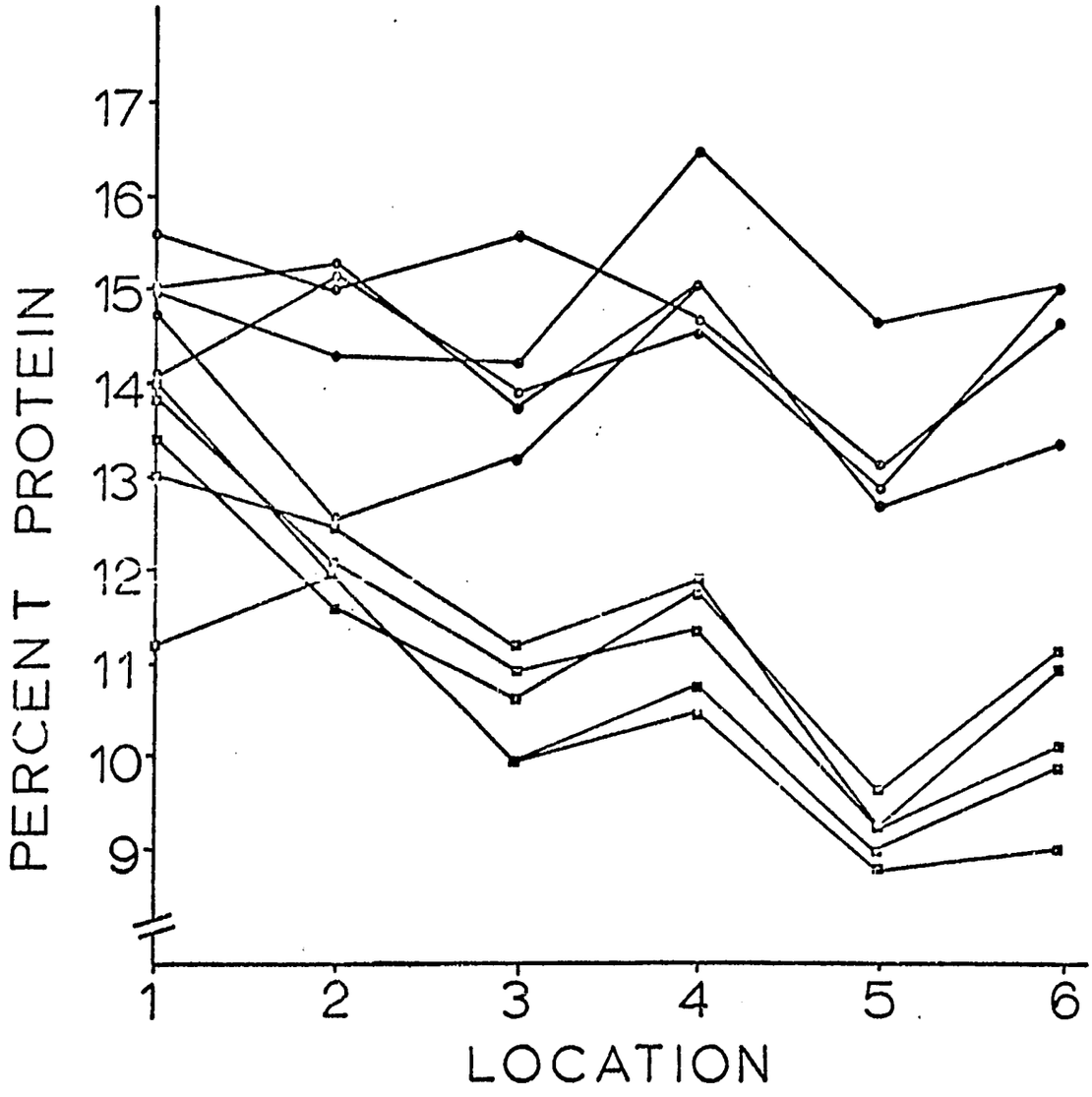
selection for percent protein via percent oil would tend to lower grain yield whereas selection for greater protein content per seed would tend to increase percent protein without reducing grain yield. Both seed weight and oil can be measured without destroying the seed.

The high positive correlation between days to flower and percent lysine of protein and high negative correlation between days to flower and percent protein indicate that selection for these traits should be done within maturity groups.

The significant genotype-location interaction for percent protein, percent lysine of protein, seed weight, and protein per seed appeared to be small interactions between genotypes of similar magnitude rather than large changes in rank at different locations. Figures 1.1, 1.2, 1.3 and 1.4 show that there were no antagonistic interactions between the best five and poorest five genotypes across all locations for percent protein, percent lysine of protein, seed weight, and protein per seed. This indicated that the poorest genotypes could be discarded in a screening program without serious losses of elite material.

Conclusions

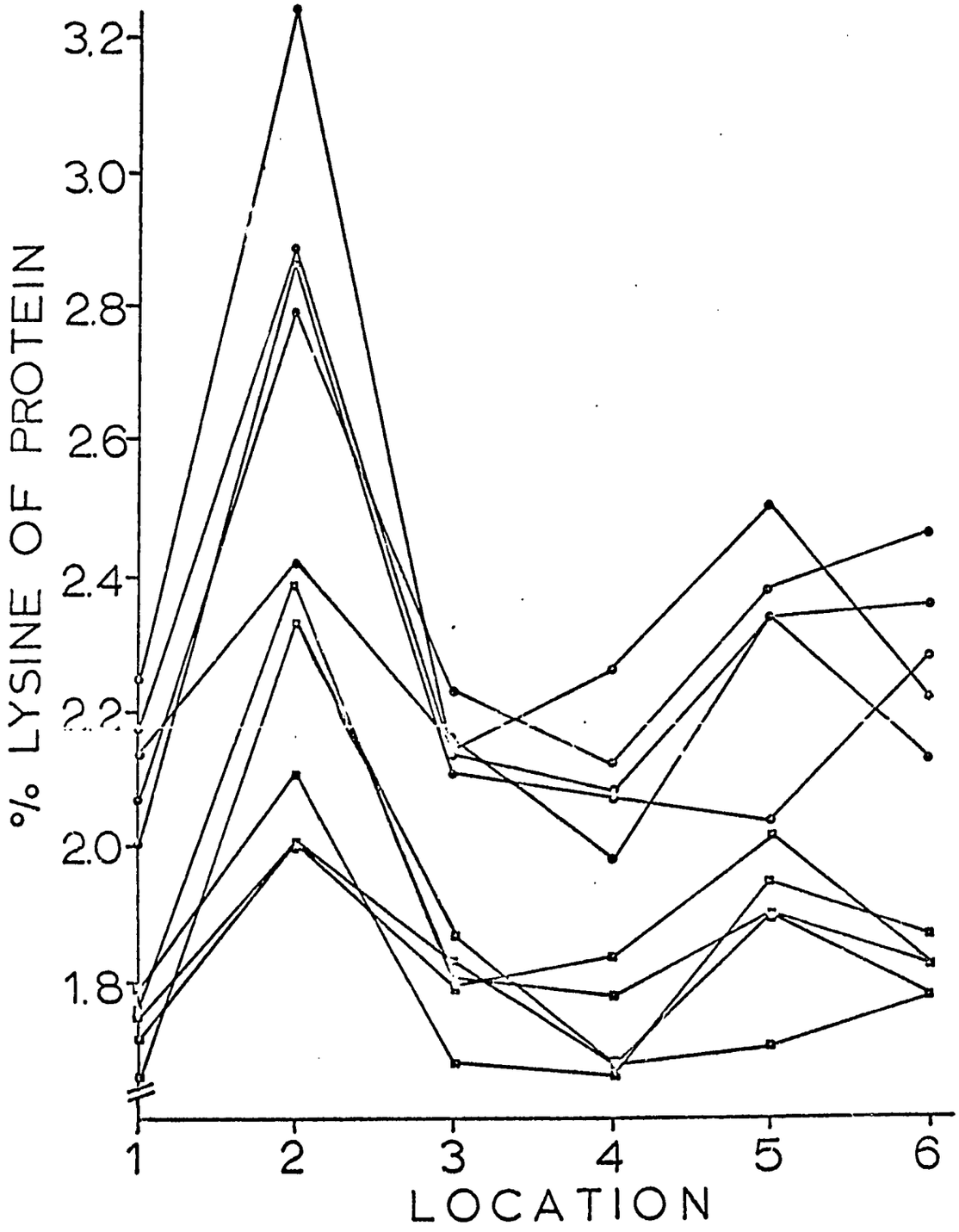
Significant second order interactions existed for all the variables studied in this investigation except lysine as a percent of protein. In general, the first order interactions were generally of similar magnitude and smaller than the second order interaction and experimental error. This allows the plant breeder to substitute locations for years without appreciably inflating the expected variance of a genotype mean. Yield was an exception to this trend with a larger genotype-year interaction than genotype-location interaction. Lysine as a percent of the



Location
1 - Lubbock, Texas
2 - Mead, Nebraska
3 - PAF, Normal Fertility

Location
4 - PAF, High Fertility
5 - SIPAC
6 - PPAC

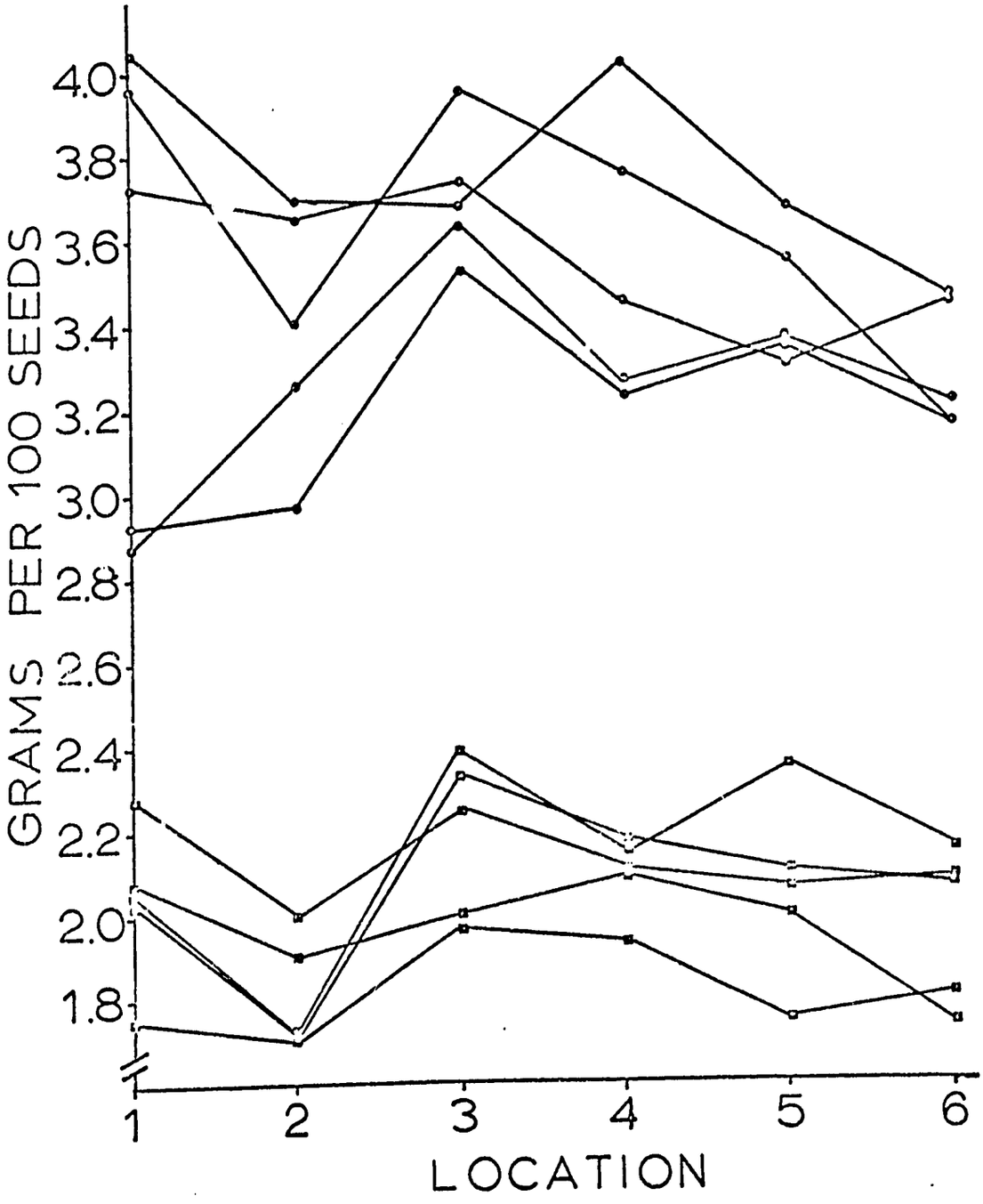
Figure 1.1. Genotype-location interaction of the five highest and five lowest genotypes for percent protein.



Location
1 - Lubbock, Texas
2 - Mead, Nebraska
3 - PAF, Normal Fertility

Location
4 - PAF, High Fertility
5 - SIPAC
6 - PPAC

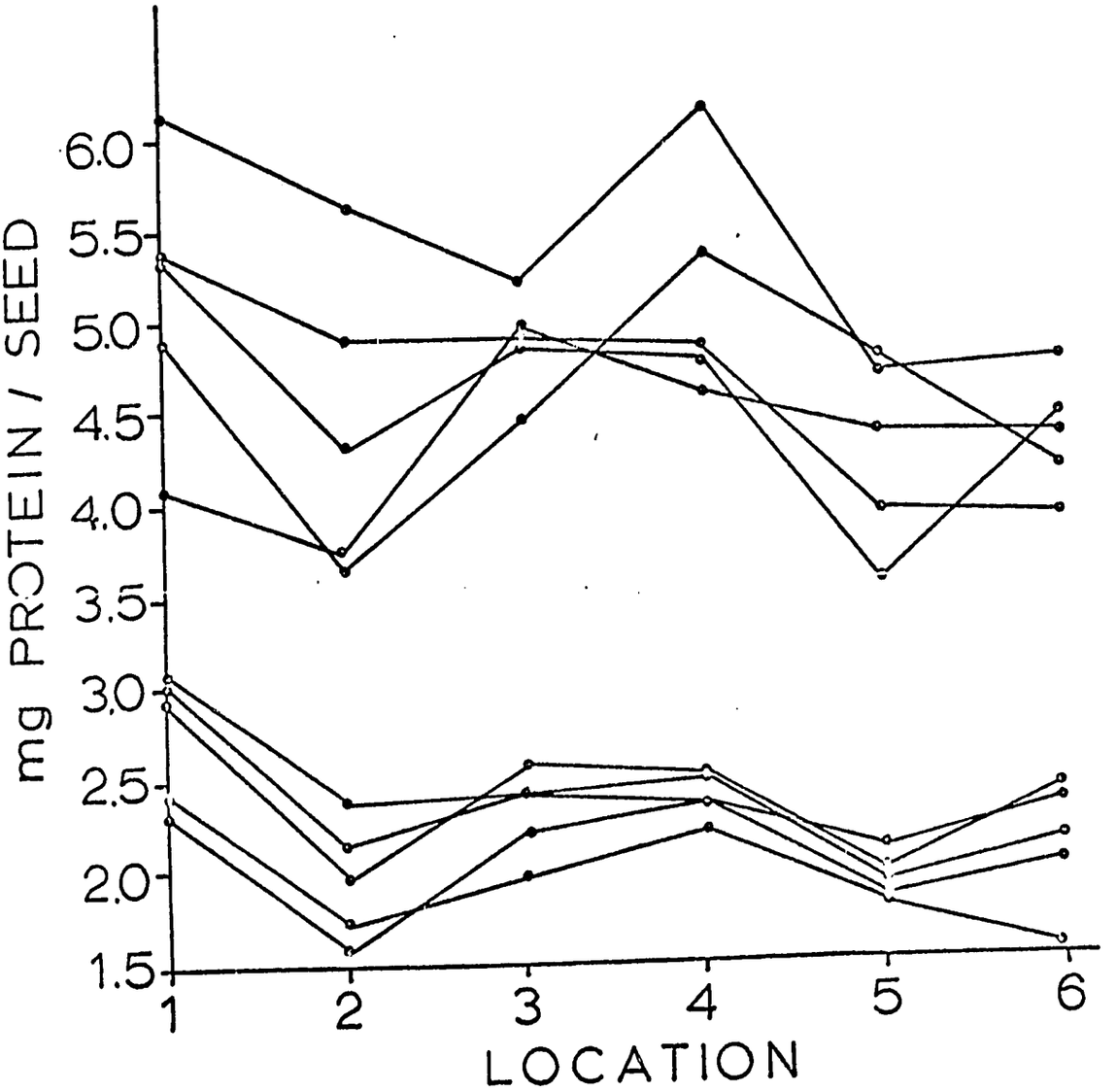
Figure 1.2. Genotype-location interaction of the five highest and five lowest genotypes for percent lysine of protein.



Location
1 - Lubbock, Texas
2 - Mead, Nebraska
3 - PAF, Normal Fertility

Location
4 - PAF, High Fertility
5 - SIPAC
6 - PPAC

Figure 1.3. Genotype-location interaction of the five highest and five lowest genotypes for 100 seed weight.



Location
1 - Lubbock, Texas
2 - Mead, Nebraska
3 - PAF, Normal Fertility

Location
4 - PAF, High Fertility
5 - SIPAC
6 - PPAC

Figure 1.4. Genotype-location interaction of the five highest and five lowest genotypes for protein per seed.

sample had non-significant first order interactions indicating locations could readily be substituted for years in a testing program and the expected variance of the genotype mean was actually reduced by increasing the number of locations. Correlations indicated that protein content could be improved by selecting for increased protein per seed without reducing yield. Percent oil and seed weight were significantly correlated with percent protein and should be useful selection tools for increasing percent protein. However, selection for greater oil would tend to lower yield whereas selection for greater protein per seed would increase percent protein without reducing yield. The positive correlation between days to flower and percent lysine of protein and negative correlation between days to flower and percent protein indicated that selection for these traits should be done within maturity groups.

These data indicate that selection can be applied effectively, for traits studied in the investigation, within the World Collection of sorghum or similar material from one environment. However, the existence of a significant second order interaction for the traits studied indicates that final evaluations should be made in more than one environment. The existence of relatively small first order interactions of nearly equal magnitude for percent protein, lysine as a percent of protein, lysine as a percent of the sample, oil, seed weight, and protein per seed indicate that years can be substituted for locations and locations can be substituted for years except in the final evaluation.

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AMINO ACID COMPOSITIONS OF WHOLE KERNEL AND ENDOSPERM PROTEIN FRACTIONS OF SORGHUM.

R. Jambunathan and E. T. Mertz

In the 1971 annual report no. 8 of this project we described a procedure for the fractionation of sorghum proteins into five different solubility fractions using the procedure of Landry and Moureaux (1970). We were able to obtain good nitrogen recoveries employing this procedure. Four sorghum lines grown at the Purdue Agronomy Farm were used in this fractionation procedure: IS0062 (925006) and IS3982 (025042), which were yellow and light yellow in color respectively; and IS2283 (925074) and IS 6992 (025154) which were red brown and brown in color respectively. From the results of catechin equivalent determinations, samples IS0062 and IS3982 were classified as low tannin lines while IS6992 and IS2283 were classified as high tannin lines. Whole grains of each line were soaked in cold water for two hours and separated into germ, pericarp and endosperm by means of a scalpel. The separated endosperms were air dried, defatted and stored in screw capped bottles.

The fractionation procedure has been described in detail in our last annual report. Both the whole kernels and the endosperms were fractionated using various solvent systems and the average of the results obtained are presented in Tables 1 and 2 respectively. The average weight gain of rats obtained when the whole grains were fed to six rats for a period of four weeks is also shown in Table 1.

The distribution patterns obtained for the whole grain protein fractions and the possible influence of the tannins on the distribution of proteins have been discussed in detail in the 1971 annual report. A preliminary report of these studies has been published (Jambunathan, Misra and Mertz, 1972) and a detailed report is in press (Jambunathan and Mertz, 1973) at the present time. Results from tables 1 and 2 indicate that the distribution of endosperm proteins is similar to the distribution obtained from the whole kernel protein fractionations except that the differences in nitrogen distribution in fractions II and III of the sorghum endosperms between high and low tannin sorghums is not as great as exhibited by the whole grain fractions. Also the total percentage of nitrogen soluble in fractions II and III combined of the endosperm is higher than the total percentage of the nitrogen obtained for fractions II and III of the whole grain.

Amino acid composition of the whole grain, endosperm and the protein fractions from each were obtained by employing a Beckman Model 121 Amino Acid Analyzer. The samples and the fractions were hydrolyzed by refluxing in an excess of 6N HCl. The excess HCl was removed in vacuo using a rotary evaporator. The evaporated sample was dissolved in a pH 2.2 buffer and was applied to the ion exchange columns of the amino acid analyzer. The amino acid composition of the whole grain, endosperm and their protein fractions are given in Tables 3 through 9.

Table 3 gives the amino acid composition of the whole grain and endosperms of the four sorghum lines. When individual amino acid values

for the four sorghum grains or endosperms are compared between lines, no major differences seem to exist. It may be noted that the tryptophan content of the whole grain is more than one percent in all samples Tryptophan was determined by ion exchange chromatography after barium hydroxide hydrolysis according to the method of Slump and Schreuder (1969). The results indicate that sorghum grain may not be deficient in tryptophan. The values for cystine and methionine are probably low since no effort was made to protect them against oxidation during hydrolysis. The amino acid values of whole grain samples for these four lines fall within the range of the average values reported for 522 lines.[1] It is also clear from table 3 that the endosperm contains lesser amounts of lysine, arginine, glycine and higher amounts of glutamic acid, proline, alanine, leucine, isoleucine and phenylalanine, which indicates differences in the amino acid composition of the germ and pericarp in the distribution of amino acids relative to the endosperm.

Table 4 gives the amino acid composition of the saline soluble (fraction I) proteins of the whole grain and endosperm. This protein fraction has good distribution of lysine and arginine with lesser amounts of glutamic acid, proline, alanine, isoleucine, leucine and phenylalanine as compared to the whole grain and endosperm amino acid distribution. From the amino acid distribution, they seem to be good quality proteins though the fraction obtained from the high tannin sorghum samples appears to be lower in lysine and other essential amino acids. This may be related to

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[1] Reported on page 2 of this report.

the lower nitrogen recovery of fraction I from the high tannin samples. The isoleucine to leucine ratio ranges from 0.5 to 0.6 for these protein fractions.

Table 5 gives the amino acid composition of the isopropanol soluble (fraction II) proteins. The low tannin fraction of the grain and endosperm contains higher amounts of arginine, aspartic acid, threonine, glutamic acid, valine, isoleucine, leucine and tyrosine as compared to the high tannin protein fractions. No definite pattern appears in the distribution of lysine, histidine and phenylalanine.

The distribution of amino acids of proteins soluble in isopropanol containing mercaptoethanol (fraction III) is given in table 6. The low tannin protein fractions are higher in arginine, proline and glycine contents while other essential and non-essential amino acids do not exhibit any fixed pattern of distribution among the high and low tannin protein fractions.

The distribution of amino acids in fraction IV are given in table 7. The low tannin protein fractions of whole grain and endosperm samples IS0062 and IS3982 contain higher amounts of histidine and proline, and lesser amounts of arginine, aspartic acid, serine, alanine and phenylalanine as compared to the high tannin protein fractions. Other amino acids including lysine do not have any common pattern of distribution. In the endosperm protein fractions, the high tannin protein fractions contain more lysine, glutamic acid, glycine, valine, isoleucine and tyrosine as compared to the low tannin protein fractions.

The amino acid composition of fraction V of the whole grain and endosperm proteins is given in table 8. The distribution of essential amino acids do not show any major resemblances between the high and the low tannin protein fractions though there seems to be some similarities when the whole grain and the endosperm amino acids are considered separately. In the amino acid composition of whole grain protein fractions, the low tannin samples contain higher amounts of histidine and lower amounts of arginine, serine, glutamic acid and tyrosine. In the endosperm amino acid distribution, the low tannin samples contain higher amounts of lysine and arginine and lesser amounts of glutamic acid, proline, alanine, leucine, tyrosine and phenylalanine.

Table 9 gives the amino acid composition of the residues of the whole grain and endosperm samples obtained at the end of the fractionation procedure. Though the distribution of the amino acids indicate that it is a good quality protein both in the case of whole grain and endosperm samples, the protein percentage of the residue was very low, being in the range of 0.4 to 1.1 percent protein.

The distribution of amino acids indicate that among the five solubility fractions of sorghum, fractions II and III are very poor quality proteins, while fraction I is a very good quality protein. Fractions IV and V are also good quality proteins though not as good as the proteins of the first fraction. Thus, to improve the overall nutritional quality of sorghum, attempts should be made to decrease the percentage of fraction II and III and increase the percentage of fraction I simultaneously. Increasing the distribution

percentage of fraction IV and V would also enhance the nutritional quality of sorghum. It appears from the results that the fractionation studies can supplement or substitute for the rat growth studies to a certain extent in the screening procedure to identify nutritionally good quality sorghum.

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Table 1. Nitrogen Distribution in Sorghum Whole Grain.*

Fraction	IS0062	IS3982	IS6992	IS2283
I (Saline)	16.8	15.4	5.6	4.1
II (Isopropanol)	18.4	10.6	5.9	2.5
III (Isopropanol + 2-Mercaptoethanol)	18.9	18.2	11.5	13.8
IV (Borate buffer + 2-Mercaptoethanol)	6.1	6.4	15.0	17.3
V (Borate buffer + 2-Mercaptoethanol + Sodium Dodecyl Sulfate)	29.9	34.2	54.8	49.3
Total Nitrogen Extracted	90.1	84.8	92.8	87.0
Wt. Gain Per Rat Per Week**	8.7	7.2	1.8	-0.2
Percent Protein	11.2	12.6	9.2	7.7

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* Percent of soluble nitrogen.
** Six rats, four weeks duration.

Table 2. Nitrogen Distribution in Sorghum Endosperms.*

Fraction	IS0062	IS3982	IS6992	IS2283
I (Saline)	8.0	6.6	2.8	2.9
II (Isopropanol)	19.9	13.6	9.2	13.3
III (Isopropanol + 2-Mercaptoethanol)	35.1	29.7	27.0	28.1
IV (Borate buffer + 2-Mercaptoethanol)	6.4	9.2	10.6	11.2
V (Borate buffer + 2-Mercaptoethanol + Sodium Dodecyl Sulfate)	26.5	24.0	45.0	35.7
Total Nitrogen Extracted	95.9	83.1	94.6	91.2
Percent Protein	10.0	8.6	9.5	7.2

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* Percent of soluble nitrogen.

Table 3. Amino Acid Composition of Sorghum Whole Grain and Endosperm.*

	Whole Grain				Endosperm			
	IS0062	IS3982	IS6992	IS2283	IS0062	IS3982	IS6992	IS2283
Lysine	2.8	2.5	2.6	2.3	1.8	2.2	2.1	2.1
Histidine	2.3	2.3	2.2	2.1	2.4	2.7	2.1	2.5
Arginine	4.5	4.0	4.5	3.6	3.5	3.7	3.6	3.2
Aspartic Acid	7.7	6.7	7.2	7.0	8.4	8.0	8.0	6.4
Threonine	3.3	3.3	3.3	3.2	3.5	3.7	3.4	3.2
Serine	4.2	4.4	4.5	4.4	4.9	5.0	4.7	4.6
Glutamic Acid	22.8	21.8	22.1	20.8	27.5	26.4	26.0	22.9
Proline	7.7	7.8	6.9	6.2	8.8	9.3	8.4	7.9
Glycine	3.3	3.4	3.6	3.4	3.0	2.9	3.0	3.0
Alanine	9.2	9.2	9.5	8.5	11.1	10.8	10.8	9.5
Cystine	1.3	1.5	1.3	1.5	1.4	2.1	0.7	1.2
Valine	5.1	5.2	5.4	4.8	5.7	5.7	5.6	4.9
Methionine	1.7	1.8	1.9	1.3	1.2	1.5	1.4	1.6
Isoleucine	4.0	4.0	4.3	3.8	4.6	4.6	4.7	4.0
Leucine	13.2	13.2	13.7	12.2	17.1	16.3	16.4	14.2
Tyrosine	4.3	4.5	4.7	4.4	5.3	5.3	5.1	4.4
Phenylalanine	5.1	5.0	5.4	4.9	6.0	6.0	6.0	5.2
Tryptophan	1.3	1.3	1.2	1.4				

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* Grams per 100 grams protein.

Table 4. Amino Acid Composition of Fraction I of Sorghum Whole Grain and Endosperm.*

	Whole Grain				Endosperm			
	IS0062	IS3982	IS6992	IS2283	IS0062	IS3982	IS6992	IS2283
Lysine	5.1	5.4	3.7	3.9	5.0	5.1	3.0	3.0
Histidine	2.2	2.3	1.3	1.4	1.9	1.9	1.1	1.0
Arginine	8.6	7.6	6.6	5.3	6.8	7.8	3.1	3.2
Aspartic Acid	11.3	9.7	11.5	11.3	9.7	9.3	4.6	5.2
Threonine	3.4	3.6	2.4	2.4	4.6	4.2	1.9	2.0
Serine	4.0	4.5	3.1	2.9	4.5	3.9	1.8	2.0
Glutamic Acid	12.8	13.6	8.4	9.6	10.9	15.1	7.5	8.1
Proline	4.0	5.0	4.3	2.3	4.9	6.0	2.0	1.7
Glycine	4.4	5.3	3.9	4.4	5.9	4.0	2.6	2.9
Alanine	4.9	5.6	4.1	3.7	6.2	7.2	3.4	3.7
Cystine	1.6	1.4	0.5	0.8	2.8	3.7	0.8	0.6
Valine	4.4	5.0	3.1	3.1	4.9	5.3	2.6	2.8
Methionine	1.1	1.6	1.0	1.0	0.5	0.9	1.0	0.8
Isoleucine	2.7	3.0	1.9	2.1	3.5	3.5	1.8	2.0
Leucine	4.8	5.6	3.2	3.6	6.7	6.9	3.5	3.6
Tyrosine	2.5	2.8	1.8	2.0	3.2	3.3	1.5	1.3
Phenylalanine	2.7	3.9	1.9	2.0	3.4	3.2	1.7	1.7

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* Grams per 100 grams protein.

Table 5. Amino Acid Composition of Fraction II of Sorghum Whole Grain and Endosperm.*

	Whole Grain				Endosperm			
	IS0062	IS3982	IS6992	IS2283	IS0062	IS3982	IS6992	IS2283
Lysine	0.3	0.3	0.4	0.3	0.2	0.3	0.3	0.2
Histidine	1.3	1.3	1.1	1.6	1.2	1.7	1.1	1.4
Arginine	2.0	1.9	1.2	1.2	1.7	2.2	1.8	1.5
Aspartic Acid	7.3	6.8	6.1	5.6	8.5	8.0	7.3	7.1
Threonine	2.8	3.2	2.3	2.3	3.3	3.5	2.7	2.6
Serine	3.9	4.7	3.7	3.8	4.8	5.1	4.4	3.8
Glutamic Acid	30.5	32.6	26.8	24.0	35.7	38.6	32.0	30.9
Proline	10.1	9.4	7.4	10.8	9.9	10.4	9.4	8.7
Glycine	1.2	1.7	1.2	1.3	1.5	1.6	1.4	0
Alanine	12.5	14.0	10.8	10.3	13.9	15.5	12.6	13.9
Cystine	0.7	0.9	0.3	0.3	0.8	1.4	1.1	0.3
Valine	5.1	5.5	4.2	3.6	5.2	6.1	4.2	5.0
Methionine	1.1	2.3	1.3	1.1	0.8	2.2	1.6	0.9
Isoleucine	4.2	4.5	3.8	2.8	4.4	5.0	3.5	4.4
Leucine	18.8	20.7	16.8	14.1	21.7	23.4	19.5	18.0
Tyrosine	5.3	6.0	4.1	3.8	6.2	6.7	5.5	5.2
phenylalanine	4.6	6.8	5.9	4.2	6.6	7.2	6.0	5.6

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* Grams per 100 grams protein.

Table 6. Amino Acid Composition of Fraction III of Sorghum Whole Grain and Endosperm.*

	Whole Grain				Endosperm			
	IS0062	IS3982	IS6992	IS2283	IS0062	IS3982	IS6992	IS2283
Lysine	0.1	0.2	0.2	0.1	0.2	0.2	0.3	0.2
Histidine	2.2	1.4	1.4	1.8	1.4	1.7	1.5	1.7
Arginine	1.6	1.6	1.5	1.1	1.7	1.8	1.4	1.4
Aspartic Acid	6.3	6.1	7.5	6.2	6.5	6.7	6.6	6.4
Threonine	2.7	3.0	2.9	2.5	2.7	3.2	2.5	2.8
Serine	4.6	5.0	5.0	4.5	4.4	5.5	4.2	4.9
Glutamic Acid	32.4	31.9	36.6	30.3	31.2	34.8	31.1	33.6
Proline	9.5	9.7	9.0	7.9	9.9	11.0	9.1	10.7
Glycine	1.6	1.7	0.6	0.8	1.4	1.7	1.2	0
Alanine	13.7	13.8	15.9	13.5	12.5	14.4	12.7	15.3
Cystine	1.2	1.2	0.7	0.6	1.4	1.6	1.0	0.8
Valine	5.0	5.1	5.4	4.5	4.8	5.4	4.9	4.8
Methionine	1.6	1.9	1.2	1.1	1.2	1.4	0.9	1.2
Isoleucine	4.6	4.7	5.3	4.2	4.4	5.1	4.7	4.8
Leucine	19.9	20.7	23.1	18.7	19.2	22.4	19.7	20.8
Tyrosine	4.4	4.4	4.7	4.7	5.1	4.9	3.6	5.7
Phenylalanine	5.7	7.6	8.1	6.1	6.6	7.9	6.8	6.5

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* Grams per 100 grams protein.

Table 7. Amino Acid Composition of Fraction IV of Sorghum Whole Grain and Endosperm.*

	Whole Grain				Endosperm			
	IS0062	IS3982	IS6992	IS2283	IS0062	IS3982	IS6992	IS2283
Lysine	2.7	1.8	3.0	2.6	1.1	1.3	2.3	2.5
Histidine	6.9	7.8	4.9	4.4	7.4	7.1	5.8	6.4
Arginine	5.5	4.2	6.3	6.0	3.6	3.3	5.7	6.2
Aspartic Acid	4.1	2.9	5.2	6.0	1.6	2.2	5.2	4.5
Threonine	5.6	6.0	5.2	4.8	6.1	5.2	5.8	7.4
Serine	4.1	3.9	4.4	4.5	3.4	3.2	4.7	4.5
Glutamic Acid	15.0	15.3	14.6	16.9	15.6	17.0	17.9	18.0
Proline	12.7	10.7	8.6	8.9	14.2	15.1	12.6	12.7
Glycine	5.5	5.5	5.3	6.0	5.0	4.8	5.9	6.2
Alanine	4.9	4.2	6.0	6.4	3.9	4.2	6.1	6.0
Cystine	1.7	0.6	0.8	1.9	2.4	1.3	1.2	2.1
Valine	5.3	5.1	5.0	4.9	4.3	4.7	5.3	5.5
Methionine	1.4	1.3	1.4	1.4	1.1	1.2	1.5	1.6
Isoleucine	3.1	2.8	3.1	3.2	2.4	2.6	3.3	3.2
Leucine	7.7	7.7	8.1	8.6	7.5	7.5	8.9	7.0
Tyrosine	2.9	1.4	2.1	2.8	1.2	1.5	1.7	1.7
Phenylalanine	4.2	3.1	5.2	4.5	2.0	2.3	3.3	3.1

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* Grams per 100 grams protein.

Table 8. Amino Acid Composition of Fraction V of Sorghum Whole Grain and Endosperm.*

	Whole Grain				Endosperm			
	IS0062	IS3982	IS6992	IS2283	IS0062	IS3982	IS6992	IS2283
Lysine	3.2	2.3	2.6	2.0	3.6	3.6	2.7	3.3
Histidine	3.1	2.7	2.4	2.6	3.1	3.2	2.6	3.1
Arginine	3.7	3.5	4.2	3.9	4.9	4.9	4.7	4.7
Aspartic Acid	8.0	6.8	7.1	7.4	7.7	8.4	8.4	7.8
Threonine	4.1	4.2	4.1	4.1	4.7	5.0	4.3	5.0
Serine	4.2	4.5	4.8	5.0	4.6	4.4	4.3	5.4
Glutamic Acid	22.2	21.4	22.8	26.3	21.4	22.3	25.7	23.2
Proline	7.9	6.9	8.4	6.2	7.3	8.2	8.8	8.5
Glycine	3.5	3.1	3.5	4.3	4.0	4.1	3.4	4.3
Alanine	8.5	10.0	10.1	10.1	8.9	9.6	11.1	9.9
Cystine	0	0	0	0	0	0.2	0.2	0
Valine	5.2	5.7	5.6	5.6	6.0	6.5	6.3	5.9
Methionine	1.5	1.4	2.0	2.0	1.9	2.2	1.9	2.4
Isoleucine	4.3	4.5	4.5	4.7	4.6	4.9	5.1	4.5
Leucine	13.4	14.9	14.8	16.4	13.3	14.2	16.3	14.7
Tyrosine	4.5	3.4	4.7	5.1	3.0	3.3	4.0	6.3
Phenylalanine	5.5	7.1	6.2	6.6	5.2	6.1	6.4	7.9

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* Grams per 100 grams protein.

Table 9. Amino Acid Composition of Residue of Sorghum Whole Grain and Endosperm.*

	Whole Grain				Endosperm			
	IS0062	IS3982	IS6992	IS2283	IS0062	IS3982	IS6992	IS2283
Lysine	4.6	5.8	4.6	4.7	4.1	4.1	4.3	4.6
Histidine	3.5	4.2	3.2	2.7	3.4	3.1	2.7	2.8
Arginine	5.1	6.7	6.9	7.1	5.2	5.5	5.6	6.0
Aspartic Acid	7.3	8.8	9.3	9.9	6.8	7.5	7.8	7.2
Threonine	4.2	5.7	4.8	4.9	3.7	4.5	4.1	2.6
Serine	4.2	4.9	5.6	5.6	4.1	4.0	4.3	4.6
Glutamic Acid	11.7	12.7	13.6	12.4	12.0	11.3	10.8	11.1
Proline	5.8	5.7	4.5	4.0	12.3	4.7	4.5	4.4
Glycine	6.1	7.3	4.0	7.6	5.8	6.0	6.1	4.0
Alanine	5.5	6.5	7.0	7.2	5.4	5.4	5.7	5.9
Cystine	2.0	0.7	1.9	1.7	1.9	0.7	1.0	1.4
Valine	5.4	6.3	6.1	6.2	5.5	5.3	5.2	5.3
Methionine	1.7	2.1	1.5	1.5	1.5	1.3	1.4	1.5
Isoleucine	3.7	4.3	4.0	4.4	3.7	3.5	3.5	3.7
Leucine	8.0	8.9	8.9	7.1	7.8	7.4	7.1	8.1
Tyrosine	3.8	4.1	4.3	4.1	3.3	3.5	3.4	3.5
Phenylalanine	4.1	4.7	4.8	4.9	3.5	3.8	3.9	4.2
Percent Protein	1.1	0.9	0.7	0.6	0.5	0.5	0.5	0.4

* Grams per 100 grams protein.

EVALUATION OF RAPID SCREENING PROCEDURES FOR IDENTIFYING HIGH LYSINE AND HIGH PROTEIN LINES OF SORGHUM.

R. Jambunathan and E. T. Mertz

One of the research objectives of the sorghum project at Purdue is to identify sorghum lines with improved protein quality and quantity utilizing both chemical and biological methods. Since lysine is the most deficient amino acid in sorghum, it is necessary to screen for lysine and also for protein content of sorghum in order to achieve the above objective. Therefore, the method of choice would be the one which could identify the high protein and high lysine sorghum lines from a large number of samples. Also, the method should be simple, rapid, inexpensive and reliable. Since it is difficult to have all the parameters in one given method, it becomes necessary to sacrifice one or more criteria to achieve the other desirable results.

There are a number of methods available for the determination of lysine, the most accurate being column chromatography with the amino acid analyzer. But the methods which are most accurate are also time consuming or expensive or both. In a project where the sample number is large, the breeder has to identify the best lines among the harvested samples in one season so that the most promising lines can be increased for biological evaluation the next season. So here, the method of screening should be a fast one though it may not give highly analytical results. Once the most promising lines are identified by this rapid method of screening, the validity of the results

of the method can always be checked using other more sophisticated methods. One of the rapid methods that has been used successfully for the screening of lysine and tryptophan in maize employs the reagent 2 chloro-3,5 dinitropyridine. The original method reported by Tsai et al (1972) has been modified by Villegas and Mertz (1971) and the modified method was used for the evaluation of sorghum samples for their lysine content. Villegas and Mertz have reported that up to sixty duplicate samples can be analyzed in one day using the pyridine method.

For the determination of protein, the micro Kjeldahl method has long been used as the primary standard and the nitrogen content obtained by this method is converted using a factor to express the protein content of the substance. Though this method gives accurate results, it is time consuming. Johnson and Craney (1971) reported a rapid biuret method for the determination of protein content in several cereal grains, including sorghum. Since they reported a high correlation between the biuret value and the micro Kjeldahl nitrogen for sorghum samples, this method was used in our study for the estimation of sorghum proteins.

The dye binding procedure (Udy, 1956) has been increasingly employed for the screening of various cereal crops and this method has been successfully utilized for the screening of wheat, rice, soybean and barley samples. This method is simple and rapid and can be used to screen for lysine content if the nitrogen (protein) content is available.

Even though a given sorghum line may have high lysine and high protein contents, one has to determine biological availability. This is usually

done by feeding the sample to rats. There is no single chemical method available which can substitute for rat growth studies. But in our preliminary studies with fifteen sorghum samples for which four weeks of rat feeding data were available, we observed that the percentage of nitrogen soluble in the first fraction gave an indication of the rat growth data obtained with these samples. So this method was put to test using a large number of samples.

Twenty-six lines of sorghum were chosen for the study. Rat growth data on these samples were obtained by feeding these samples to six rats for a period of two weeks. Feeding experiments were repeated at a later date in order to minimize the error that may have been caused due to the differences in rat behavior, environmental conditions, etc. A well mixed bulk sample of IS2319 was set aside for rat feeding studies and this was fed every time along with other experimental samples. The performance (weight gain) of other rats was compared each time with the weight gain of rats fed on IS2319 and an arbitrary percentage was assigned to the weight gain observed with other samples by taking the weight gain of IS2319 fed rats equivalent to 100. The results thus obtained were included as percent check in our correlation studies. Nitrogen was determined on these samples in the Biochemistry Laboratory, Shuman Chemical Laboratories at Battle Ground, Indiana and ABC Laboratories, Inc. of Columbia, Missouri. Lysine was determined by the amino acid analyzer in the Shuman Chemical Laboratory while lysine decarboxylase method was employed in the ABC Laboratory. Tannin contents of these samples were determined according to the method

of Burns (1971) and the results were expressed as catechin equivalents. Out of the 26 samples, nine had more than one percent catechin equivalents and were classified as high tannin samples. The rest were classified as low tannin samples. Only eight high tannin samples were taken into consideration for correlation analysis.

A. Lysine Determination by Pyridine Method.

This method utilizes a proteolytic enzyme to hydrolyze the proteins into free amino acids or low molecular weight peptides. The free alpha amino groups of the amino acids are complexed with copper phosphate reagent but the epsilon amino groups remain reactive. The epsilon amino group reacts with the reagent 2 chloro-3,5 dinitropyridine to form epsilon-dinitropyridyl derivative of lysine which is insoluble in ethyl acetate. The excess reagent is then removed by ethyl acetate and the absorbance of the aqueous phase determined at 400 nm.

Sorghum samples were ground in a Wiley mill to pass through a 40-mesh sieve, defatted with n-butanol and were made into a fine powder using a small ball mill (Wig-L-Bug Amalgamator). The procedure of Villegas and Mertz (1971) was followed without any modifications. In addition to the 26 samples, lysine determinations were done on 50 more sorghum samples (data not given).

The lysine values obtained with the high tannin samples were too low to be meaningful. Therefore, all the samples were dehulled according to the procedure of Blessin (1971) using 20 percent hot sodium hydroxide and the procedure was repeated for protein and lysine using the dehulled samples.

Table 5 gives the results obtained by this method for lysine and tryptophan in the whole kernel and dehulled samples. Correlations were obtained with the P x L values obtained by the above method with P x L values obtained from other methods and also with rat feeding data. Results of correlation analysis are given in Table 1.

Table 1. Correlations: Pyridine Method

	Whole Grain P x L		Dehulled Grain P x L	
	Low Tannin	High Tannin	Low Tannin	High Tannin
Weight Gain (Average)	.802**	.375	.713**	.328
Weight Gain Check (Average)	.801**	.377	.711**	.327
P x L (Shuman Lab.)	.850**	-.065	.779**	-.399
P x L (ABC Lab.)	.805**	.331	.721**	.162

** Significant at the 1 percent level.
* Significant at the 5 percent level.

Results of the correlations indicate that the results obtained with the pyridine method are highly correlated with the rat growth data and also with the P x L value obtained by other reliable methods in the low tannin samples. Though the sample size in the high tannin variety is small, the results obtained with that sample size indicate that the pyridine method for the estimation of lysine in high tannin varieties is not reliable.

Conclusion: The pyridine method for the estimation of lysine is highly reliable in the low tannin samples and excellent correlations are obtained

between the P x L values obtained by this method and the rat growth data and also with the P x L values obtained by other methods including that of the amino acid analyzer. Results obtained from limited high tannin sorghum samples indicate that the pyridine method is not useful for screening high tannin sorghum samples. Dehulling does not seem to improve the results obtained with high tannin sorghums. No comparison of the tryptophan values are made as it was not determined by any other method.

B. Estimation of Proteins by the Biuret Method.

The biuret method has long been used for the quantitative estimation of proteins in biological materials and there have been many reported modifications of the original method. The method is based on the formation of a violet color when a protein complex reacts with an alkaline copper reagent. In the Johnson and Craney method (1971) proteins from cereals are extracted with isopropyl alcohol and reacted with solid cupric carbonate and alkaline isopropyl alcohol solution to produce the color. Since the absorbance values obtained with one gram sorghum samples were very high, we used 500 mg of a finely powdered sample for the extraction and we centrifuged the final mixture instead of filtering as suggested by the authors in their article. The results obtained are given as absorbance values obtained with 500 mg of the sample in Table 5. In addition to the 26 samples, biuret reaction was also run for 50 more sorghum samples (data not given).

Results of correlations for the 25 samples (Table 2) are separated into two classes, low tannin and high tannin in order to determine whether the high tannin samples interfere with the biuret color.

Table 2. Correlations: Biuret Method with micro Kjeldahl N Determinations.

	Low Tannin	High Tannin
Micro Kjeldahl Protein:		
(Biochemistry Lab.)	0.887**	0.859**
(Shuman Lab.)	0.861**	0.720*
(ABC Lab.)	0.846**	0.855**
Average Protein	0.873**	0.819*
Weight Gain (Average)	0.597*	-0.090
Weight Gain Check (Average)	0.596*	-0.079

** Significant at the 1 percent level.
* Significant at the 5 percent level.

Results of the statistical analysis indicate that the results obtained by the biuret method are highly significantly correlated with the micro Kjeldahl protein values obtained from three different laboratories in the low tannin sorghums and the correlation obtained for the high tannin samples varies among the different laboratories, the average protein value being significant at the five percent level. Weight gain of rats and the biuret method results show significant correlation at the five percent level in the low tannin sorghums and no correlation is evident with the high tannin sorghums.

Conclusion: Results obtained by the rapid biuret method and the protein values obtained from three different laboratories agree significantly at the one percent level for the low tannin sorghums while weight gain of rats agree significantly at the five percent level. High tannin samples show varied correlations between the biuret results and the micro Kjeldahl protein value while the weight gain of rats show no significance at all. Taken together, the results of high and low tannin sorghum for proteins and weight gain of rats show a highly significant correlation to the biuret results.

C. Udy Dye Binding Method.

This method is based on the principle that each protein has a definite dye binding capacity (DBC) and for the total protein complex of a given commodity, the DBC can be quantitatively related to the total nitrogen content. The dye reacts with the basic amino acids: lysine, histidine and arginine. If we assume that the arginine and histidine contents in a given commodity remain more or less unaltered, the DBC can also be related to the lysine content of the commodity provided that samples having the same protein content are used for the analysis based on the protein value obtained by the micro Kjeldahl method. There have been many modifications of the original Udy method (1956) and various dyes such as orange G, amido black 10B and acid orange 12 have been employed. An important precaution in this method is to have all the samples ground to the same particle size, otherwise some non specific adsorption may give rise to unreliable results.

We used the following simplified procedure for the dye binding method. Two grams of acilan orange G, 15.84 grams of citric acid, 2.98 grams of $Na_2HPO_4 2H_2O$ and 0.3 gram of thymol were dissolved in water and made up to one liter. The absorbance of this solution was measured at 575 nm against water as a blank and the dye solution was diluted so as to give a reading of 0.65 absorption unit for a 10 nm light path in a spectronic 20 colorimeter. The dye was stored in the dark.

Two hundred milligrams of the finely powdered defatted sorghum sample was mixed with 15 ml of the dye solution and the mixture was stirred for four minutes in a mini-Waring blender. The solution was centrifuged and the supernatants were transferred to a cuvette and read at 575 nm against water as a blank. The difference in the absorption unit between the original dye solution and the readings obtained with the sample is an indication of the dye binding capacity of the sample in question. Duplicate analyses were done for all the 26 sorghum samples and the results are given as absorption units for 200 mg samples in Table 5. Statistical analysis between the dye binding capacity and the P x L values obtained from different laboratories and the rat weight gain are given in Table 3.

Table 3. Correlations: Udy Dye Binding Method with micro Kjeldahl N and other determinations.

	Low Tannin	High Tannin
P x L (Biochemistry Lab.)	0.465	0.194
P x L (Shuman Lab.)	0.716**	0.815*
P x L (ABC Lab.)	0.633**	0.639
P x L (Average)	0.582*	0.869**
Weight Gain (Average)	0.603*	0.272
Weight Gain Check (Average)	0.601*	0.275
P x L Dehulled Sample	0.341	-0.197
Biuret Value	0.712**	0.524

** Significant at the 1 percent level.
* Significant at the 5 percent level.

The dye binding capacity results agree significantly at the one percent level with the P x L values obtained from two different laboratories in the low tannin sorghums. There is no significant correlation between the P x L values obtained in the Biochemistry Laboratory and the dye binding capacity. The average P x L value and DBC agree significantly at the five percent level of correlation. There is a significant relation between the weight gain of rats and the DBC, the values being very close to one percent significance level. The results of the biuret method and the DBC agree very well at the one percent level of correlation.

The results of the DBC with the high tannin samples indicate that although the average P x L value is significantly correlated with the DBC at the one percent level, none of the individual values show significant correlation at this level, except the P x L value from the Shuman Laboratory which showed a correlation at the five percent level. Weight gain for rats and the biuret method results do not show any relation with the DBC of the samples.

Conclusion: The average P x L values of the low tannin sorghum samples agree with the DBC results at the five percent significance level while two out of three individual values of P x L from the different laboratories agree with the DBC results at the one percent significance level. The results of weight gain of rats and the dye binding capacity agree close to the one percent level of significance. Very good correlations between the results of the biuret method and the DBC results were observed.

There is no correlation between the weight gain of rats and the DBC values in the high tannin sorghum samples, though the average P x L value is correlated to the DBC value at the one percent significance level. The results of the DBC of the low and high tannin sorghums taken together are significantly correlated at the one percent level to the average P x L results, weight gain of rats and the biuret values.

D. Percentage of Nitrogen in the Saline Extracts of Sorghum.

The results obtained last year on 15 sorghum samples led us to believe that there may be a meaningful relationship between the percentage of nitrogen in the saline soluble fraction of sorghum and the weight gain of rats fed the same sorghum samples for four weeks. Since the average results of rat feeding trials were available for the 26 sorghum samples which were fed twice, independent of each other to six rats for 14 days, we ran correlation studies to find out whether this method can be used as a fast screening procedure for selecting nutritionally good quality sorghum samples.

Two grams of defatted finely ground sorghum samples were successively extracted at $4^{o}C$ for 60, 30 and 15 minutes with 20 ml of 0.5 M NaCl and then twice with 20 ml of water for a duration of 15 minutes each time. The supernatants were pooled together and made up to 100 ml. Nitrogen was determined by the micro Kjeldahl method on the sorghum samples and the extracts. The total nitrogen in the extracts was expressed as the percent of nitrogen solubilized from the samples taken for extraction. Results expressing the percent of saline soluble nitrogen are given in Table 5.

Correlations between the saline soluble nitrogen and the P x L value and rat weight gain are given in Table 4.

Table 4. Correlations: Saline Soluble Nitrogen (Percent) with micro Kjeldahl N and other determinations.

	Low Tannin	High Tannin
P x L (Biochemistry Lab.)	0.457	0.954**
P x L (Shuman Lab.)	0.353	0.042
P x L (ABC Lab.)	0.356	0.385
P x L (Average)	0.418	0.119
Weight Gain (Average)	0.419	0.357
Weight Gain Check (Average)	0.420	0.356
Dye Binding Capacity	0.046	0.264
Biuret Value	-0.221	0.604
P x L Dehulled Sample	0.152	-0.042

** Significant at the 1 percent level.
* Significant at the 5 percent level.

As is evident from the above table, none of the P x L values or the weight gain results of the low and high tannin sorghums show any correlation to the percent of saline soluble nitrogen except that of the P x L value obtained from the Biochemistry Laboratory for the high tannin sorghum. Taken together, except the P x L value from the Biochemistry Laboratory, other P x L values do not show any correlation. However, there is a significant correlation at the one percent level between the saline soluble proteins and the weight gain of rats. No correlation was evident between the saline soluble proteins and the biuret results, dye binding capacity or the P x L value of dehulled sorghum samples when considered either separately as low

and high tannin class.

Conclusion: The percent of the saline soluble proteins in sorghum does not show any correlation with the P x L values or the rat growth data obtained for both the high tannin and low tannin sorghum except with the P x L value obtained in the Biochemistry Laboratory. More data from other samples are needed before any definite conclusion can be drawn about the usefulness of this method as a rapid screening procedure.

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Table 5. Results of Lysine (Whole Grain and Dehulled Grain), Biuret Value, Dye Binding Capacity and the Percent of Saline Soluble Proteins Obtained for 26 Sorghum Samples.*

Sample Identity	Whole Grain			Dehulled Grain			Biuret Value (Absorbance Units)	Dye Binding Capacity (Absorbance Units)	Total Percent of Nitrogen in Saline Soluble Fraction
	Protein Percent	Lysine Percent	Tryptophan Percent	Protein Percent	Lysine Percent	Tryptophan Percent			
RS690**	9.64	1.71	0.59	10.47	1.72	0.33	0.187	0.190	12.48
025440**	9.19	0.38	0.93	9.49	1.95	0.37	0.190	0.188	4.13
025154**	9.27	0.11	0.52	9.17	1.96	0.31	0.195	0.228	5.70
NK300**	9.71	0.26	1.65	10.26	1.90	0.34	0.237	0.207	4.85
025150**	12.03	1.04	0.29	12.05	1.12	0.23	0.258	0.223	11.48
025041**	12.14	1.81	v.high	12.17	1.56	0.27	0.327	0.220	14.57
025275**	10.20	2.16	0.56	10.61	1.84	0.33	0.257	0.226	14.22
029001	12.56	1.27	0.39	12.13	1.40	0.31	0.396	0.215	12.00
NK222G	10.60	1.75	1.63	10.31	1.60	0.32	0.218	0.200	13.02
048290	14.28	1.93	v.high	14.43	2.15	0.25	0.421	0.248	15.77
025280	10.14	1.48	0.78	9.48	1.64	0.30	0.218	0.221	14.13
025274	10.77	1.72	0.45	10.98	1.78	0.30	0.213	0.200	15.71
925006	11.66	2.44	1.29	10.66	2.44	0.45	0.246	0.200	18.16
IS2319	13.02	1.88	0.48	12.77	1.57	0.20	0.371	0.249	15.86
RS610	7.53	2.19	1.24	7.84	2.04	0.16	0.149	0.192	17.45
917025	10.11	2.13	0.99	9.40	2.13	0.19	0.210	0.235	16.58
925018	12.86	1.87	0.62	13.07	1.76	0.37	0.320	0.228	13.51
025042	12.17	2.01	0.52	11.64	1.59	0.27	0.281	0.234	15.23
025134	10.69	2.01	0.42	11.44	1.62	0.24	0.211	0.206	15.13
025126	9.77	2.10	0.42	9.21	2.06	0.23	0.210	0.213	14.31
025014	11.79	1.48	1.08	12.43	1.89	0.22	0.236	0.203	10.42
025260	10.01	1.90	v.high	10.58	2.13	0.24	0.261	0.215	14.82
025158	9.86	1.32	0.93	9.87	1.87	0.20	0.241	0.206	14.56
025289	9.66	2.33	0.56	9.56	2.20	0.21	0.189	0.199	16.06
BR64**	8.75	-	0.55	9.42	2.07	0.32	0.177	0.182	2.75

* Results were not corrected for the moisture content of the samples. ** High tannin lines.

AMINO ACID COMPOSITION OF SORGHUM GRAIN AND RELATIONSHIPS BETWEEN AMINO ACIDS AND PROTEIN.

G. Srinivasan, J. D. Axtell and R. Jambunathan

Data on the amino acid composition of 522 sorghum lines from the world sorghum collection including the high and low tannin varieties were studied to estimate overall averages of each amino acid, correlations between amino acids and correlation of amino acids with protein. There were 323 lines (high protein lines) with 12.0 percent protein and above, and 199 (low protein lines) with below 12.0 percent protein.

Means and standard deviations of protein and amino acid composition of 522 lines are given in Table 1.

Table 1. Protein and Amino Acid Composition of Sorghum Grain*

Composition	Mean	Standard Deviation
Protein	12.61	1.89
Lysine	2.14	0.35
Histidine	2.01	0.20
Arginine	3.59	0.44
Aspartic Acid	7.83	0.77
Threonine	3.26	0.21
Serine	4.52	0.32
Glutamic Acid	23.22	1.99
Proline	8.16	0.89
Glycine	3.07	0.27
Alanine	9.89	0.71
Cystine	0.92	0.22
Valine	5.35	0.31
Methionine	1.03	0.47
Isoleucine	4.08	0.25
Leucine	14.27	1.21
Tyrosine	4.50	0.32
Phenylalanine	5.19	0.37
Tryptophan**	1.31	0.14

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* Weighted average for 522 lines.

The average protein content was 12.6 percent and lysine was 2.1 percent of protein. There was no significant difference in the average amino acid content between low and high protein groups.

Correlations among amino acids and between amino acids and protein are summarized in Tables 2 and 3. Lysine, histidine, arginine, threonine, serine, cystine and glycine were negatively (significantly) correlated with protein. This indicates that increasing protein content might change amino acid patterns and biological value of sorghum. The concentration of lysine increased as the concentration of histidine, arginine, aspartic acid, threonine, serine, glycine and valine increased, and as concentration of glutamic acid, proline, cystine, alanine, leucine, tyrosine and phenylalanine decreased.

A comparative study was made on correlations among amino acids between the low and high protein group. In the low protein group, protein was positively correlated with threonine and valine. Protein content increased as isoleucine and leucine concentration increased in the high protein group. A negative correlation was found between lysine and glutamic acid, alanine, tyrosine and phenylalanine in the high protein group, histidine was positively correlated with leucine in the higher protein group.

Table 2. Overall Correlations Among Amino Acids and With Protein.

	Lysine	Hist.	Arg.	Asp.	Threo.	Serine	Glut.	Prol.	Cyst.	Glyc.	Ala.	Valine	Methio.	Isol.	Leu.	Tyro.	Phenyl.
Protein	-.252**	-.128**	-.233**	.020	-.217**	-.147**	.167**	.050	-.164**	-.413**	.137**	-.069	.028	.071	.151**	.066	.094*
Lysine		.134**	.656**	.618**	.469**	.154**	-.285**	-.261**	-.123**	.693**	-.328**	.222**	.063	.002	-.380**	-.176**	-.274**
Hist.			.263**	-.048	.371**	.283**	.202**	.187**	.354**	.331**	.133**	.316**	.089*	.236**	.133**	.292**	.290**
Arg.				.355**	.514**	.281**	-.028	-.143**	.149**	.604**	-.079	.313**	.106*	.113*	-.084	.113*	-.058
Asp.					.531**	.278**	.154**	-.216**	-.124**	.412**	.102*	.411**	-.199**	.203**	.058	.159**	.008
Threo.						.708**	.421**	.076	.228**	.714**	.378**	.600**	.060	.468**	.394**	.573**	.379**
Serine							.380**	.144**	.203**	.434**	.400**	.387**	.075	.409**	.409**	.520**	.416**
Glut.								.209**	.126**	-.002	.878**	.576**	.002	.579**	.875**	.774**	.686**
Prol.									.091*	-.032	.193**	.130**	.095*	.233**	.240**	.264**	.309**
Cyst.										.179**	.096*	.145**	.329**	.152**	.153**	.263**	.226**
Glyc.											-.010	.379**	.042	.165**	-.037	.114*	-.026
Ala.												.575**	-.029	.594**	.932**	.771**	.714**
Valine													.063	.602**	.584**	.594**	.441**
Methio.														.095*	-.023	.066	.065
Isol.															.664**	.640**	.681**
Leu.																.826**	.751**
Tyro.																	.810**

* Significant at .05 level.

** Significant at .01 level.

Table 3. Correlations Among Amino Acids and with Protein in High and Low Protein Groups.

	Lysine	Hist.	Arg.	Asp.	Threo.	Serine	Glut.	Prol.	Cyst.	Glyc.	Ala.	Valine	Methio.	Isol.	Leu.	Tyro.	Phenyl.
Protein 1	-.166**	-.019	-.161**	-.005	-.109	-.110	.093	.017	-.046	-.215**	.103	.005	.070	.199*	.125*	.054	.033
Protein 2	-.250**	-.138	-.224**	-.031	-.239**	-.098	.076	.190**	-.203**	-.401**	-.014	-.208**	.011	-.006	-.013	-.002	.054
Lysine		.021	.630**	.654**	.373**	.054	-.350**	-.270**	-.240**	.674**	-.401**	.160**	.067	-.085	-.443**	-.264**	-.353**
		.268**	.678**	.594**	.599**	.294**	-.074	-.249**	-.037	.704**	-.112	.343**	.072	.191**	-.188**	.037	-.083
Hist.			.178**	-.055	.280**	.221**	.262**	.169*	.394**	.228**	.163**	.307**	.139*	.275**	.186**	.322**	.361**
			.355**	-.029	.472**	.352**	.163*	.228**	.294**	.429**	.131	.333**	.060	.190**	.050	.274**	.221**
Arg.				.377**	.476**	.262**	-.039	-.086	.113*	.610**	-.055	.304**	.122*	.121*	-.073	.098	-.051
				.346**	.540**	.282**	.066	-.250**	.157*	.564**	-.012	.322**	.107	.112	-.055	.176*	-.032
Asp.					.536**	.234**	.094	-.293**	-.153**	.434**	.038	.359**	-.251**	.131*	-.009	.093	-.061
					.566**	.376**	.284**	-.058	-.082	.480**	.242**	.534**	-.178*	.352**	.212**	.300**	.136
Threo.						.685**	.460**	.067	.284**	.645**	.385**	.555**	.039	.450**	.417**	.570**	.401**
						.734**	.460**	.102	.128	.804**	.459**	.697**	.087	.534**	.453**	.641**	.406**
Serine							.412**	.161*	.258**	.346**	.412**	.356**	.065	.367**	.428**	.513**	.412**
							.388**	.118	.162**	.554**	.438**	.444**	.092	.502**	.440**	.566**	.465**
Glut.								.179**	.248**	-.024	.878	.580**	-.047	.585**	.875**	.781**	.705**
								.282**	.009	.198**	.872**	.613**	.056	.591**	.868**	.764**	.638**
Prol.									.132*	.002	.169**	.112	.156**	.202**	.224**	.244**	.248**
									.044	-.087	.248**	.175*	.051	.299**	.285**	.310**	.434**
Cyst.										.121*	.190**	.164**	.162**	.246**	.262**	.366**	.356**
										.174*	.055	.115	.449**	.042	.045	.156*	.100
Glyc.											-.065	.314**	.070	.115*	-.083	-.056	-.047
											.226**	.526**	.030	.302**	.187**	.301**	.092
Ala.												.570**	-.142**	.600**	.936**	.773**	.731**
												.624**	.088	.588**	.919**	.766**	.672**
Valine													.081	.622**	.591**	.580**	.449**
													.056	.565**	.607**	.642**	.444**
Methio.														.092	-.105	.043	-.027
														.111	.064	.101	.161*
Isol.															.682**	.621**	.650**
															.637**	.683**	.747**
Leu.																.825**	.769**
																.836**	.709**
Tyro.																	.836**
																	.734**

* Significant at the .05 level.
** Significant at the .01 level.
1 = correlations of high protein group.
2 = correlations of low protein group.

INFLUENCE OF SUPPLEMENTAL NITROGEN ON THE RELATIONSHIP BETWEEN TANNIN CONTENT AND *IN VITRO* DRY MATTER AND NITROGEN DISAPPEARANCE IN *SORGHUM BICOLOR* (L.) MOENCH GRAIN.

R. E. Schaffert, D. L. Oswalt and V. L. Lechtenberg

Tannin in sorghum grain has been shown to adversely effect grain utilization by ruminant and monogastric animals. However, the tannin does have a production advantage with regard to bird-resistance, inhibition of pre-harvest seed germination, and weathering resistance. *In vitro* dry matter disappearance (IVDMD) techniques were employed in this investigation to determine their potential use as a screening device for improved nutritive value by plant breeders. An *in vitro* system would be less expensive, much faster, and require a smaller sample than commonly used in *in vivo* screening techniques. The *in vitro* system was also used to gain insight as to whether reduced feeding value of high tannin sorghum was due to inhibition of bacterial growth or due to the reduction in digestibility of the plant protein presumably caused by the formation of a biologically unavailable tannin-protein complex.

Review of Literature

Tannins have long been recognized as a general protein denaturant. Strumeyer and Malin (1969) demonstrated that an ethanol extract from sorghum grain contained oligomeric condensed tannins of the leucocyanidin group with various degrees of polymerization. This tannin extract inhibited the activity of alpha amylase, esterase, and carboxylase enzymes.

Currently, tannin in grain sorghum is generally considered to have an adverse effect on digestibility by both monogastric and ruminant animals, even though some controversy concerning this matter exists in the literature. Handler and Baker (1944) and Glick and Joslyn (1970a) showed that the addition of tannic acid at 1.0 to 8.0% of the ration reduced the weight gains of rats. Ringrose and Morgan (1940) demonstrated a marked growth repression in chicks when tannic acid was fed as 2% of the ration.

Chang and Fuller (1964) fed sorghum as 50% of the diet to chicks and found that varieties with high levels (2.0%) of tannin resulted in growth retardation similar in magnitude to that caused by feeding equivalent levels of tannic acid. Fuller, Potter, and Brown (1966) concluded that when the tannin in grain sorghum exceeded 0.8% of the total diet, chick growth was retarded. Connor et al. (1969) observed that increases in tannin from sorghum grain depressed feed intake and reduced feed efficiency when the grain was fed to growing chicks. Rostango (1972), feeding diets with suboptimal protein quantity to growing chicks, demonstrated that bird-resistant (high tannin) sorghums

resulted in poorer weight gains and feed conversions than normal sorghums (low tannin). In the chick feeding investigations mentioned above, corn rations were superior to both the low tannin and high tannin sorghum rations with respect to weight gain and feed conversion. In contrast, Stephenson, York, and Bragg (1967, 1968) showed no differences in chick growth between high and low tannin sorghum varieties when sorghum comprised not more than 60% of the ration. Damron, Prine, and Harms (1968) found no differences between corn, high tannin sorghum, and a low tannin sorghum diets when fed to growing chicks. In these comparisons one-half of the corn in the ration was replaced with sorghum. They also noted that feed intake for the high tannin sorghum diet was greater than for the low tannin sorghum and corn diets although the feed efficiency was less. Halloran and Maunder (1971) reported no differences in acceptability by chicks between normal sorghum, brown sorghum (high tannin), and corn, but poorer feed conversion was observed for the brown sorghum.

Chang and Fuller (1964) also noted a slight depression in protein digestibility of rations containing high tannin sorghum. Rostango (1972) found that the amino acid availability of the sorghum hybrid RS610 (low tannin) was decreased when tannic acid was added to the ration of growing chicks. He also found that high tannin sorghum varieties had approximately 66% lower "availability" of amino acids than low tannin sorghum varieties. Weight gain and feed efficiency of high and low tannin sorghum diets significantly improved when the essential amino acids were supplemented to the levels found in corn, but supplementation did not completely overcome the effect of the tannin.

Nawar et al. (1970) showed significant variations in digestibility of dry matter and protein between varieties of sorghum grain fed to rats. Oswalt (personal communication, 1972) determined that most of the varieties, reported by Nawar et al. (1970), with low dry matter digestibility (DMD) and low protein digestibility also had high tannin content. DMD ranged from 48.9 to 76.8% compared to 88.1% for rations containing 20% casein. A two-fold difference was observed in available protein. Oswalt (1972) determined that the low levels of available protein were associated with high levels of tannin in the grain. Glick and Joslyn (1970b) found that rats fed diets containing various plant tannins were found to excrete more fecal nitrogen than controls. They also concluded that proteins from enzymatic or other endogenous origins rather than dietary casein appeared to constitute a major portion of the excreted nitrogen compounds.

McGinty (1969) found differences in digestibility of sorghum varieties when fed to steers. High tannin varieties had lower dry matter and protein digestibilities than low tannin varieties. These differences were attributed to the tannin found in the pericarp and testa of the sorghum kernel. IVDMD and *in vivo* DMD were highly correlated and of similar magnitudes for eight sorghum varieties. IVDMD ranged from 30 to 67% and *in vivo* DMD ranged from 50 to 71%. Stallcup and Davis (1962) conducted digestion trials for a high tannin hybrid, ARK 614, with steers. Both DMD and protein digestibility were lower than those associated with low tannin sorghums.

Driedger and Hatfield (1972) found that lambs fed soybean meal treated with 10% Tara tannin had greater daily weight gains, feed

efficiencies and nitrogen balances than lambs fed soybean meal without tannin. The improved performance was attributed to an alteration of the amino acid pattern of the soybean meal reaching the absorptive site. The tannin prevented degradation of the protein by the rumen microorganisms in the first stages of digestion.

The objectives of this experiment were to determine the potential of the IVDMD technique to evaluate the biological value of sorghum grain and to determine if high tannin sorghum grain has a poorer digestibility than low tannin sorghum due to toxic effects or due to the formation of a non-digestible tannin-protein complex.

Materials and Methods

Samples. The samples of sorghum grain used in this investigation were grown during 1970 at the Purdue University Agronomy Farm, Lafayette, Indiana. The grain was dried in the panicle at a temperature between 43 and 60° C to about 10 to 12% moisture. The grain was threshed and ground in a Wareing blender.

Tannin Determination. Tannin content was estimated by the Vanillin-hydrochloric acid method described by Burns (1963, 1971). Tannin estimates were expressed as catechin equivalents per 100 g dry grain weight and should not be confused with percent tannin in the grain.

In Vitro Dry Matter Disappearance. IVDMD was determined by modifying the two-stage technique for in vitro digestion of forage crops proposed by Tilley and Terry (1963). The standard procedure was to add 20 ml of phosphate carbonate buffer (pH 6.8-7.0) and 5 ml of strained rumen fluid to 250 mg of dry, uniformly ground grain in a 100 ml anaerobic

digestion tube. The tube was incubated at 39° C for 48 hours. The tubes were swirled by hand three or four times during the interval to insure that the bacteria acted upon all the material. At the end of 48 hours, 2 ml of 6 N HCl and 0.1 g of pepsin were added and incubated aerobically at 39° C for an additional 24 hours. At the end of the second incubation the material was filtered through Whatman No. 5 filter paper, dried to constant weight at 100° C and weighed. Percent IVDMD was determined by the following formula:

$$\%IVDMD = 100 - 100[(Residue - Rumen\ fluid\ blank)/Sample\ dry\ weight]$$

The standard procedure for determining IVDMD was further modified by; (1) adding 15 mg of urea (for a 250 mg sample) with the buffer and rumen fluid and, (2) by a second incubation period with fresh buffer and rumen fluid. This was accomplished by centrifuging the tubes after 48 hours, discarding the supernatant and resuspending the residue in buffer solution and fresh rumen fluid (and urea if included in the first incubation). The samples were incubated anaerobically at 39° C for an additional 48 hours before the addition of pepsin.

In Vitro Nitrogen Loss (IVNL). IVNL was determined by measuring the amount of nitrogen in the dry grain and the amount of nitrogen in the residue after incubation by the Kjeldahl procedure (AOAC, 1960). Percent IVNL was calculated in a manner analogous to percent IVDMD. Protein is reported as 6.25 times the percent nitrogen.

Experimental Procedure. One experiment was conducted on two separate sets of material to study the influence of added nitrogen in the form of urea on the relationship between tannin content and IVDMD. The first

set of material consisted of 24 commercial and experimental lines and hybrids. The second set of material consisted of 10 India Sorghum (IS) lines selected from the World Collection of Sorghum (IS0062, IS0075, IS0184, IS0418, IS0616, IS2288, IS5437, IS7125, IS8165 and IS8361), Redlan B, and the 10 hybrids between Redlan A and each IS line. Duplicate determinations were made for tannin, expressed as catechin equivalents per 100 g dry grain, IVDMD without urea, and IVDMD with urea. Simple regressions and correlations for the mean values of tannin and for IVDMD were determined.

The second experiment consisted of determining IVNL with and without urea for two low tannin lines (IS0062 and IS0418), two high tannin lines (IS0616 and IS8165), and their respective Redlan hybrids. Each determination was completed in duplicate. The data were analyzed considering the eight genotypes as a fixed variable, the two levels of urea (0, 15 mg) as a fixed variable and the duplicates as an estimate of experimental error. The genotype source of variation was partitioned into entries (four IS lines) with three degrees of freedom, treatments (four lines verses four hybrids) with one degree of freedom and entry by treatment interaction with three degrees of freedom. Differences between means were determined by using the Newman-Keuls' test (Steel and Torrie, 1960).

A third experiment consisted of comparing the effect of two separate anaerobic incubations with only one anaerobic incubation. Two levels of urea (0, 15 mg) were used. The same genotypes used in experiment two were used in this experiment. The statistical analysis was the same as for experiment two except that IVDMD was determined in

more than one laboratory run (different days) and runs were considered a random variable.

A fourth experiment consisted of determining IVNL after two anaerobic incubations for one low tannin line (IS0062) and one high tannin line (IS8165). Each entry was replicated four times. A four gram sample was used in a 250 ml flask. After the final incubation, the samples were centrifuged and dried rather than filtered.

Results and Discussion

In the first experiment tannin values ranged from 0.4 to 13.7 catechin equivalents. IVDMD for the first set of material ranged from 35 to 80% without urea and 78 to 96% with urea. In the second set of material tannin values ranged from 0.2 to 7.5 catechin equivalents. IVDMD ranged from 43 to 81% without urea and 78 to 95% with urea. The regression equations with IVDMD as the dependent variable (Y) and tannin as the independent variable (X) with their squared correlation coefficients are presented in Figure 2.1. The differences in maximum IVDMD with and without urea indicates that there is an insufficient amount of nitrogen present in the grain for adequate bacterial growth necessary for maximum dry matter disappearance. Schmid, Marten and Roth (1969) observed similar effects when urea was added to corn grain. The differences in the slope of the lines with and without urea suggests that tannin is not inhibiting microbial breakdown of the sorghum grain directly but rather indirectly by reducing the amount of nitrogen available for bacterial growth and rapid substrate digestion. If tannin inhibited bacterial action appreciably, then no increase in IVDMD would be expected by adding urea.

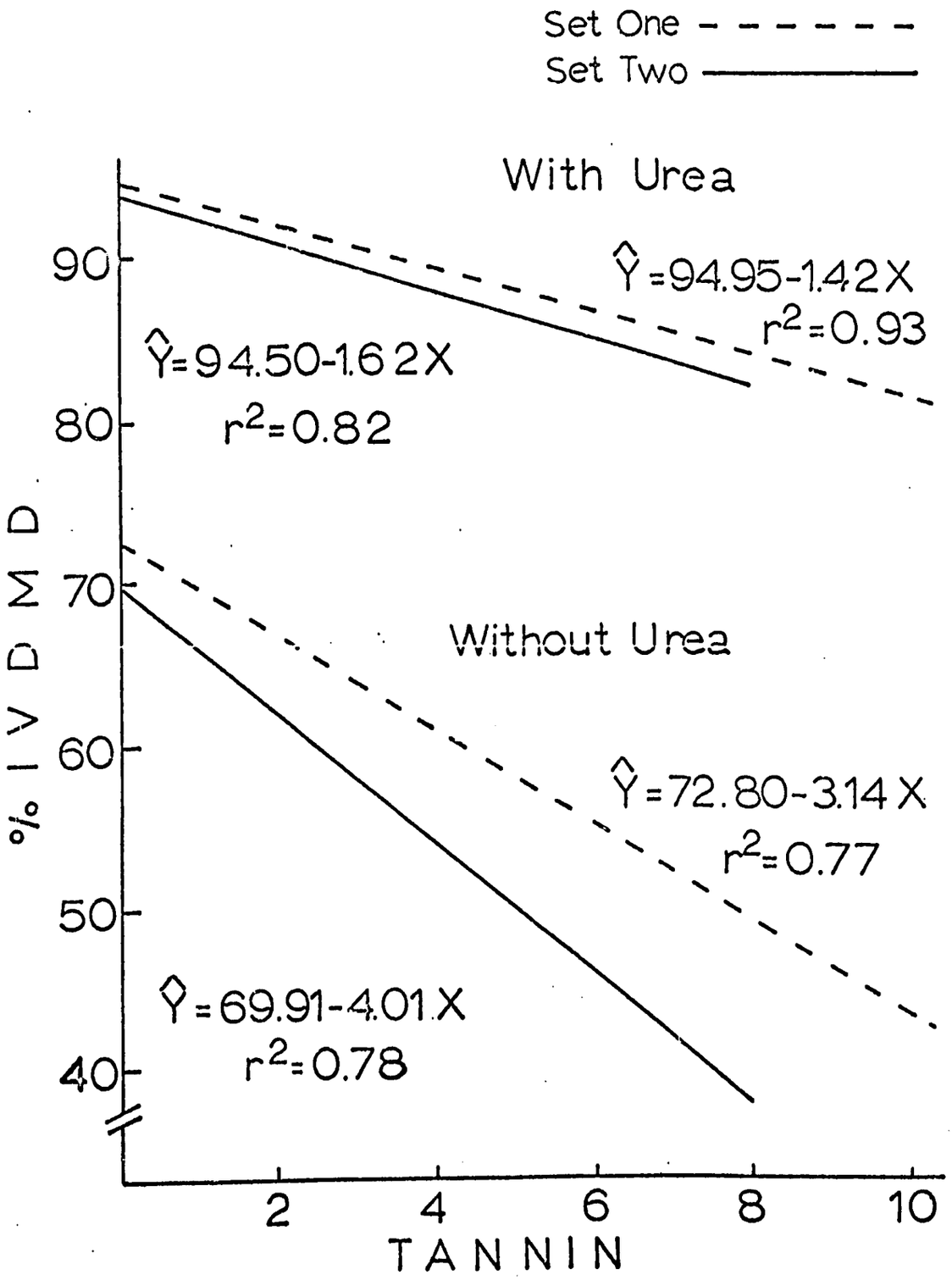

Figure 2.1. Effect of supplemental nitrogen on the relationship between tannin and IVDMD.

The relationships between tannin in the grain, IVNL, and IVDMD are shown in Tables 2.1 and 2.2. In all cases high tannin content was associated with low IVDMD and IVNL. The difference between lines and hybrids is partially due to the increase in tannin for the two high tannin entries, but the difference in IVDMD and IVNL between IS0062 and Redlan × IS0062 cannot be attributed to differences in tannin only. The differences may have been due to polyphenolyic compounds other than those detected by the Vanillin-HCl method, possibly other pigments in the pericarp. IS0062 has a colorless pericarp whereas its hybrid has a red pigmented pericarp inherited from the Redlan parent, also a low tannin line. IS0418 has a red pigment in the pericarp and is not different from its hybrid in this respect.

The reduction in IVNL due to increased levels of tannin partially explains the differences in IVDMD. Assuming the nitrogen in the grain to be in the form of protein and the differences in IVNL to be caused by differences in protein digestibility, part of the difference in IVDMD and possibly <u>in vivo</u> dry matter digestibility is actually due to differences in protein digestibility. Chang and Fuller (1964), McGinty (1968) and Nawar et al. (1970) reported lower protein digestibilities in high tannin sorghum than in low tannin sorghum for chicks, cattle and rats, respectively.

The effects of one versus two anaerobic incubations are presented in Tables 2.3 and 2.4. Urea appears to have no effect with two incubations and two incubations without urea gave IVDMD values equivalent to one incubation urea. The second addition of rumen fluid probably supplied sufficient nitrogen for bacterial growth and breakdown of

Table 2.1. Analysis of variance for eight grain sorghum genotypes with and without the addition of urea for IVDMD and IVNL.

Source of Variation	df		Mean Squares	
			% IVDMD	% IVNL
Genotypes	7		760.7**	461.5**
Entries		3	1616.7**	863.9**
Treatments+		1	287.4**	403.3**
Entries × Treatments		3	63.5**	78.6**
Level of Urea	1		4982.5**	259.9**
Genotype × Urea	7		66.8**	49.3**
Error	16		3.2	6.14

* Significant at 5% level of probability.

** Significant at 1% level of probability.

\+ Lines versus hybrids.

Table 2.2. List of means of eight genotypes for tannin (catechin equivalents), percent protein, percent IVDMD, percent IVNL, and percent non-digestible protein.

Genotype	Tannin (Catechin Equivalents)	Protein	Percent IVDMD*	Percent IVNL*	Percent Non-Digestible Protein
IS0062	0.29	13.78	89.1a	91.8a	1.13
IS0418	0.27	12.11	82.1b	86.5b	1.60
IS8165	4.28	11.26	73.1c	75.1c	2.80
IS0616	5.93	14.20	57.4e	77.7c	4.78
Means of Lines (Treatment 1)	2.69	12.83	75.4	82.8	2.58
Redlan × IS0062	0.30	13.12	82.0b	87.8b	1.60
Redlan × IS0418	0.22	13.12	82.7b	86.5b	1.66
Redlan × IS8165	6.34	10.30	60.2d	64.6d	3.65
Redlan × IS0616	7.46	13.81	52.8f	63.8d	5.00
Mean of Hybrids (Treatment 2)	3.58	12.58	6.94	75.7	2.98
With Urea			84.8	82.1	
Without Urea			59.9	76.4	

* Any two genotype means within a column not followed by the same letter differ at the 5% level of significance according to Newman-Keuls' test (Steel and Torrie, 1960).

Table 2.3. Analysis of variance for the four IVDMD experiments.

Source of Variation	One *in vitro* Incubation				Two *in vitro* Incubations			
	Without Urea		With Urea		Without Urea		With Urea	
	df	Mean Square	df	Mean Square	df	Mean Square	df	Mean Square
Genotypes	7	2246.0**	7	567.5**	7	85.6**	7	84.7**
Entries	3	4805.8**	3	1253.5**	3	197.1**	3	181.3**
Treatments+	1	823.0**	1	159.7**	1	2.0	3	0.1
Entries × Treatments	3	160.6**	3	17.5	3	4.3	1	16.5**
Runs	4	47.0*	3	51.8**	1	282.6**	1	237.1**
Genotypes × Runs	28	29.6*	21	18.0**	7	2.1‡	7	1.5‡
Error	40	16.4	32	4.9	16	1.9	16	1.9

* Significant at 5% level.

** Significant at 1% level.

\+ Hybrids versus lines.

‡ Genotypes × runs pooled with within runs for estimate of error term.

Table 2.4. Table of genotype means* and standard errors for the four IVDMD experiments.

Genotype	Tannin Content	Percent IVDMD			
		1 Incubation		2 Incubations	
		Without Urea	With Urea	Without Urea	With Urea
IS0062	0.29	80.7a	95.4a	94.0a	95.6a
IS0418	0.27	68.6b	92.8a	93.0a	93.2a
IS8165	4.28	55.4c	85.6b	88.1b	89.4b
IS0616	5.93	46.0d	77.7c	83.3c	82.7d
Mean of Lines (Treatment 1)	2.69	62.7	87.9	89.6	90.2
Redlan × IS0062	0.30	71.5b	92.9a	93.2a	93.7a
Redlan × IS0418	0.22	69.3b	92.3a	93.8a	94.0a
Redlan × IS8165	6.34	43.0d	81.2bc	85.7c	86.9c
Redlan × IS0616	7.46	41.3d	72.5d	83.9c	86.2c
Mean of hybrids (Treatment 2)	3.58	56.3	84.7	89.1	90.2
Grand Mean		54.5	86.3	89.4	90.2
Standard Deviation		5.44	4.24	1.40	1.34
SE Genotype Mean		1.72	1.50	0.70	0.67
SE Treatment Mean		0.86	0.75	0.35	0.34

* Any two genotype 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).

dry matter. These results suggest that tannin does not inhibit the microbial breakdown of the grain directly but rather indirectly by reducing the amount of nitrogen available for bacterial growth. The difference in IVDMD with added urea, two incubations, or added urea and two incubations between the high and low tannin grain was only seven to eleven percentage units and indicates that the true digestibility between high and low tannin grain is small and affects digestibility by reducing nitrogen availability. It is also possible that tannin directly inhibits bacterial action and the first incubation removed the tannin allowing the bacteria added in the second incubation to operate normally.

The IVNL after two anaerobic incubations was 68.8% for IS0062 and 54.8% for IS8165. These IVNL values are lower than for one incubation but the magnitude of the differences are nearly equal. The lower IVNL values may have resulted because of the use of centrifugation instead of filtering to separate the soluble and insoluble fraction. This indicates there definitely was an insoluble and presumably an undigestible protein fraction in the high tannin sorghum. This fraction represented 2.8 to 5 percent of the dry weight.

Estimates from various studies have indicated that the range of percent tannin in high tannin sorghum grain is between 2.0 and 2.5%, (Chang and Fuller, 1964). Assuming tannin to be insoluble and non-digestible and to be complexed with the insoluble protein fraction, nearly half the 7.4% difference in IVDMD between IS8165 and IS0062 or 11.8% between IS0616 and IS0418 (Table 2.4 average of last three columns) can be accounted for by differences in tannin and non-digestible protein.

This is a reasonable assumption because the tannin (polymeric polyphenol) and protein (polypeptide) are most likely bound together to form an unavailable complex.

Conclusions

The results of these investigations indicated that the *in vitro* technique may be successfully used in a screening and plant breeding program to detect differences in biological value of sorghum grain. These findings also showed that a large portion of the difference in dry matter disappearance of high and low tannin sorghum grain was due to lower protein solubility in the high tannin grain. Significant differences within the low tannin sorghum varieties indicated that some factor or factors other than tannin estimated by the Vanillin-HCl method was causing differences in nutritive value.

The IVDMD technique did detect significant differences within high tannin sorghum varieties, within low tannin sorghum varieties, and between high and low tannin varieties. The IVDMD differences between high and low tannin varieties positively correlate with monogastric and ruminant *in vivo* results published in the literature. This alone makes the IVDMD technique an important tool for selection in a plant breeding program, however, more investigations are needed to correlate *in vitro* results within the low tannin varieties or within the high tannin varieties to *in vivo* results. When the IVDMD technique is used to estimate the nutritive value of a grain, urea should be added to the system since it reduced the experimental error mean square nearly 75%.

The findings from this investigation indicate that differences in *in vivo* digestibility frequently reported between high and low tannin sorghum may be accounted for in part by the non-digestible tannin and the non-digestible protein fraction. Approximately 50% of the difference in IVDMD between high tannin and low tannin sorghum grain was accounted for by differences in tannin content and insoluble protein. This alone could account for the reduction in feed efficiency frequently reported in feeding trials. This might also explain some of the controversy with regard to the feeding value of rations containing high and low levels of tannin. High tannin rations containing adequate protein, that is, enough to form a complex with the tannin and enough to meet the requirements of the animal, could produce animal gains equal to low tannin ration, but rations with the high tannin would be expected to have a lower feed efficiency for the reason mentioned above. A better understanding of the nature of the chemistry between tannin and non-digestible protein would help explain this phenomenon. The unaccountable difference in IVDMD could in fact be due to some toxic effect of the tannin.

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COMPARATIVE STUDIES OF THE UTILIZATION OF BIRD RESISTANT AND NON-RESISTANT SORGHUM GRAIN BY CHICKS.

W. D. Armstrong, J. C. Rogler and W. R. Featherston

Studies were conducted on the performance of chicks fed diets containing the bird resistant sorghums BR64 and IS8260 as compared with chicks fed diets containing the non-resistant sorghums RS610 and RS671 and to observe the effects on chick performance and the incidence of leg anomalies of various dietary supplements. Feeding bird resistant sorghum grains BR64 and IS8260 resulted in significantly ($P < 0.05$) poorer growth and feed efficiency as compared with that noted when non-resistant sorghum grains RS610 and RS671 were fed. Supplementation of bird resistant sorghums BR64 and IS8260 with methionine resulted in a marked improvement in chick growth and feed efficiency to levels comparable to those noted with the non-resistant sorghums. Supplementation with choline on an equal methyl group basis failed to show the response observed with methionine. Methionine supplementation did not overcome the growth depressing effects noted when 1.0% commercial tannic acid was added to the sorghum RS617 diet. Additions of 1.0% polyvinylpyrrolidone, which binds vegetable tannins and is not absorbed, overcame the depressing effects of commercial tannic acid additions to sorghum RS617 diets as well as the tannin provided by the bird resistant sorghum BR64. The results of one experiment which demonstrates the response of chicks to these various dietary treatments are shown in Table 1.

Table 1. Effect of Tannic Acid, Methionine and Polyvinylpyrrolidone Supplementation of Sorghum Grains on Weight Gain and Feed Conversion of Chicks.

Treatment	Weight gain (g)	Feed/gain ratio (g)
1. Sorghum RS671	234^{bc}	1.94^{ce}
2. Trt 1 + 1% tannic acid	125^{d}	2.46^{a}
3. Trt 1 + 0.15% methionine	296^{a}	1.75^{f}
4. Trt 1 + 1% tannic acid + 0.15% methionine	151^{d}	2.24^{b}
5. Trt 1 + 1% polyvinyl-pyrrolidone	242^{bc}	1.88^{e}
6. Trt 1 + 1% tannic acid + 1% polyvinylpyrrolidone	207^{c}	2.01^{cde}
7. Sorghum BR64	154^{d}	2.51^{a}
8. Trt 7 + 1% polyvinyl-pyrrolidone	248^{b}	2.04^{cd}
9. Trt 7 + 0.15% methionine	277^{a}	2.06^{cd}

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

An increased incidence and severity of a leg abnormality was noted as a result of methionine supplementation of the bird-resistant sorghums BR64 and IS8260. A similar increased incidence and severity of the leg abnormality was not observed when the non-resistant sorghum diets were supplemented with methionine. Dietary supplementations of lysine, carnitine or a mineral mix containing calcium, manganese and zinc failed to alleviate the problem.

It is assumed that the higher tannin content of the bird resistant sorghums are related in some way to the poorer performance observed in chicks fed bird resistant sorghum diets as compared with chicks fed non-resistant sorghum diets. Since these tannins are located in the pericarp and testa layer of the seed, studies have been conducted on the effect of pericarp removal on the performance of chicks fed bird resistant and non-resistant sorghum grain diets. As can be seen from the data presented in Table 2, removal of the pericarp resulted in a significant ($P < 0.05$) improvement in weight gain of chicks fed the bird resistant sorghum IS8260 whereas no improvement in weight gain was noted when the pericarp was removed from the non-resistant sorghum RS671. Feed conversion was improved by the pericarp removal from both grains; however, the improvement was much greater with the bird resistant sorghum IS8260. The improvement in feed conversion which resulted from the removal of the pericarp from the non-resistant sorghum RS671 can be explained by the lower fiber content of the grain with the pericarp removed. These results suggest that the protein quality of the two grains is not greatly different once the detrimental effects of the tannins have been removed.

Table 2. Effect of Pericarp Removal from Sorghum Grains on Weight Gain and Feed Conversion of Chicks.

Treatment	Weight gain (g)	Feed/gain ratio (g)
Sorghum RS671	232^a	2.03^b
Sorghum RS671 (dehulled)	218^a	1.85^c
Sorghum IS8260	128^c	2.69^a
Sorghum IS8260 (dehulled)	204^a	1.85^c

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

EFFECT OF SUPPLEMENTAL PROTEIN ON THE NUTRITIVE VALUE OF HIGH AND LOW TANNIN *SORGHUM BICOLOR* (L.) MOENCH GRAIN FOR THE GROWING RAT

R. E. Schaffert, D. L. Oswalt and J. D. Axtell

Tannins from many sources have been recognized for a long period of time to adversely influence the biological value of a diet. More recently it has been shown that the lower nutritive value is associated, at least in part, with lower protein digestibility.

Chang and Fuller (1964) showed that sorghum with a high tannin content caused growth retardation in chickens similar in magnitude to that caused by equivalent levels of tannic acid. They also demonstrated that the growth retardation occurred in direct proportion to the level of tannin in the diet. A slight reduction in protein digestibility was also observed in diets containing high levels of tannin. Vohra, Kratzer and Joslyn (1966) concluded that tannin in the diet reduced feed intake and nitrogen retention in growing chicks. Connor et al. (1969) observed depressed feed intake and reduced feed efficiency when high tannin sorghum grain was fed to chicks.

Glick and Joslyn (1970a) demonstrated that the depression in growth rate of rats fed diets containing tannin was due to other factors in addition to reduced feed intake. Glick and Joslyn (1970b) concluded that endogenous protein rather than dietary protein was the major

source of increased fecal nitrogen of rats fed tannins. Tamir and Alumot (1970) explained that increased fecal nitrogen, from growing rats fed diets that included tannin from carobs, was due to nonspecific binding of protein with tannins.

Tamir and Alumot (1969) showed that digestive enzymes were denatured due to a nonspecific protein-tannin binding and that the enzyme activity could be restored in some cases with the addition of polyvinyl pyrrolidone which replaced the protein in the binding with tannin. Schaffert (1972, section II of this thesis) showed that the reduced dry matter disappearance from in vitro incubation of high tannin sorghum grain, when compared to that from low tannin sorghum grain, was partially due to decreased nitrogen utilization associated with a non-digestible protein-tannin complex.

McGinty (1968) observed reduced dry matter digestibility (DMD) when high tannin sorghum was fed to steers compared to DMD of low tannin sorghum. The protein digestibilities of the high tannin sorghum were approximately half those of low tannin sorghum grain. He also showed that in vitro dry matter disappearance and in vitro gas production were highly correlated with in vivo digestibility in steers.

The objectives of this experiment were: (1) to determine if protein supplementation should be used in evaluating the biological value of sorghum grain, (2) to determine if protein supplementation would give similar results for sorghum with high and low tannin, and (3) to determine if protein supplementation would give similar results in sorghum samples with different levels of protein.

Materials and Methods

The solutions to these objectives were sought by feeding four levels of protein supplementation, with low and high tannin sorghum grain each with a low and high level of protein in the grain to weanling rats.

Rat feeding. Male weanling rats[1] ranging in weight from 45 to 65 g and averaging 56 g were divided into groups of six and placed into individual cages. The experimental diets were fed to each group of rats for 13 days. Feed consumption was determined for each group of rats at the end of the feeding trial. Feed spilled by the rat was returned to the feeder each day. The weight gain of each rat was determined, but the average weight gain per six rats was used in the analysis. Protein efficiency ratios (PER, grams of gain per gram of protein consumed) and feed efficiency ratios (FER, grams of feed consumed per gram of gain) were calculated.

Diets. Each 100 g of the basal diet consisted of 79 g of grain sorghum, 4 g of mineral mix[2], 2 g of vitamin mix[3], and 15 g of corn starch. The starch was replaced with 5, 10, and 15 g of soybean meal (53.25% protein) to give four levels (0, 5, 10, and 15 g) of protein supplementation. Four different grain sorghum genotypes were used; IS2549 (low tannin, low protein), IS0062 (low tannin, high protein), IS6992 (high tannin, low protein) and IS1210 (high tannin, high protein).

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[1] Rat feeding trials were conducted by Shuman Chemical Laboratory, Battle Ground, Indiana.

[2] Hawk-Oser salt mixture number three, Nutritional Biochemicals, Inc., Cleveland, Ohio.

[3] Vitamin supplement, General Biochemicals, Chagrin Falls, Ohio.

The crude protein levels of the sorghum grain, soybean meal and mixed rations were determined by multiplying 6.25 times the percent nitrogen determined by the micro-Kjeldahl technique. The amino acid content of the grain samples and the soybean meal were determined with an automatic Beckman Model-120C ion-exchange resin chromatography amino acid analyzer. The protein and essential amino acids required for rat growth taken from Mertz (1969) and those provided in each diet are presented in Table 3.1.

Experimental Design. Due to a limitation in the number of rat feeding units only eight of the sixteen treatment combinations could be fed at one time. Each eight treatments fed at the same time constituted a laboratory run. In order to minimize the loss of information, this experiment was considered as a 2^4 pseudofactorial and runs were confounded with the third order interaction of the pseudofactorial, actually the second order interaction of the $2 \times 2 \times 4$ factorial. The factors considered were two grain protein levels, two grain tannin levels, and four soybean meal protein levels which were considered as two pseudofactors (soybean meal at zero and five percent of the ration, and soybean meal as zero and ten percent of the ration to give the four levels of soybean meal supplementation). The method of confounding and analysis of variance described by Cochran and Cox (1957) was used. This procedure gave full information on all the main effects and first order interactions and two-thirds information on the second order interaction. The entire experiment was conducted in two replications. Tannin in the grain, protein in the grain and supplemental protein were all

Table 3.1. Protein and essential amino acid requirements of the rat and protein and essential amino* acid provided by each ration expressed as a percent of dry feed.

Component of Ration	Low Tannin								High Tannin								Weanling Rat Requirement+
	IS2349				IS0392				IS6492				IS1210				
	% Soybean Meal				% Soybean Meal				% Soybean Meal				% Soybean Meal				
	0	5	10	15	0	5	10	15	0	5	10	15	0	5	10	15	
Protein	8.08	10.21	13.10	17.48	10.53	13.39	15.78	18.71	7.46	10.30	12.55	15.50	8.46	11.50	14.30	17.30	16.0
Arginine	0.29	0.55	0.77	0.98	0.43	0.65	0.86	1.08	0.35	0.57	0.78	1.00	0.34	0.56	0.78	0.99	0.2
Histidine	0.16	0.23	0.30	0.38	0.19	0.26	0.33	0.40	0.16	0.24	0.31	0.38	0.20	0.27	0.34	0.41	0.4
Isoleucine	0.33	0.46	0.58	0.71	0.39	0.51	0.64	0.76	0.29	0.41	0.53	0.66	0.33	0.45	0.57	0.70	0.5
Leucine	1.16	1.37	1.57	1.78	1.43	1.63	1.84	2.05	0.94	1.15	1.36	1.57	1.16	1.37	1.57	1.78	0.9
Lysine	0.19	0.36	0.52	0.69	0.24	0.40	0.57	0.73	0.18	0.34	0.50	0.67	0.17	0.33	0.50	0.66	1.0
Methionine plus Cystine**	0.14	0.21	0.28	0.35	0.24	0.31	0.38	0.49	0.22	0.28	0.32	0.42	0.26	0.33	0.40	0.46	0.6
Phenylalanine plus Tyrosine	0.76	1.02	1.28	1.53	0.95	1.21	1.47	1.72	0.64	0.90	1.16	1.42	0.83	1.09	1.35	1.61	0.9
Threonine	0.35	0.45	0.55	0.66	0.33	0.43	0.53	0.64	0.23	0.33	0.43	0.53	0.25	0.35	0.45	0.56	0.5
Valine	0.43	0.56	0.70	0.83	0.55	0.68	0.81	0.94	0.36	0.50	0.63	0.76	0.41	0.54	0.67	0.81	0.4

* Tryptophane was not measured due to breakdown during hydrolysis.

** Methionine plus cystine were underestimated in the amino acid analysis due to some loss during hydrolysis.

+ Mertz (1969).

considered as fixed effects. Interaction sources of variation were pooled with the experimental error mean square to make tests of significance when they were not significant at the 0.25 probability of a Type I error. Differences between means were tested at the 0.05 probability of a Type I error with the Newman-Keuls' test (Steel and Torrie, 1960).

Results and Discussion

The analysis of variance for weight gain, FER, PER and feed consumed are presented in Table 3.2. The effect of tannin and the effect of soybean meal supplementation were significant for all four variables.

The effects of tannin, grain protein, and soybean meal supplementation on weight gains are illustrated in Figure 3.1. The low tannin genotypes had significantly greater gains at the zero, five, and ten percent soybean meal supplementation than the high tannin genotypes. The difference was not significant at the 15% supplementation level. This indicated that the amount of digestible protein was not significantly different between the high and low tannin genotypes at this level of soybean supplementation, however, the data in Table 3.1 indicate that lysine was still limiting when the diet was supplemented with 15% soybean meal. Weight gains were significantly different for all levels of protein supplementation. The lack of any interactions indicated that weanling rats fed rations with zero, five or ten percent soybean meal can be used to evaluate the biological value of the sorghum and still detect differences between high and low tannin genotypes. The coefficients of variation of weight gain for each group of six rats shown in Table 3.3 were reduced 33 to 77% by the addition of five percent soybean meal.

Table 3.2. Analysis of variances for weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER), and feed consumed on the average of six rats for 13 days.

Source of Variation	df	Mean Squares			
		Weight Gain	FER	PER	Feed Consumed
Replications	1	99.9*	13.6	0.03	382.9*
Tannin (T)	1	419.0**	929.9**	1.20**	625.7**
Grain Protein (G)	1	15.0	1.0	1.52**	855.9**
Soybean Protein (S)	3	3332.3**	1586.0**	2.94**	386.2**
T × G	1	16.2	2.5	0.06	446.2*
T × S	3	17.7	696.7**	0.12	7.6
G × S	3	0.8	4.0	0.04	61.9
T × G × S	2	2.3	0.2	0.02	19.7
Runs+	2	79.7*	48.3	0.10	61.9
Error	14	26.1	52.3	0.06	77.6
Pooled Error		19.2	37.4	0.05	60.8
Standard Error		4.4	6.11	0.71	7.8

* Significant at the 0.05 level of probability.

** Significant at the 0.01 level of probability.

+ Runs were confounded with T × G × S.

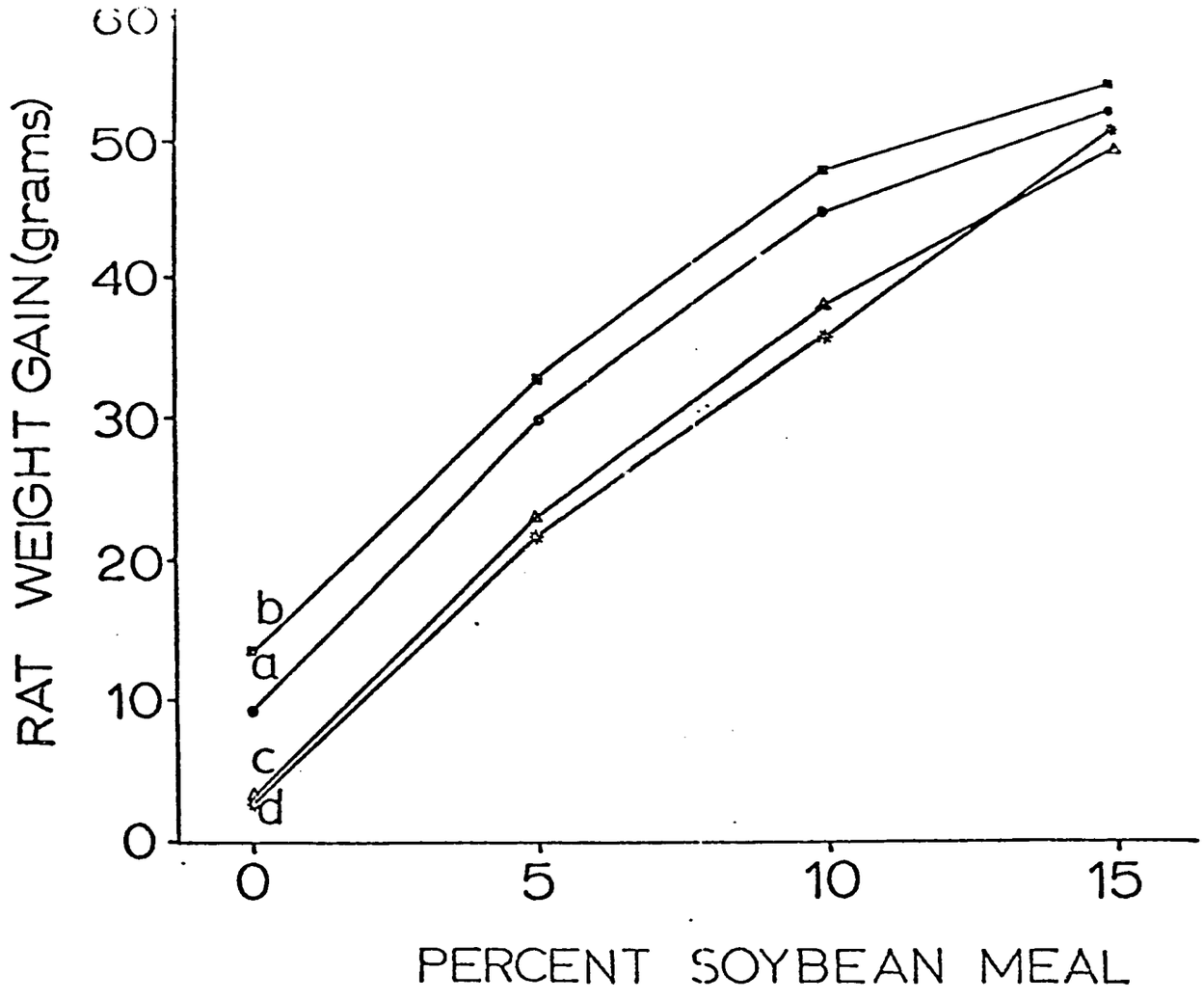

Figure 3.1. Average weight gain per rat for 13 days. a = IS2549 (low tannin, low protein), b = IS0062 (low tannin, high protein), c = IS6992 (high tannin, low protein), and d = IS1210 (high tannin, high protein).

Table 3.3. Coefficient of variation percentages of rat weight gain for the sorghum genotypes at each level of protein supplementation.

Percent Soybean Meal in Ration	Sorghum Genotypes			
	IS0062	IS2549	IS1210	IS6992
0	67.6	60.1	149.4	273.6
5	45.4	24.5	49.7	61.8
10	40.4	18.1	47.8	32.9
15	45.9	27.0	41.8	54.6

The differences in feed efficiency between the high and low tannin genotypes shown in Figure 3.2 were significant only for the non-supplemented ration. The non-supplemented ration had significantly poorer feed efficiency than the three supplemented rations which were not significantly different from each other. The significant tannin-supplementation interaction was due to the difference in slope for the high and low tannin genotypes between the zero and five percent protein supplementation. The results of Tamir and Alumot (1970) indicated that the differences in feed efficiency were caused by reduced protein digestibility in the high tannin sorghum.

There were no significant differences at the .05 level of significance between the high and low tannin sorghums for PER shown in Figure 3.3, but the difference was significant at the 0.15 level of probability for the non-supplemented ration and the differences were significant at the 0.20

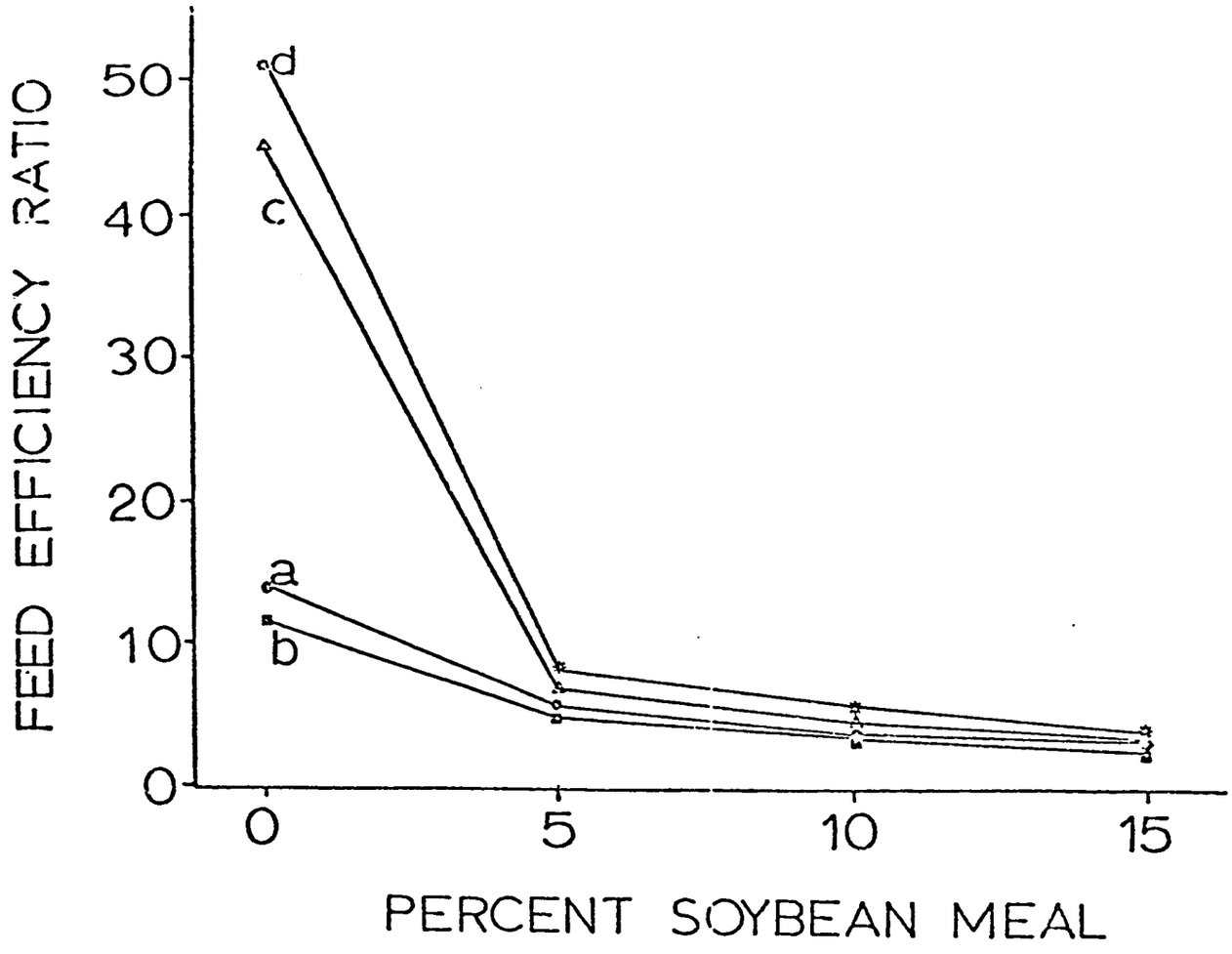

Figure 3.2 Feed efficiency ratios for rats fed 13 days. a = IS2549 (low tannin, low protein), b = IS0062 (low tannin, high protein), c = IS6992 (high tannin, low protein), and d = IS1210 (high tannin, high protein).

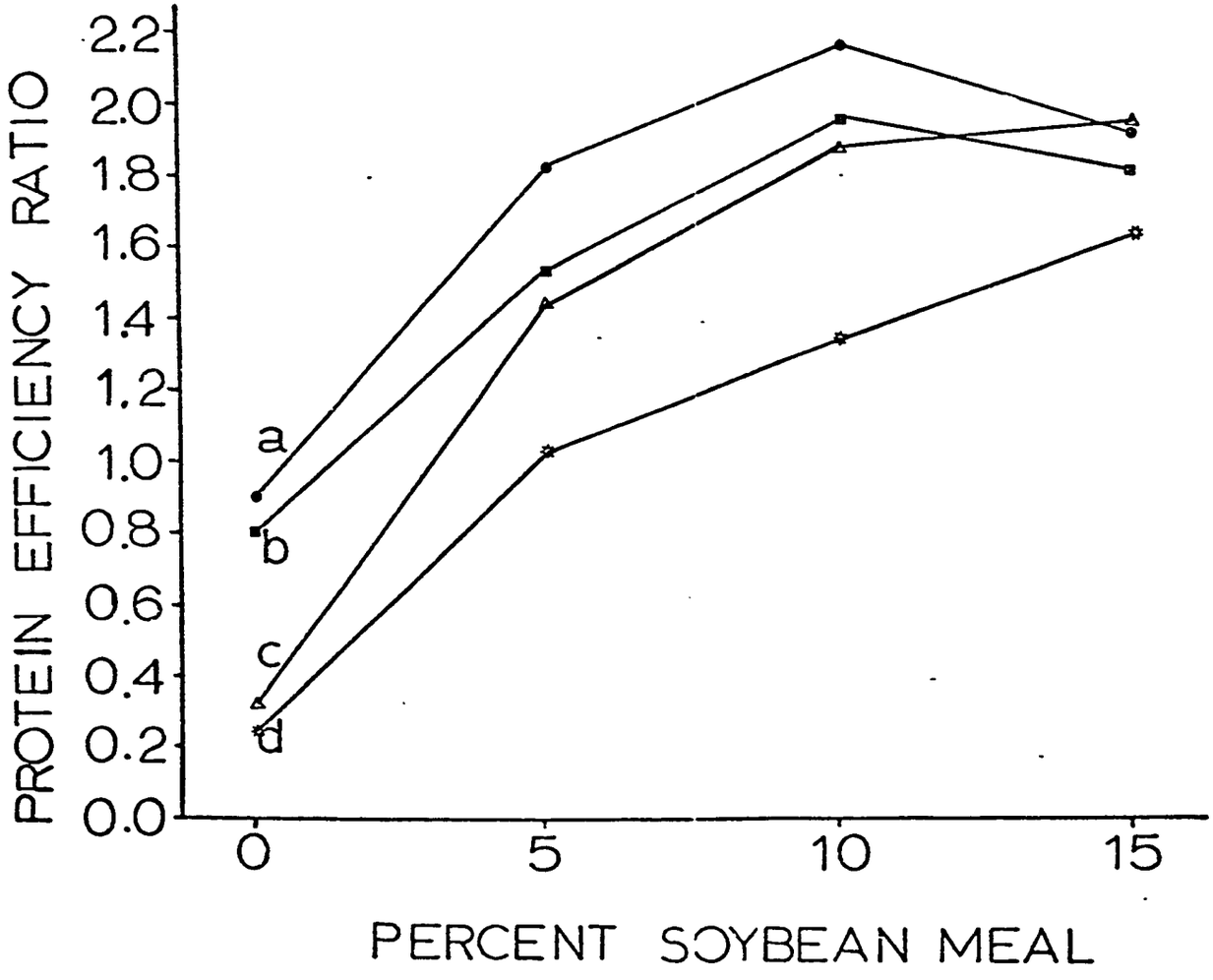

Figure 3.3 Protein efficiency ratios for rats fed 13 days. a = IS2549 (low tannin, low protein), b = IS0062 (low tannin, high protein), c = IS6992 (high tannin, low protein), and d = IS1210 (high tannin, high protein).

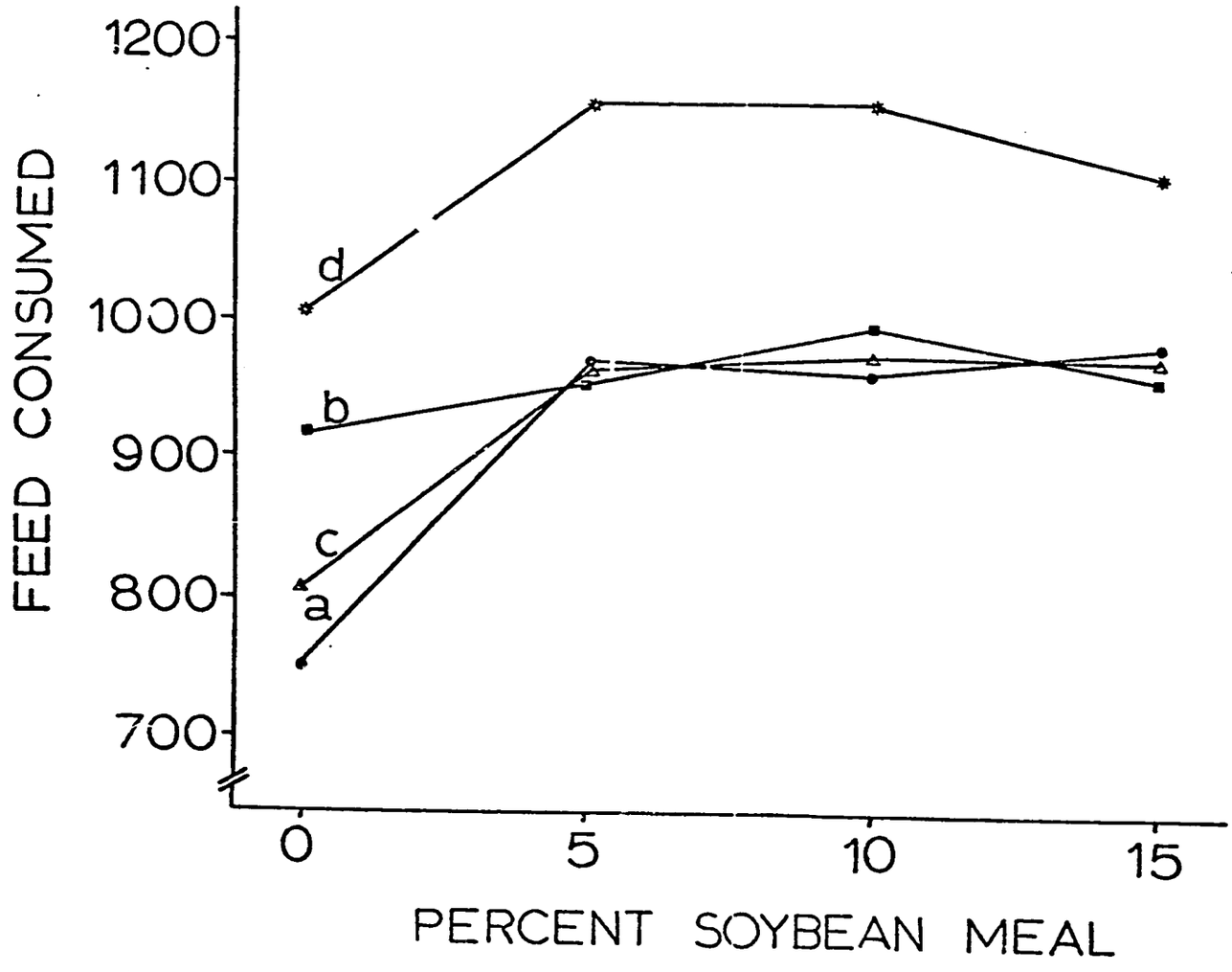

Figure 3.4. Grams of feed consumed by six rats in 13 days. a = IS2549 (low tannin, low protein), b = IS0062 (low tannin, high protein), c = IS6992 (high tannin, low protein), and d = IS1210 (high tannin, high protein).

level of probability for the five and ten percent supplementation. The rations with ten and fifteen percent soybean meal had significantly higher PER than the non-supplemented ration. There were no significant interactions for the PER values.

The significant effect of both grain tannin and grain protein in the analysis of variance for feed consumption was due to only one of the high tannin genotypes, IS1210, shown in Figure 3.4. The amino acid composition of this genotype was not appreciably different from the other genotypes. Later examination of this genotype revealed that it has a waxy endosperm, that is, the amylose had been replaced with amylopectin. This may have been the factor that caused increased feed consumption, and may have influenced the feed efficiency and protein efficiency ratio, but was not associated with increased weight gain. The other high tannin sorghum was intermediate in feed consumption. This indicated that the difference in performance between the high and low tannin sorghum was due to factors other than feed intake.

Conclusions

These data indicate that in general high tannin sorghum grain had poorer weight gains, poorer feed efficiency and lower PER values. There was also little effect of tannin on feed consumption by the weanling rats. One high tannin sorghum had greater feed consumption but that may have been due to the high amylopectin waxy endosperm. The lack of antagonistic interactions with supplemental protein for weight gain, feed efficiency, PER values, and feed consumption indicate that protein from soybean meal can be supplemented in a

biological evaluation program without changing the relative performance of different sorghum genotypes. The coefficients of variation for weight gain were reduced when the ration was supplemented with soybean meal. Significant differences between genotypes for weight gain were observed at intermediate levels (5 and 10% soybean meal) of supplementation but not at the higher level (15%). The low tannin grain sorghum with higher protein content produced greater gains, had better feed efficiency and protein efficiency than the low protein sorghum but the differences were not significant. Differences in protein efficiency within the high tannin and within the low tannin grain sorghums were greatest at intermediate levels of soybean meal supplementation. In final summary, the biological evaluation of sorghum grain would be of the greatest value at intermediate levels of protein supplementation, although the introduction of amino acids from the soybean meal might complicate the evaluation in some sorghum lines.

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A COMPARISON OF THREE-ROW AND ONE-ROW PLOTS FOR THE EVALUATION AND SELECTION OF LINES FOR PROTEIN CONTENT.

D. L. Oswalt

The coefficient of variabilities and error mean squares for yield, days to flower and height as reported in our annual report no. 8 of 1971 showed that both these statistics were reduced by evaluating the middle of a three-row plot as compared to a one-row estimate. The range in height of the 43 genotypes studied was 120 to 360 cm with an average of 245 cm. Data in Table 1 shows that protein data are also influenced significantly by differences due to variations in competition in three-row plots and among genotypes in one-row plots.

Table 1. A Comparison of One-Row with Three-Row Plots for Screening for Protein Quality (Averages of 43 Genotypes in Three Replications for One Year).

Protein Yield	One-Row Plot	Middle Row of Three-Row Plot
kg/ha	718	740
Error mean square	38441	23371
Coefficient of variability (%)	27.3	20.7
Percent Protein		
Average	10.95	11.48
Error mean square	.6794	.4671
Coefficient of variability (%)	7.5	6.0

This indicates the need to border against genotype interaction as well as height differences.

In Figures 1 through 4 the line with a slope of 1 represents identical responses from a one-row and three-row plot for a given genotype. The dotted lines on either side suggest significantly different values as determined by estimates of the LSD (.05) for each plot size. Points outside these limits have been identified by genotype code numbers. A comparison of the genotypes outside the limits in the four figures indicates that especially lines 1 and 41 compete best with themselves and lines 12 and 26 compete best with other genotypes. Table 2 contains the protein and yield data for these four lines showing the comparison of the individual row information.

Table 2. Protein Yields, Grain Yields and Percent Protein of Four Lines Which Showed the Extremes Indicated by Types of Competition in One-Row and Three-Row Plots (Means of Three Replications).

	Genotype				
Grain Yield kg/ha:	1	41	12	26	$LSD_{(.05)}$
One-row plot	1210	3273	11340	10450	2850
West of three-row plot	3800	6440	6420	6380	2950
Middle of three-row plot	4620	7010	7030	6670	2220
East of three-row plot	3960	6480	8810	6610	2820
Percent Protein:					
One-row plot	11.10	12.10	9.70	10.37	1.32
West of three-row plot	10.03	16.77	10.77	11.70	1.26
Middle of three-row plot	10.83	13.03	10.63	11.57	1.11
East of three-row plot	10.53	16.10	10.76	11.33	1.24
Protein Yield kg/ha:					
One-row plot	135	320	1110	1080	310
West of three-row plot	380	780	670	740	340
Middle of three-row plot	467	720	750	780	250
East of three-row plot	410	890	930	750	310

These data demonstrate the variability among genotypes when competing against themselves and against other genotypes. The number of lines outside the limits stress the necessity for multi-row plot use even within limited height groups. The genotypes that show strong ability to compete favorably with other genotypes, as indicated in the figure by being to the lower right of the suggested limits, would tend to dominate by increased seed production in heterogeneous populations. These data show the overall advantage for protein estimates of data collected from the middle row of three-row plots which more nearly simulate line use in single genotype competition. One-row evaluations tend to underestimate the percent protein potential of a given line as indicated by a shift to the left in Figure 3.

FIGURE 1. PLANT HEIGHT

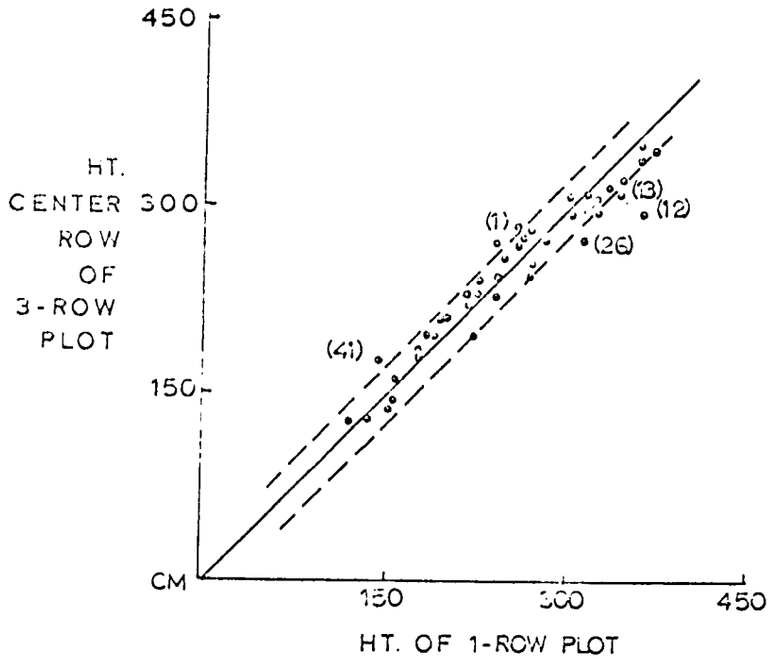


FIGURE 2. GRAIN YIELD

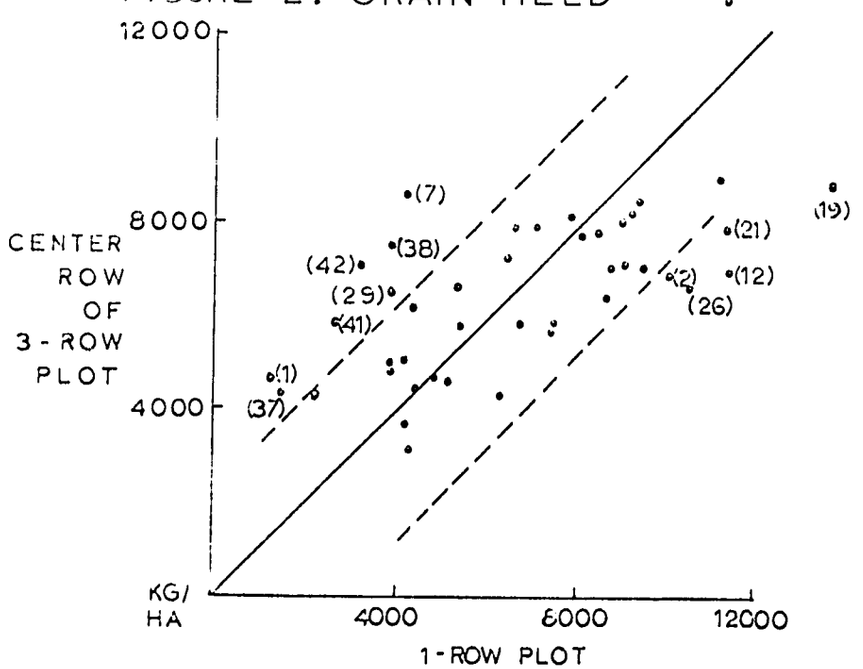


FIGURE 3. PERCENT PROTEIN

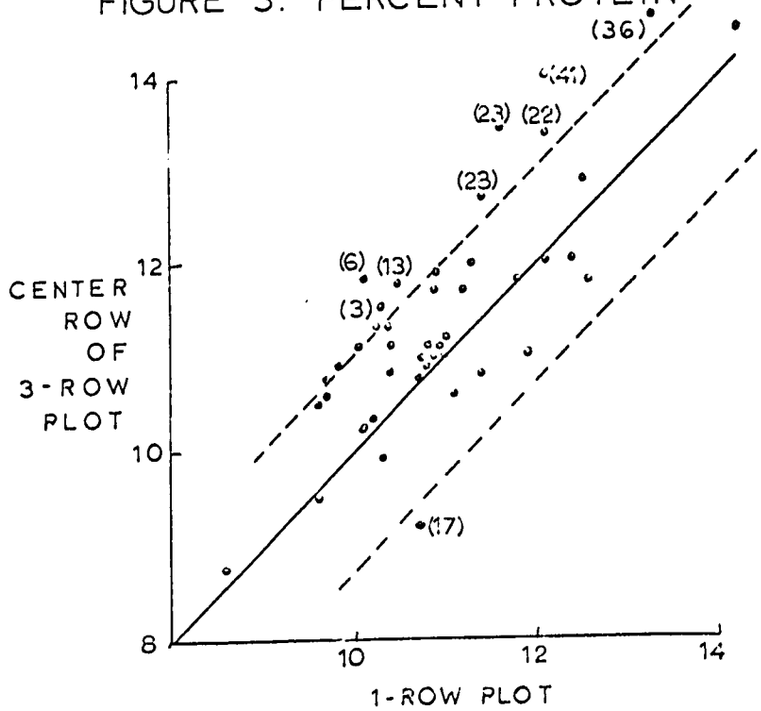
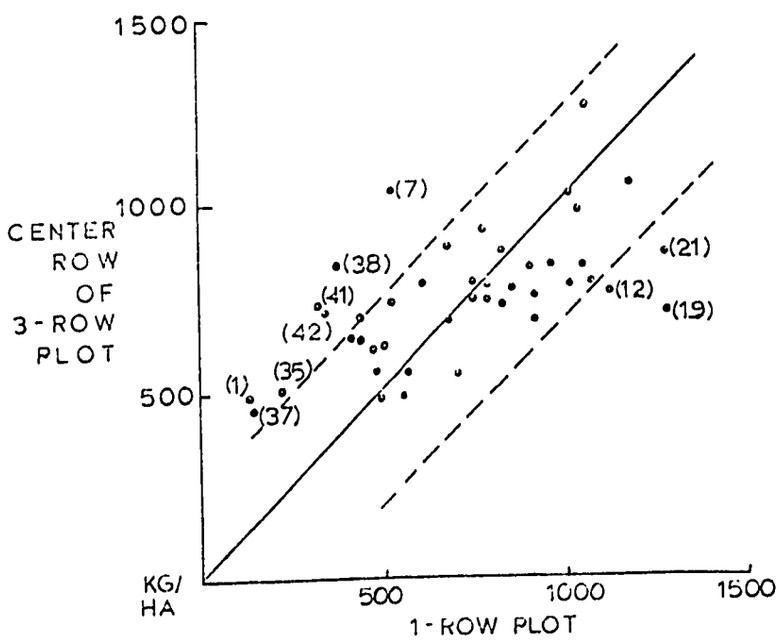


FIGURE 4. PROTEIN YIELD



PRELIMINARY RESULTS OF THE FIRST PURDUE-AID INTERNATIONAL PROTEIN YIELD AND QUALITY TRIAL.

D. L. Oswalt, R. C. Pickett and J. D. Axtell

The summary of data and criteria for selection of the first 20 entries were reported in our annual report no. 8 of 1971. Sets containing three-row plots, replicated three times of 20 selected lines and four checks were randomized by eight varieties in each of the three height groups and sent to 32 locations at their request. It is reported by the cooperators that 22 sets have been planted to date, many of which are not yet harvested. Estimates of disease resistance are given in Table 1 as tested in 1972 at Yuma, Arizona for "new milo disease", at Tucson, Arizona for charcoal stalk rot (macrophomina phaseoli), and at Berclair Head Smut Nursery near Beeville, Texas for downy mildew, head smut, bacterial stripe, grey leaf spot, rust and zonate leaf spot.

Correspondence indicates generally good adaptation of entries in this trial. From one location in Northeast Brazil (M.A. Faris of Fortaleza, Ceara) nine entries were selected on the basis of their yield and desirability and entered into 20 variety trials within Brazil in anticipation of the release of those continuing to show high yield potentials. Insufficient yield data has been returned to date to include a summary with this report regarding overall performance of this trial, but will be included in the 1973 report. A second set of entries is being currently selected.

Table 1. Disease Ratings of First Purdue-AID International Protein Yield Trial Entries in 1972 (Study 298).

Identification Entry	Source	New[1] Milo Disease	Charcoal[2] Rot	Downy[3] Mildew Rep (1)	(2)	Head[4] Smut Rep (1)	(2)	Bacterial[5] Stripe	Grey[6] Leaf Spot	Rust[7]	Zonate[8] Leaf Spot
1	954114	2.5D	1.00	0	0	0	0	2	2	3	3
2	954255	2.0	.55	0	0	0	0	1	3	4	4
3	954063	1.0	2.75	0	0	0	0	1	2	3	2.5
4	932127	1.0	2.35	5	2	0	0	1	3	4	4
5	RS610	3.0D	.84	4	0	1	4	1	2	4	4
6	BB64	3.0D	.55	7	2	0	0	1	2.5	3	4
7	IS7822	2.5D	1.90	10	2	0	0	3	2.5	3	4
8	954062	1.0	2.59	0	0	0	0	2	2	2	4
9	932296	3.0D	1.54	0	1	0	0	1	2	4	4
10	IS8361	1.5	2.25	2	2	0	0	1	2	4	4
11	932075	1.0	1.89	1	0	0	0	1	2	4	4
12	956036	1.5D	1.25	4	1	0	0	2	3	4	4
13	954130	1.0	2.55	0	0	0	0	1	2	4	3
14	954206	1.0	1.16	1	0	0	0	1	2	4	4
15	954164	1.0	1.94	2	0	0	0	1	2	4+	3.5
16	NK300	1.0	2.30	4	0	0	0	1	2	4	4
17	IS2319	1.0	1.11	0	0	0	0	1	2	4	4
18	954104	1.5D	.97	0	0	0	0	1	2	4	4
19	932062	1.0	1.27	0	0	0	0	1	3	4	3
20	IS9198	1.0	-	0	0	0	0	1	2.5	3	2.5
21	IS7579	1.0	1.15	0	0	0	0	1	2.5	4	3
22	IS9569	1.0	1.24	0	0	0	0	1	-	-	-
23	932027	1.0	1.38	0	0	0	0	1	2.5	4	3
24	IS4225	1.0	2.77	10	1	0	0	1	2	4	4

[1]Yuma, Arizona by Robert Voigt. 1972. 1 = no disease, 5 = most plants dead, D = entry segregating for resistance and susceptibility.

[2]*Macrophomina phaseoli* disease evaluation at Tucson, Arizona by Robert Voigt. 1972. 0 = no infestation to 4 = severe infestation, - = too late in maturity to evaluate.

[3]Berclair Head Smut Nursery near Beeville, Texas by R. A. Frederiksen. 1972. Represents number of plants diseased out of an average of 15-20 plants in each replication.

[4]Same as 3.

[5,6,7,8]At same location as 3. 0 = disease absent, 4 = severe disease with over 25% of leaf area destroyed.

HIGH PROTEIN, HIGH LYSINE RANDOM MATING POPULATIONS

R. E. Schaffert, K. Kofoid and J. D. Axtell

In 1971, 174 lines from the world sorghum collection, tested for restoration capabilities, yield, protein, and amino acid content, were crossed on segregating genetic ($\underline{ms}_3$) male sterile populations.[2] The lines were crossed on the appropriate R or B population. Two crosses were made from each line and the resultant hybrid seed from each cross was composited. A twenty-foot row of the F_1 hybrid was then grown in Puerto Rico in 1971. Three individual plants in each row were selfed and the S_1 seed from each cross was composited. Thirty grams of composited S_1 seed was then bulked into the appropriate population.

The crosses were placed into populations depending upon characteristics of the lines. Populations 1, 3 and 5 were composed of crosses of restorer lines and the populations 2, 4 and 6 were for the crosses of non-restorer lines. Populations 1 and 2 were composed of lines having high total protein yield potential. Populations 3 and 4 were composed of lines having a high lysine content. Populations 5 and 6 were composited from the remaining lines. There are 53 lines in population 1 ranging in value from 480 to 1030 kg/ha of protein.[1] There are 13 lines in population 2 ranging from 640 to 840 kg/ha of protein.[1] There are 30 lines in population 3 with lysine values ranging from .288 to .332 (g lysine/100 g sample) and/or lysine (percent

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[1] Based on 1971 Lafayette data.
[2] Nebraska population NP2B and NP3R were used as sources of the $\underline{ms}_3$ gene.

of protein) values ranging from 2.47 to 3.36.[1] There are 13 lines in population 4 with lysine values ranging from .289 to .333 (g lysine/100 g sample) and/or lysine (percent of protein) values ranging from 2.47 to 3.34.[1] There are 81 lines in population 5 and 23 lines in population 6.

The populations were planted in isolation at Lafayette in 1972 and allowed to random mate. Two plantings were made in each population. These plantings were two weeks apart. One week after the earliest types reached anthesis, male sterile plants were identified and tagged. Identification and tagging continued until approximately 300 plants were tagged in the B populations and 500 to 600 plants in the R populations.

Each head was threshed individually and split into three portions. The first portion contained all of the seeds up to 10 grams per head, the second portion contained the remainder up to 10 grams per head and the third portion contained the remainder of each head up to 30 grams per head.

A second generation of random mating for all six populations is being obtained at Sete Lagoas in Brazil during the period of November 1972 to February 1973. A composite of populations 1 and 2 and another containing 3 and 4 are being random mated in Puerto Rico by Dr. Orrin Webster during the 1972-73 winter months. A third generation of random mating for all eight of these populations will be obtained in 1973 at Lafayette, Indiana.

[1] Average values.

RATE OF SEED GERMINATION AS A MEANS FOR EVALUATING SORGHUM LINES FOR THEIR CATECHIN EQUIVALENT CONTENT AND AS AN ESTIMATE OF THEIR NUTRITIVE VALUE.

H. A. Ibrahim and D. L. Oswalt

Grain sorghum varieties differ in their grain color, ranging from white to purple to dark brown according to the kind and amount of pigments (phenolic compounds) present. A high level of some classes of phenolic compounds as determined by catechin equivalents (tannins) has been associated with some bird and weathering resistance and also with reduced protein availability in rat growth trials. Furthermore, it is not possible to predict rat growth response based on grain color, percent crude protein, lysine content and other amino acid content alone. Therefore, it is important to find a simple biological method (less costly than rat feeding) to measure nutritional quality, thus this consideration of seed germination (lettuce seed) as associated with other work of measuring the inhibition or promotion of plant chemicals (Khan, 1966) was undertaken.

Maxson and Rooney (1972) compared two methods of tannin analysis for grain sorghums; the Modified Vanillin-Hydrochloric acid (MV-HCl) and the urea extraction followed by reaction with Ferric Ammonium Sulfate (FAS). These two methods require equipment, such as a centrifuge, chemical reagents, pH meter and a colorimeter which in some cases are not available to the breeders in the developing nations. This work was directed

toward finding some alternative biological means for evaluating nutritional quality in grain sorghum.

Germination one of the assays used to measure the effect of plant hormones, auxins and the inhibitors or promotors of the plant growth regulators. The process of germination was used to measure the effect of the complex compounds of the grain sorghum polyphenols.

Materials and Methods

Lettuce seed (var. Great Lakes) was used for the germination process. Lettuce was chosen because the seeds are small in size, it germinates rapidly and uniformly in 18 hours assuming uniform seed size and it is white seeded and light insensitive.

Ten grams of ground grain sorghum were soaked in 25 ml of distilled water at room temperature for one hour with continuous stirring. The color of the ground grain mixture in water was observed and ranked from one to ten. The mixture was filtered and the filtrate measured to find the water retention capacity of the ground sorghum. One ml of the filtrate was used to germinate the lettuce seed on filter paper in 5 cm petri dishes. The petri dishes were kept in an air conditioned room at 75° F. Germination in water was used as a control. The percent germination was determined at intervals of 18, 24, 30 and 40 hours. A trial with five replications (each petri dish considered as one replication which contained 50 lettuce seeds) was conducted to measure the difference among 44 lines which were selected because they had been previously tested for nutritive quality using rat

performance trials. Catechin equivalent values were also known on these samples.

The percent germination of sorghum seed was conducted for 32 of these 44 lines using 3 ml distilled water in 10 cm petri dishes on filter paper. The percent germination was determined after 30 hours.

The amount of weathering was determined by a visual count of the number of weather damaged grains per hundred. Three hundred seeds were observed for each line. Gallotannic acid in different concentrations was used to find its effect on the germination process of lettuce seed.

The pericarp of three lines was removed using hot 20 percent sodium hydroxide for 10 minutes and repeated washing in distilled water, then neutralized by acetic acid (Blessin, 1971). Both dehulled and grain with the pericarp were evaluated by rat trials and this method of lettuce seed germination.

Results and Discussion

Table 1-A presents the mean values of six different measurements for 44 sorghum lines and two measurements for 32 lines. There are great variations among sorghum lines for most of the characters. The water extract of some lines such as IS2319, 932152, IS3935 or IS1295 inhibits the germination of lettuce seed completely for more than 40 hours, while some others, like IS8165 and NK300, do not show any sign of inhibition of lettuce seed germination.

In rat trials these lines showed variations ranging from a gain in weight of 216 percent to a loss of weight of -48 percent over the check. The same variability exists for catechin equivalent values ranging from 0.10 to 7.84.

Table 1-B shows the correlation coefficients between some of those measurements. They are as follows:

1) There is a highly significant positive correlation between catechin equivalents and the germination of lettuce seed on the water extract of grain sorghum after 30 or 40 hours.
2) A highly significant negative correlation exists between the rate of rat gain and the germination of lettuce seed; i.e., a highly significant negative correlation between the nutritive value of sorghum lines and its ability to stimulate lettuce seed germination.
3) There is a highly significant negative correlation between catechin equivalents and rat gain.
4) The sorghum seed germination does not correlate with the lettuce seed germination or the amount of tannin present in the seed, but it is affected by the amount of weathering of the seed.
5) There is a highly positive correlation between the capacity of the ground seed to retain water and its content of the polyphenols measured as catechin equivalents.
6) Wet ground grain color is highly correlated with the amount of catechin equivalents present in the grain. The darker the mixture of grain and water the greater the amount of polyphenols present. This does not correlate strongly with the weathering of the seed.

Table 1-A. The Mean Values of Different Measurements and their Simple Correlations for 44 Sorghum Lines.

Identification	Lettuce seed germination 30 hrs. %	Lettuce seed germination 40 hrs. %	Rat wt. gain % of check+	Catechin Equiv.	Water Retention %	Sorghum Germi-nation %	Weathering %	Wet Grain Color++
IS2319	0.0	0.0	100	.63	62.0	52	10	2
025035	0.0	0.8	60	.42	56.0	62	12	1
IS2942	14.4	21.2	21	5.53	68.4	80	0	10
IS3983	5.6	11.2	60	.45	58.0	46	34	3
IS0219	4.8	20.8	69	.41	58.0	50	26	2
IS7193	1.6	12.8	29	.35	60.0	50	10	2
IS6992	30.4	58.4	22	4.34	70.4	78	0	9
IS8165	53.6	65.6	27	5.10	68.0	80	0	9
Rx8165	19.6	41.6	22	5.40	62.4	70	0	9
Mx8165	28.8	45.6	30	3.70	62.4	60	0	9
RS690	47.0	62.4	28	6.35	65.2	50	2	9
IS0104	0.8	6.4	41	.52	59.2	32	18	2
IS2500	0.8	4.8	70	.18	65.2	60	6	4
IS0175	18.4	35.2	43	.37	56.0	44	22	2
954066	11.2	24.8	48	.45	56.0	50	16	1
Tx2527	3.2	10.8	31	.94	56.0	22	60	4
954039	0.0	0.8	54	.51	59.0	58	20	5
IS0129	2.4	7.2	8	.58	54.4	14	62	2
IS3568	0.8	8.0	27	.30	59.2	86	8	2
IS3485	16.0	36.8	69	2.95	65.2	64	2	9
IS3687	31.2	60.8	56	1.10	54.8	42	2	5
IS3979	11.2	17.6	15	.94	57.2	56	18	10
IS0508	4.8	7.2	69	.10	58.8	68	16	3
954179	2.4	7.2	61	.38	54.8	56	18	1
954111	9.6	41.6	13	.40	53.6	58	24	1
954239	2.4	8.4	31	5.10	53.2	28	14	5
954130	0.0	10.4	44	.28	55.6	40	10	1
027231	1.6	23.2	54	.26	58.8	50	10	1
IS0075	0.8	19.2	61	1.10	55.6	30	36	6

Table 1-A. Continued.

Identification	Lettuce seed germination 30 hrs. %	40 hrs. %	Rat wt. Gain % of Check+	Catechin Equiv.	Water Retention %	Sorghum Germi-nation %	Weathering %	Wet Grain Color++
029004	16.8	31.2	72	.11	58.8	6	12	5
IS3935	0.0	0.8	57	.11	61.6	18	4	2
NK300	59.2	80.8	35	4.11	65.2	84	0	9
IS1295	0.0	0.8	216	.50	53.6	-	-	7
IS0062	0.0	5.6	206	.70	52.0	-	-	1
954109	1.6	7.2	133	.23	57.6	-	-	1
954126	1.6	7.2	124	.15	64.7	-	-	1
IS3925	0.8	2.4	107	.17	54.0	-	-	1
IS9527	7.2	28.0	-39	.96	55.2	-	-	1
IS2283	27.2	56.8	-38	6.81	65.7	-	-	10
IS9457	0.8	4.8	157	.33	52.0	-	-	5
932152	0.0	0.0	103	.24	54.2	-	-	2
IS0083	2.4	3.2	118	.58	57.2	-	-	7
IS8260	27.2	56.0	-31	7.84	64.0	-	-	10
IS0345	2.4	7.2	-48	.43	48.0	-	-	7

Table 1-B. Correlation Coefficients (r)

Lettuce seed germination after 30 hours	-.44**	.72**	.39**	.42*	-.45**
Lettuce seed germination after 40 hours	-.56**	.56**	.41**	.33	-.41*
Catechin equivalents	-.53**	X	.41**	.40*	-.43*
Seed grain color	-	.70**	-	-	-.42*
Sorghum seed % germination	-	-	-	x	-.51**

** Significant at the 1 percent level.

* Significant at the 5 percent level.

+ IS2319 considered to be 100% check in rat gain. The mean of lettuce seed germination in water in both counts is 98.6%.

++ Index of color of wet ground grain: 1 = white, 2 = white chalky or dirty white, 3 = white yellow 4 = yellow, 5 = light red or reddish, 6 = light purple, 7 = red, 8 = purple, 9 = light brown 10 = brown.

Selected regressions are presented in Figure 1 and 2.

Table 2 shows the effect of dehulling in reducing the catechin equivalent concentration. The dehulled data shows the same relationship (r = 0.93) between catechin equivalents and germination of lettuce seed with a water extract of sorghum seed as was found for the 44 samples.

Table 2. Correlation Coefficients Between Percent Lettuce Seed Germination and Catechin Equivalent Using Three Sorghum Lines and Their Dehulled Counterparts.

Lines	Catechin Equiv.	Lettuce Seed Germination 40 hrs.
IS2319	0.61	0.8
IS2319 (dehulled)	0.03	0.0
IS0129	0.52	2.4
IS0129 (dehulled)	0.11	3.2
IS6992	4.27	68.0
IS6992 (dehulled)	0.01	20.0
(r) values - catechin equivalents		+ 0.93**

** Significant at the .01 level.

Table 3 shows the effect of tannic acid concentrations on the germination of lettuce seed compared with the effect of catechin compounds of sorghum seed. After 18 hours the lettuce seed started to germinate. There was a decrease in the germination with each increase of tannic acid, but after 30 hours the tannic acid concentrations of 0 to 2 percent did not show any significant reduction in lettuce seed germination while eight

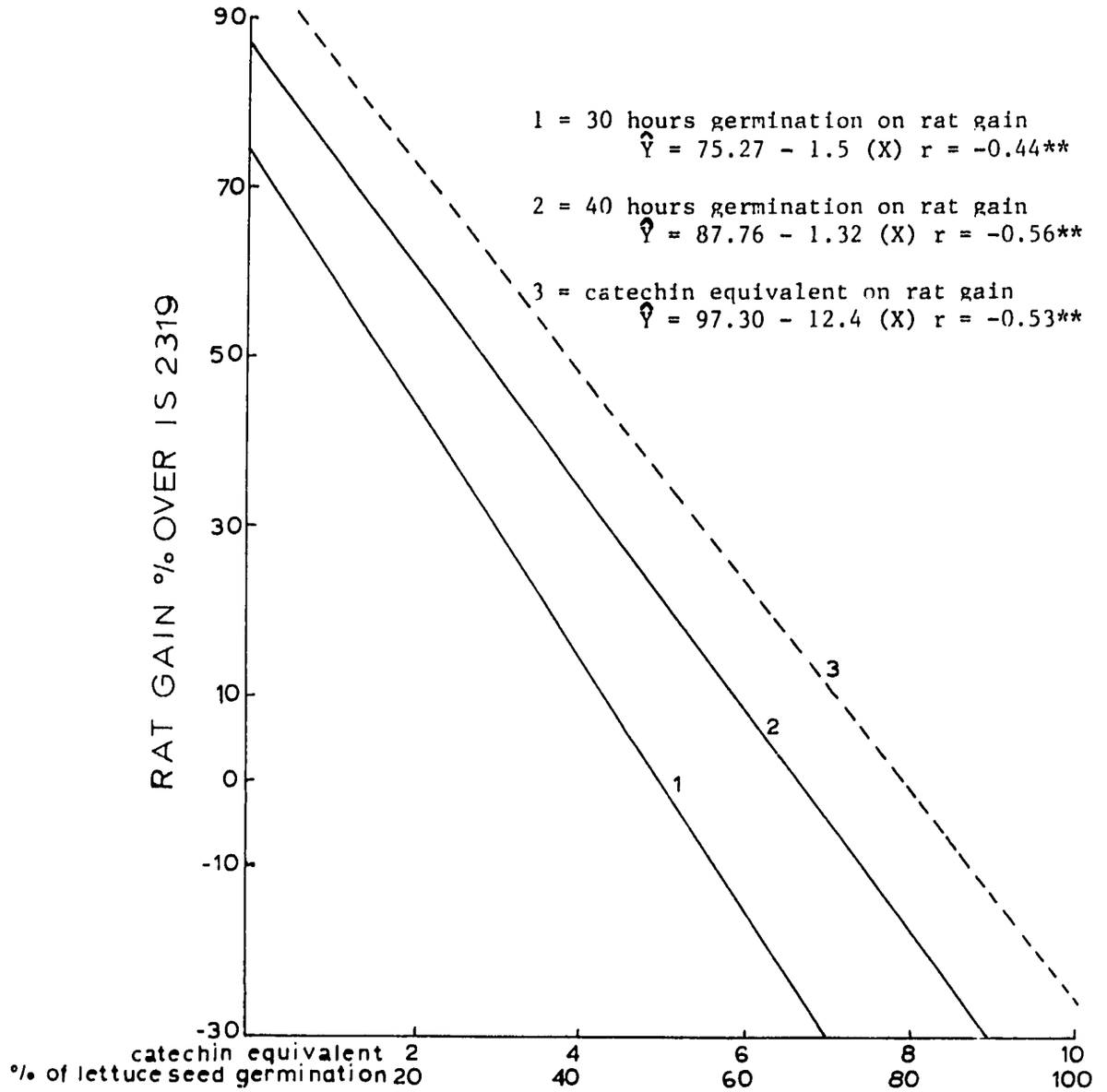
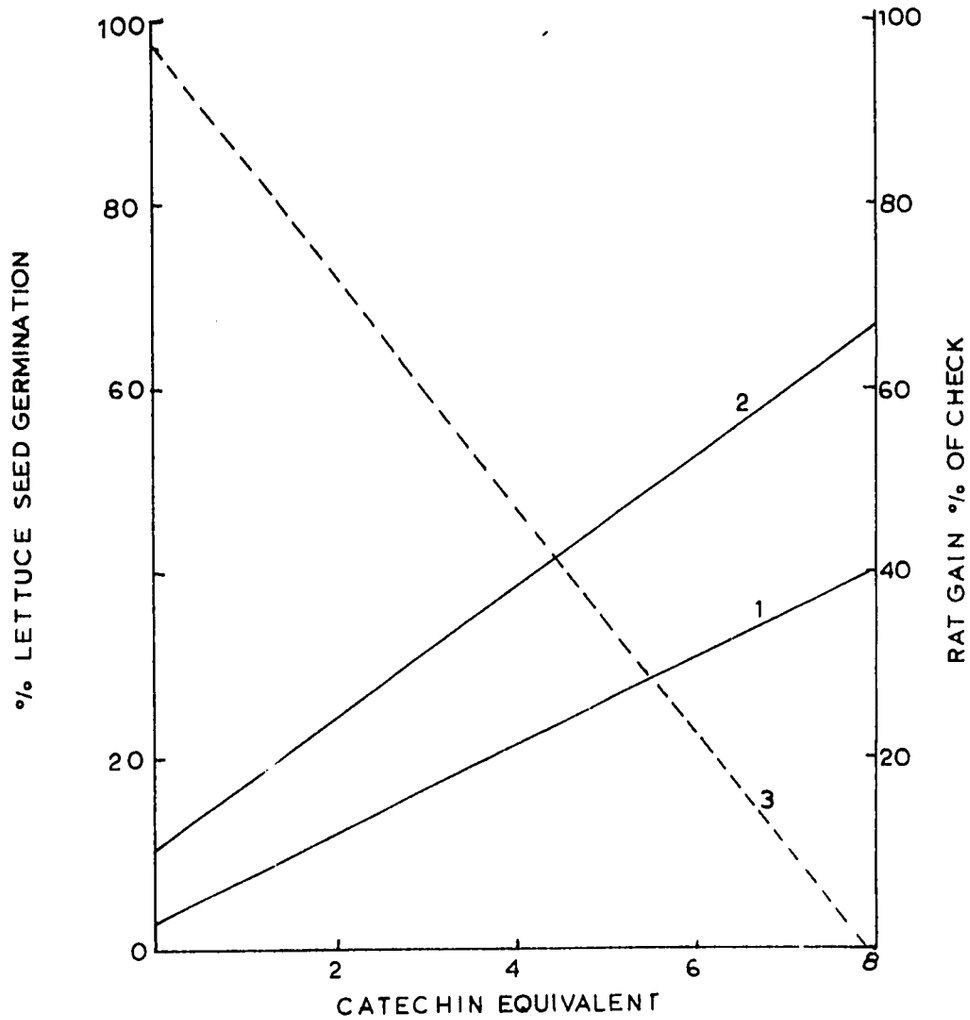


Figure 1. Regression of Lettuce Seed Germination and Catechin Equivalent on the Rat Gain Percentage.



1 = Catechin equivalent on 30 hours germination
$\hat{Y} = 2.66 + 5.02\ (X)\quad r = 0.72^{**}$

2 = Catechin equivalent on 40 hours germination
$\hat{Y} = 10.32 + 7.02\ (X)\quad r = 0.56^{**}$

3 = Catechin equivalent on rat gain
$\hat{Y} = 97.30 - 12.4\ (X)\quad r = -.053^{**}$

Figure 2. Regression of Catechin Equivalents on the Lettuce Seed Germination and Rat Gain Percent of Check.

percent tannic acid inhibited completely the germination process. This showed a reverse relationship between the effect of catechins from sorghum on lettuce seed germination and the effect of tannic acid. From this it can be concluded that tannic acid in sorghum seed is below the two percent level or the catechin equivalent measurement is a measure of other phenolic substances associated with tannin which can influence the germination of lettuce seed as well as the rat gain. This point especially requires more work for clarification.

Table 3. Effect of the Concentration of Tannic Acid and Selected Sorghum Filtrates on the Rate of Lettuce Seed Germination.

Tannic Acid			Selected Sorghum Filtrates	
Concentration Percent	Lettuce Seed Germination 18 hrs.	30 hrs.	Catechin Equiv.*	Lettuce Seed Germination After 30 hrs.
	%	%		%
Water	95.0	100.0	water	98.6
0.125	91.0	99.0	0.11	0.0
0.25	92.0	98.0	0.26	1.6
0.5	67.0	92.0	0.58	2.4
1.0	67.0	94.0	1.10	0.8
2.0	57.0	86.0	2.95	16.0
4.0	22.0	58.0	4.34	30.4
8.0	0.0	0.0	6.35	47.0
L.S.D. at five percent	12.2	9.7		
L.S.D. at one percent	16.6	13.2		

Values under the same lines are not significant according to the L.S.D.

* Catechin equivalents of some selected lines and its effect on lettuce seed germination.

In conclusion, this method of using lettuce seed germination for the estimation of grain sorghum nutritive value may have use for screening the large numbers of lines or advanced selections in countries where the laboratory facilities are scarce. This technique of using a biological assay for measuring the grain nutritive quality needs more work using other biological systems such as growth of yeast, fungi or roots of higher plants, etc.

Summary

The testing of lettuce seed germination with water extracts of sorghum grain can be considered as a measure of catechin equivalents since there is a highly significant positive correlation between them. Since the catechin equivalent content affects the nutritive value of sorghum lines as shown in the rat feeding trials this germination method can be a measure in general terms for the nutritive value of sorghum lines since it shows a highly significant negative correlation.

The color of the ground grain and water mixture can be an indicator of catechin equivalent values but not always, especially for the darker grains.

The catechin equivalent values are not only a measure of the presence of tannic acid but are also a measure of some other substances influencing the germination of lettuce seed as well as the feeding quality of sorghum lines.

This line of research by using lettuce seed rate of germination as a biological assay requires more work for refinement and for answering many

questions about sorghum grain quality as a food or feed.

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