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COMPONENTS OF NUTRITIONAL QUALITY IN GRAIN SORGHUM

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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. 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 presents 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.

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COMPONENTS OF NUTRITIONAL QUALITY IN GRAIN SORGHUM[1]

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R. Jambunathan and G. Srinivasan.[2]

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

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[1] Contribution from the Purdue University Agric. Exp. Station, Lafayette, Indiana, 47907. Journal paper no. _____. Supported by the U.S. Agency for International Development under contract csd-1175 entitled "Inheritance and Improvement of Protein Quality and Content in Sorghum bicolor (L.) Moench"

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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 the

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

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* 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

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 dependant 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.

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 Line 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 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.

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,

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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Figure 1. Essential Amino Acids

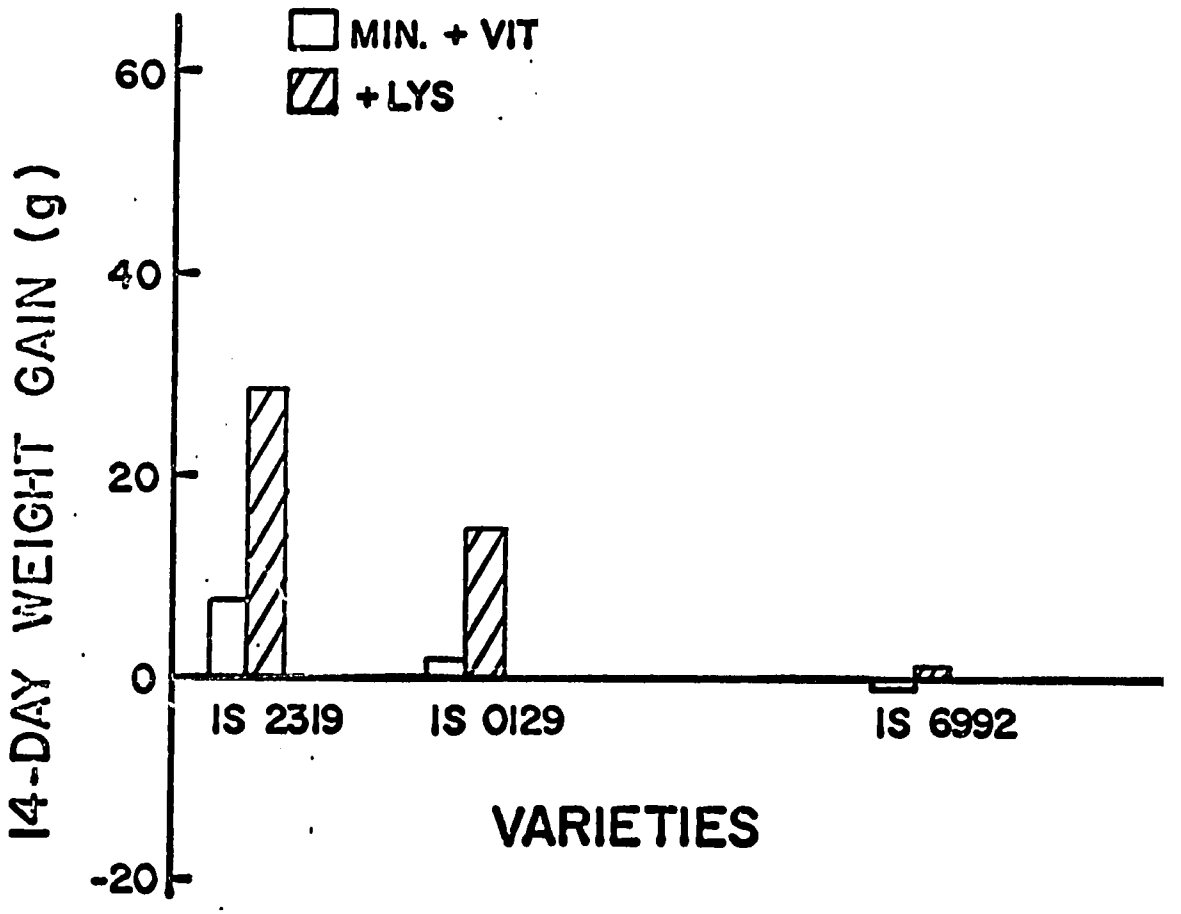

Figure 2. Rat Weight Gains on Iso-nitrogenous Samples of Supplemented Grain Sorghum Lines.

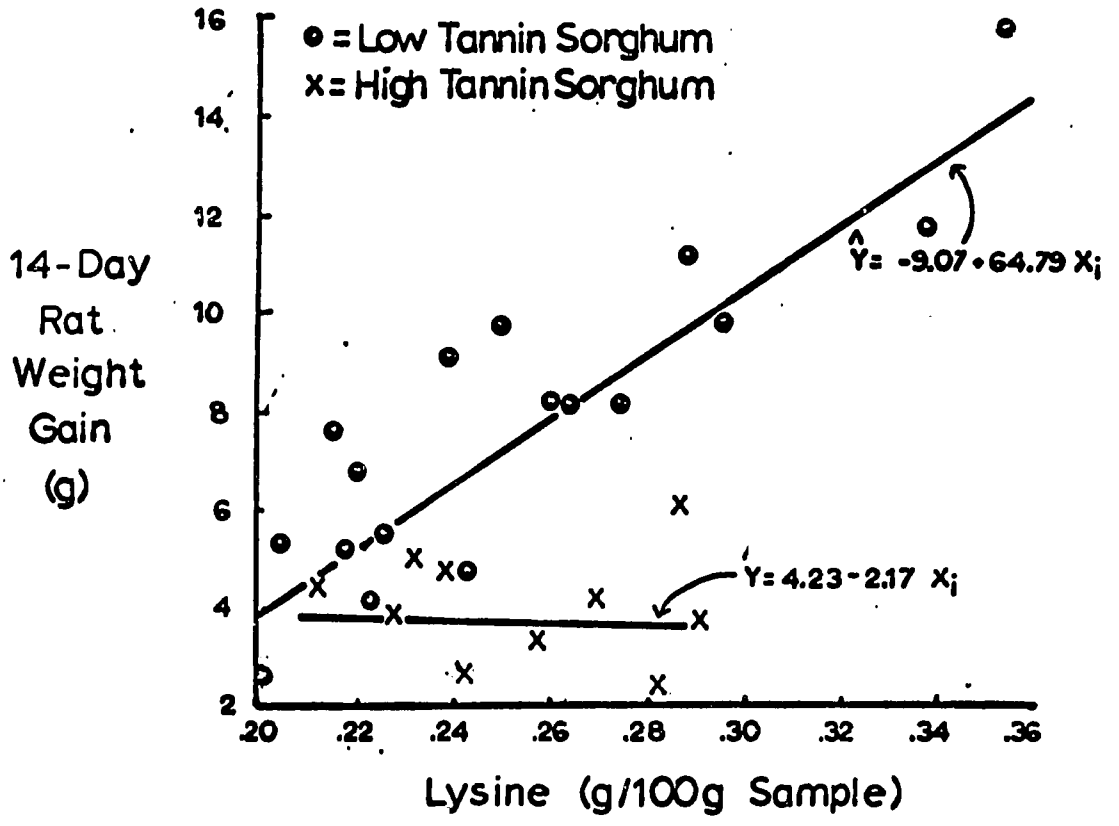

Figure 3. Relationships of Rat Weight Gain with Lysine Content in High and Low Tannin Sorghum Lines.

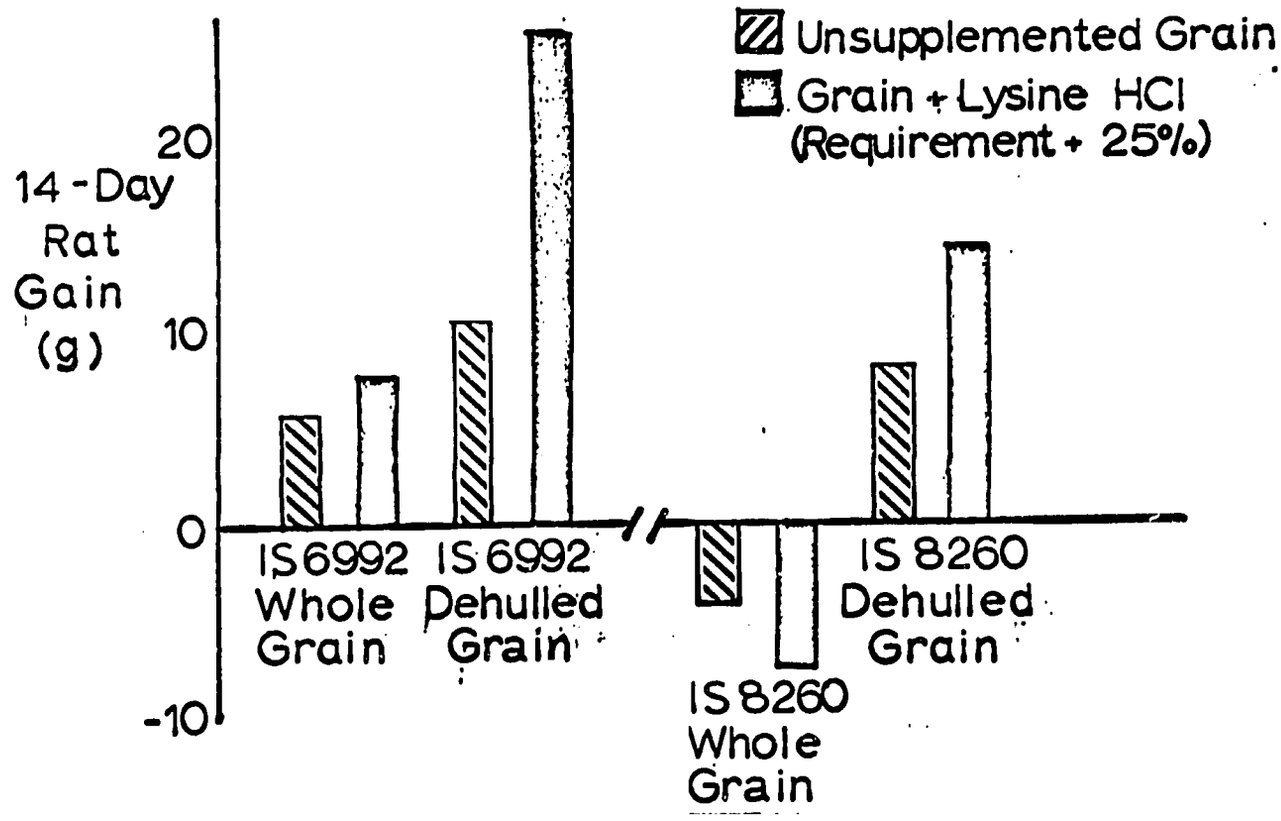

Figure 4. Lysine Supplementation of Whole Grain and Dehulled High Tannin Sorghum Lines.

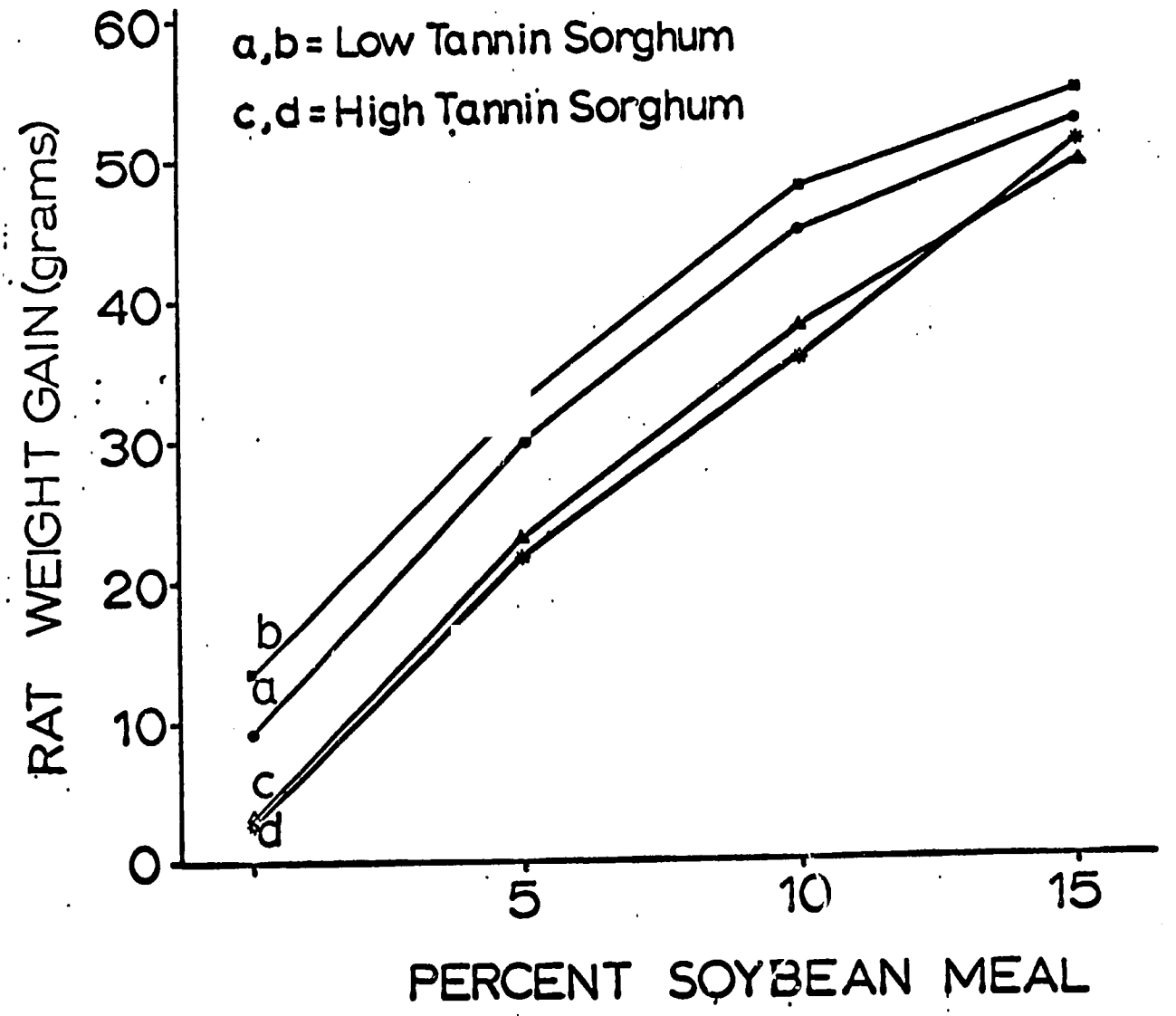

Figure 5. Average Weight Gain Per Rat for 13 Days.

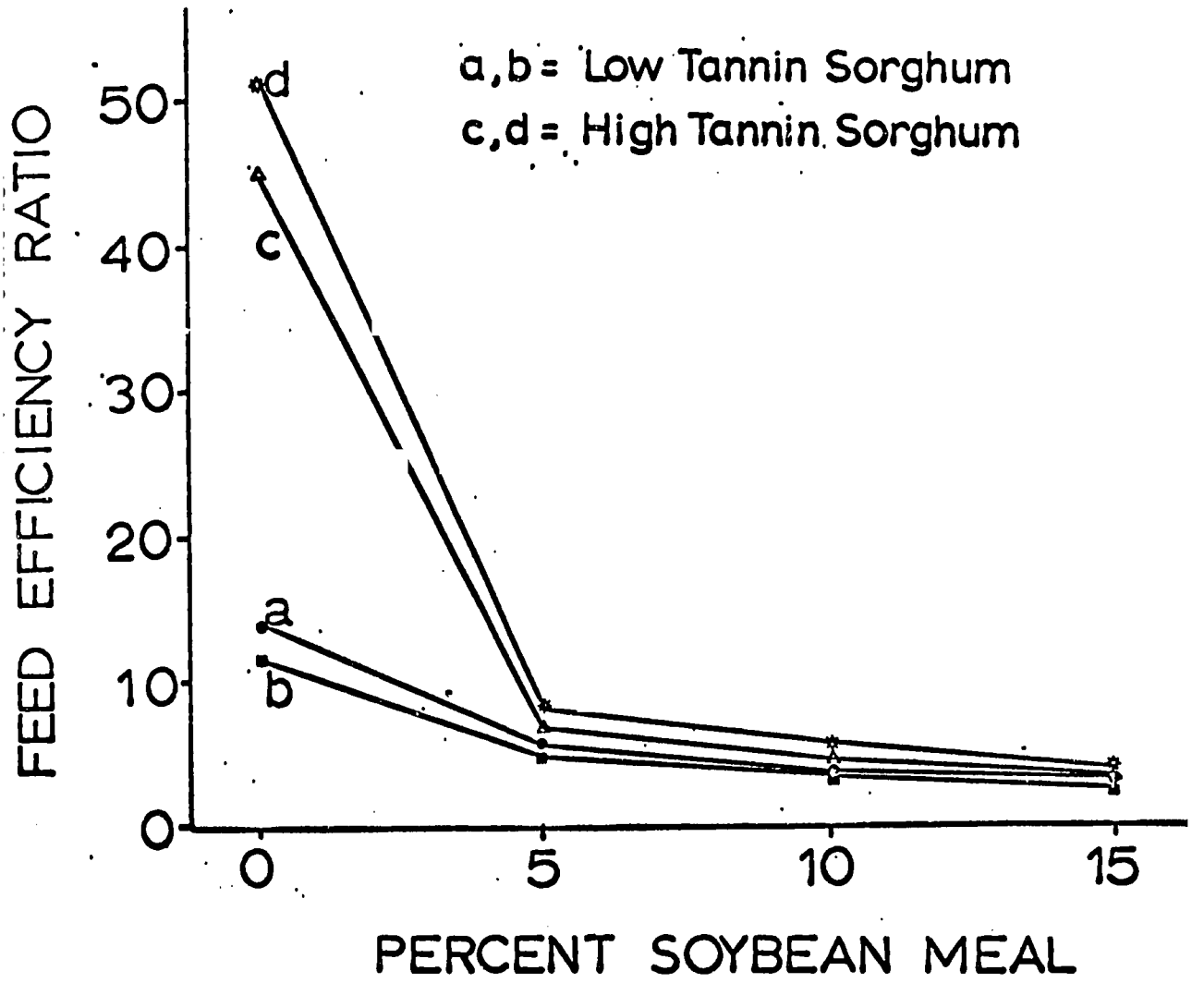

Figure 6. Feed Efficiency Ratios for Rats Fed 13 Days.