AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY

1. SUBJECT CLASSIFICATION

A. PRIMARY: Agriculture — AF30-0170-0000

B. SECONDARY: Plant breeding--Cereals--Sorghum

2. TITLE AND SUBTITLE

Genetic improvement of biological efficiency and protein quality in Sorghum

3. AUTHOR(S)

Axtell,J.D.

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1974	22p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS

Purdue

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*

(Presented at 29th Annual Corn and Sorghum Industry-Research Conf.,Chicago)

9. ABSTRACT

10. CONTROL NUMBER

PN-RAA-442

11. PRICE OF DOCUMENT

12. DESCRIPTORS

Lysine
Mutations
Proteins
Sorghum

13. PROJECT NUMBER

14. CONTRACT NUMBER

CSD-1175 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

11/13/74 Paper presented at the American Seed Trade meetings in Dec., 1974 - Chicago

John

GENETIC IMPROVEMENT OF BIOLOGICAL EFFICIENCY AND PROTEIN QUALITY IN SORGHUM[1]

J. D. AXTELL

PURDUE UNIVERSITY, WEST LAFAYETTE, INDIANA

Grain sorghum is an important human food in many parts of the world especially in Africa and Asia. Unfortunately, the nutritional quality of most of the grain sorghum varieties being used today is poor relative to that of other major cereals. The purpose of this report is to summarize our research on some of the problems associated with the poor nutritional quality of sorghum, and to review some of our recent research in this area. This report is not intended to provide experimental details which are available by reference from technical publications.

We have been studying two major components of nutritional quality in grain sorghum. First, 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

---

[1] Journal paper no._____, Purdue University Agricultural Experiment Station, Department of Agronomy. Research supported by the United States Agency for International Development under a contract entitled "Inheritance and Improvement of Protein Quality and Content in Sorghum".

are referred to generically as "tannins". Second, the protein quality of sorghum is limited by the low lysine content of the grain which reflects the high prolamine content of the endosperm. If one looks at the essential amino acid composition of sorghum grain, in comparison with monogastric nutritional requirements, it is obvious that lysine is deficient and also that there is great excess of leucine. The methionine content of sorghum is low, but considering the cystine content of 1.5%, the overall sulfur amino acid content approaches that of the weanling rat requirement. The tryptophan content of sorghum seems to be adequate in contrast to the low tryptophan content in normal maize.

TANNINS

Based on this data one would predict that the biological value of any particular variety of sorghum grain would be directly proportional to its lysine content. This, in fact, is true for low tannin sorghum genotypes. The data in Figure 1, however, illustrates that lysine is not the first limiting component of biological value for a group of high tannin sorghum lines from the world collection (Axtell et al. 1972). There is an important interaction between tannin content and protein quality in sorghum which is not found in any of the other major cereals. Cummings and Axtell (1973) demonstrated this experimentally by feeding whole seed and dehulled high tannin sorghum grain IS 8260 as shown in Figure 2. Rat growth is poor for the whole grain IS 8260 with and without supplemental lysine. In contrast, dehulling IS 8260

grain improves biological value substantially, and also allows a significant rat growth response to the addition of supplemental lysine. A similar technique was used in this study to determine the quantitative effects of tannins on biological value. Using varying proportions of high tannin sorghum mixed with its dehulled low tannin counterpart we were able to provide diets containing a range in tannin content which were essentially isogenic comparisons. Data from this study is shown in Figure 3. The biological value as measured by rat weight gain is significantly reduced by increasing levels of tannin in the diet. We can conclude from this data that tannins reduce the biological value of grain sorghum for monogastric animals. The cause of this reduction is not completely understood. Our present hypothesis is that the seed proteins become complexed or bound with the tannins of the whole grain, and that the complexed proteins are substantially less available for utilization by monogastric animals. It is very important to recognize this tannin interaction with protein quality when assessing the biological value of any grain sorghum variety, since failure to consider the biological effects of tannins has led to substantial confusion in past studies of sorghum nutritional quality.

HIGH LYSINE SORGHUM - ETHIOPIA

In 1972 we initiated two approaches which we hoped would lead to the identification of sorghum lines with increased lysine content. The first of these approaches involved cross sectioning of approximately

9,000 lines of sorghum from the world collection, and screening these sectioned kernels for floury endosperm lines (Singh and Axtell, 1973). Using this procedure we identified 62 floury endosperm lines and evaluated each for lysine and protein content. Most of these floury endosperm lines were normal for lysine content in relation to their protein content. There were two floury endosperm lines, however, which had a high lysine content at relatively high levels of protein. IS 11167 and IS 11758 were both collected from Wollo Province in Ethiopia during the 1960's. The protein and lysine content of these two lines was subsequently verified in Puerto Rico, and the average of these determinations is shown in Table 1 in comparison with the average for several hundred normal sorghum lines from the world collection. The significant point is that lysine content when expressed as percent of sample is approximately double that of normal sorghum.

The reason for this increase in lysine can be understood by looking at the distribution of endosperm proteins by solubility fractionation using the Landry-Moureaux procedure (1970). The distribution of protein in the five solubility fractions for normal sorghum is shown in Table 2, along with the lysine content of each of the five fractions (Jambunathan and Mertz, 1973). Over 50% of the endosperm protein in sorghum is present in fractions II and III, which are both lysine-poor fractions. Fractions II and III are generally referred to as the prolamine fractions. Note that fractions I and V are relatively high in lysine. The nitrogen distribution in normal and high lysine sorghum endosperm

tissue using the same procedure has been obtained by Jambunathan (unpublished data). The high lysine seed used in this determination is an average of five high lysine lines collected from farmers fields in Ethiopia, whereas the normal control was from a normal variety grown in the same environment. There is a substantial reduction in the amount of protein in fractions II and III among the high lysine lines, accompanied by an increase in the proportion of protein in the lysine-rich fractions I and V. The reduction in prolamine from approximately 55% to 39% largely accounts for the increased lysine content of the high lysine Ethiopian lines. The high lysine content of these Ethiopian lines is controlled by a single gene which has been designated hl. The biological value of the Ethiopian high lysine sorghum grain in comparison with normal has been reported (Singh and Axtell, 1973). Data obtained by feeding high lysine and normal samples at isonitrogenous levels (10% protein) to weanling rats in a 28-day trial demonstrated that rats fed the high lysine ration increased in weight approximately two to three times more than rats fed the normal control ration.

The kernel phenotype of the Ethiopian high lysine varieties is partially dented. This suggested the possibility that there may be a block in the biosynthesis of starch from water soluble polysaccharides similar to that found in shrunken, sugary, or brittle endosperm mutants in maize. The data in Table 4 shows the carbohydrate composition of both high lsyine lines and a normal control. There is no change in the quantity of reducing sugars and only a slight increase in the

amount of sucrose in the high lysine lines. Water soluble polysaccharides content is similar to that of normal, and there is only a two to three percent reduction in starch which can be accounted for by the slight elevation in the levels of sucrose. We concluded, therefore, that there is no disruption in starch synthesis from water soluble polysaccharides in hl genotypes and that it may be possible to identify plump seeded high lysine kernels in an appropriate genetic background.

Dallas Oswalt and I travelled to Wollo Province, Ethiopia, in November of 1973 to see if we could find these same high lysine varieties under cultivation, and if possible find out how the people were using them. In fact, we first found them growing within one or two kilometers of the site where they were originally collected, thanks to some excellent records and notes from Dr. Jack Harlan. Seeds of the high lysine varieties were collected in cooperation with Dr. Brhane Gebrekidan, who is in charge of the sorghum breeding program at Alemaya, and Mr. Gebisa Ejeta, a graduate assistant from Alemaya, who is now studying at Purdue. The high lysine types were always found growing in mixtures with normal sorghum varieties--a standard practice for most of the sorghum cultivated in Ethiopia. The local farmers could recognize and identify the high lysine varieties and reported to us that it was used primarily for roasting in the late dough stage. They also recognized that the yield of this variety was lower than that of their normal sorghum varieties, but they included it in the mixture because they liked the taste of the roasted grain.

HIGH LYSINE SORGHUM-INDUCED MUTATION

The second approach initiated in 1972 was the use of chemical mutagen treatments, specifically diethyl sulfate, to try to induce a mutant which would increase the lysine content of the sorghum grain. D. P. Mohan screened 23,000 M_3 heads during his Ph.D. thesis research at Purdue for the occurrence of opaque kernel mutants. All heads from treated seeds were bagged each generation to prevent outcrossing. Seeds from each M_3 head were placed over a light box and examined for segregating opaque kernels. Mohan identified approximately 475 heads segregating for opaque kernels, and both classes of seed from each segregating head were analyzed for protein and lysine content. The percent increase in lysine of these 475 putative opaque mutants is shown in Table 5. Most of the opaque mutants were normal in lysine content, but there were 33 with at least a 50% increase in lysine. The mutant from this group that appears to have the most promise at this time is P-721. The average protein and lysine content of P-721 opaque seed and normal sib seed is shown in Table 6. There is an approximately 60% increase in lysine in the P-721 mutant grain.

The inheritance of this opaque mutant is not yet clearly understood, but it appears to be controlled by a single gene which expresses partial dominance in the triploid sorghum endosperm. In some cases, F_2 segregation ratios fit a 1:1 ratio, but in others there is a deviation in favor of more opaque and fewer vitreous kernels. This ratio would be expected if two or three doses of the opaque gene produced

opaque kernel phenotypes, whereas one dose of the opaque gene in the triploid endosperm is recessive to normal vitreous expression. We expect that this ratio will vary with genetic background.

A comparison of the biological value of the Ethiopian high lysine sorghum with the newly recovered mutant P-721 is presented in Table 7. This data was obtained from two different feeding trials using a standard ANRC casein diet as a check which allows comparison between trials. RS 610 was used as a normal sorghum control in the IS 11758 trial, whereas normal sib seed of mutant P-721 was used as a normal control in the P-721 evaluation. All rations were isonitrogenous (10% protein) and were fed to weanling rats for 28 days. Feed efficiency ratios and protein efficiency ratios of both high lysine mutants appear to be similar, and we conclude from this preliminary experiment that the protein quality of both sources of high lysine sorghum germplasm is essentially the same. That is, both sources of high lysine germplasm give an approximately two-fold increase in protein quality in comparison with the casein control.

Since mutant P-721 was recovered in a photoperiod insensitive, three-dwarf line with relatively broad agronomic adaptability, this mutant may be most useful in temperate sorghum growing regions of the world. The increased protein content of the Ethiopian high lysine varieties, on the other hand, may provide useful sorghum germplasm for use as a protein supplement or weanling food for young children which is needed in many parts of Africa and Asia.

FUTURE RESEARCH

If we look at the prolamine content of the major cereals we can see that they fall into three rather distinct groups, as shown in Figure 4. Since the protein quality of cereals is inversely related to their prolamine content, these groups increase in protein quality as they decrease in prolamine content. This figure also shows the changes in prolamine content associated with high lysine mutat in three of these cereals. These changes appear to occur in two steps from higher to lower prolamine content. Dr. Oliver Nelson (1974) has suggested that this may be coincidence or it may mean that the regulation of prolamine synthesis is controlled by two major genes in these cereals. If the latter interpretation proves to be correct, it should be possible to find a "second-step" mutatation in sorghum (or corn) which will reduce the prolamine content to approximately 10%, with further concomitant increases in protein quality. This would provide us with a cereal grain which combines the photosynthetic efficiency and stress tolerance of sorghum with the protein and nutritional quality of oats or rice.

We can speculate still further into the future in our search for a more "perfect cereal" and ask the following question. Will it be easier to breed for increased photosynthetic efficiency in rice or oats or to take the alternative approach and breed for increased nutritional quality in sorghum and corn which already have the genetic machinery for a more efficient photosynthetic pathway? The answer will depend on

which biochemical process is easiest to manipulate genetically, which in turn can only be decided when we understand the genetic control of these important biochemical pathways in cereal grains.

LITERATURE CITED

1. Axtell, J. D., D. L. Oswalt, E. T. Mertz, and R. C. Pickett. 1974. Components of nutritional quality in grain sorghum. Proceedings CIMMYT-Purdue International Symposium on Protein Quality in Maize. El Batan, Mexico. (In press)

2. Cummings, D. P. and J. D. Axtell. 1973. Effect of tannin content of sorghum grain on nutritional quality. Research Progress Report on Inheritance and Improvement of Protein Quality and Content in Sorghum. Purdue University, pp. 85-111.

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

4. Landry, J. and T. Moureaux. 1970. Heterogeneite des glutelines du grain de mais: Extraction selective et composition and acides amines des trois fractions isolees. Bull. Soc. Chim. Biol. 52:1021.

5. Nelson, O. E. Jr., 1974. Interpretive summary and review. Presented at the workshop on genetic improvement of seed proteins, National Academy of Sciences, Washington, D. C.

6. Singh, Rameshwar and J. D. Axtell. 1973. High lysine mutant gene (hl) that improves protein quality and biological value of grain

sorghum. Crop Science 13:535-539.

Table 1. Protein and lysine concentration of whole grain samples of high lysine and normal sorghum lines.

Protein Composition	High Lysine Lines		Normal
	IS 11167	IS 11758	
Protein (%)	15.70	17.20	12.70
Lysine (% of protein)	3.33	3.13	2.05
Lysine (% of sample)	0.52	0.54	0.26

Table 2. Protein and lysine distribution in normal sorghum endosperm.

Solubility Fraction*	Protein (%)	Lysine (% of protein)
I	7.3	5.1
II	16.8	0.3
III	32.4	0.3
IV	7.8	1.2
V	25.3	3.6

* Landry-Moureaux procedure.

Table 3. Protein distribution in normal and Ethiopian high lysine sorghum endosperm.

Solubility Fraction*	Protein (%) Normal	Protein (%) High Lysine (hl)
I	6.33	13.58
II	19.16	16.54
III	36.26	23.02
IV	6.65	4.00
V	25.60	33.86

* Landry-Moureaux procedure.

Table 4. Carbohydrate composition of whole kernel samples of Ethiopian high lysine and normal sorghum lines.

Carbohydrate Composition	High lysine lines		Normal
	IS 11167	IS 11758	
Reducing sugars	0.38	0.32	0.34
Sucrose	3.08	2.61	1.03
Total sugars	3.46	2.93	1.34
Water soluble polysaccharides	0.91	1.01	1.11
Starch (% of sample)	58.90	57.80	60.80
Total carbohydrates	63.27	61.74	63.25

Table 5. Distribution of lysine increases (% of sample) of 475 putative opaque endosperm sorghum mutants derived from diethyl sulfate treatments.

Percent increase in lysine	Number of putative opaque mutants
0 - 9	135
10 - 19	114
20 - 29	85
30 - 39	53
40 - 49	25
> 50	33

Table 6. Protein and lysine composition of high lysine opaque mutant P-721 and normal sib grain.

	Protein (%)	Lysine (% of protein)	Lysine (% of sample)
P-721 opaque	13.9	3.09	0.432
Normal sib	12.9	2.09	0.270

Table 7. Biological value of isonitrogenous (10% protein) diets of Ethiopian (IS 11758) and P-721 high lysine sorghums and their respective normal checks, RS 610 and normal sib grain in a 28-day weanling rat feeding experiment.

	Weight Gain (g) (28 days)	FER	PER	% of Casein
IS 11758	40	5.8	1.7	58
RS 610	16	10.5	0.9	32
P-721	33	5.7	1.8	64
Normal Sib	11	10.6	0.9	32

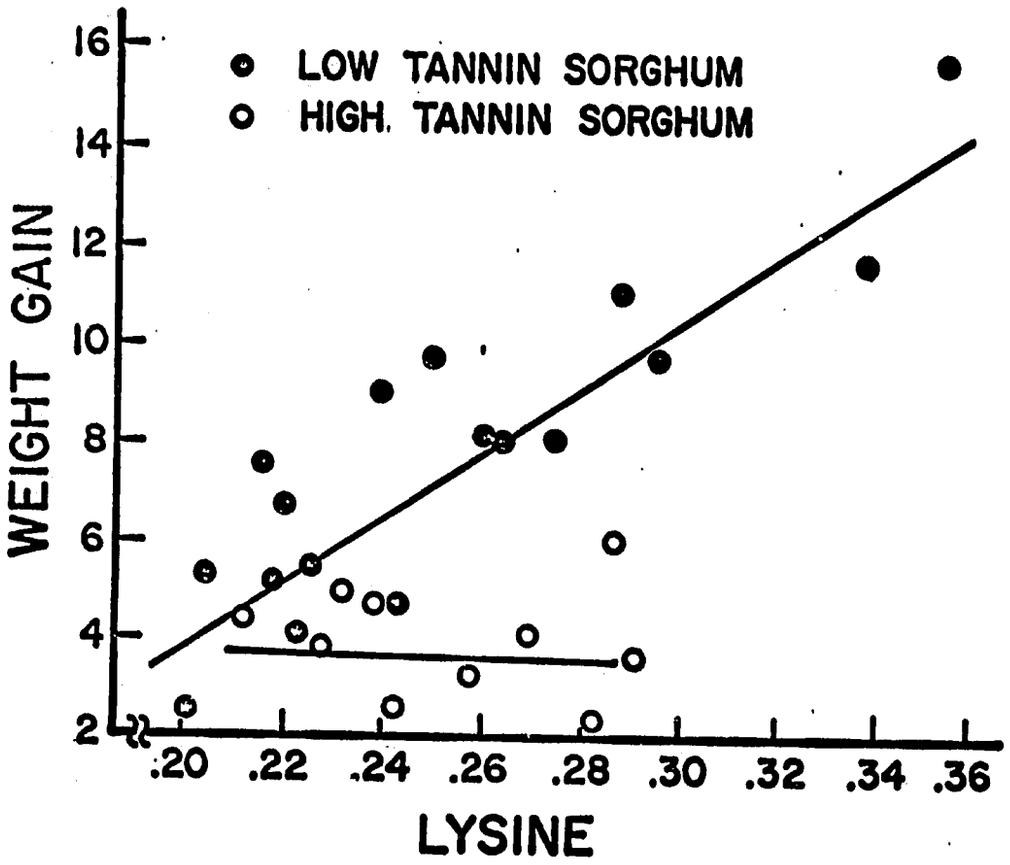

Figure 1. Relationship of biological value to lysine concentration (g/100g sample) in high and low tannin sorghum lines from the world collection in a 14-day weanling rat feeding experiment.

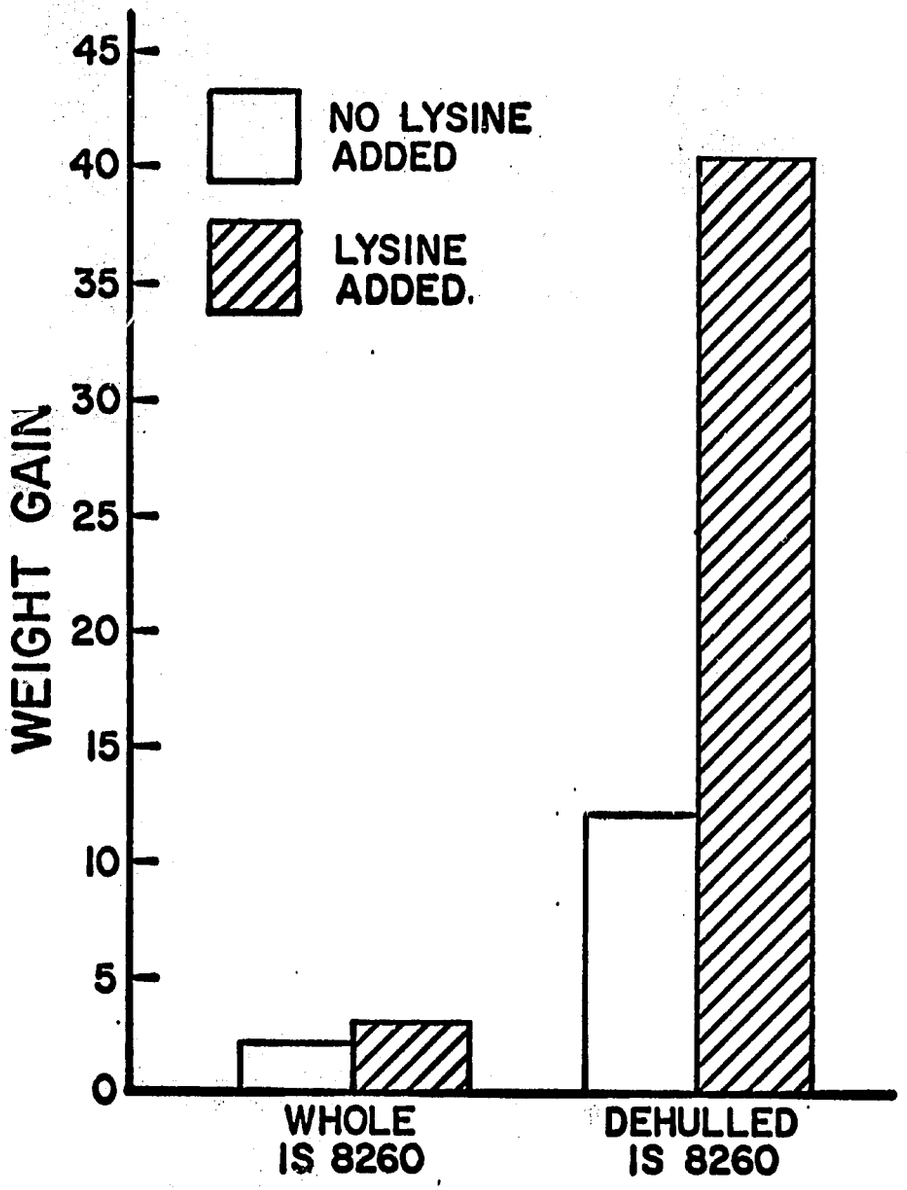

Figure 2. Biological value of whole and dehulled grain of high lysine sorghum line IS 8260 when fed to weanling rats with and without lysine supplementation in a 14-day feeding experiment.

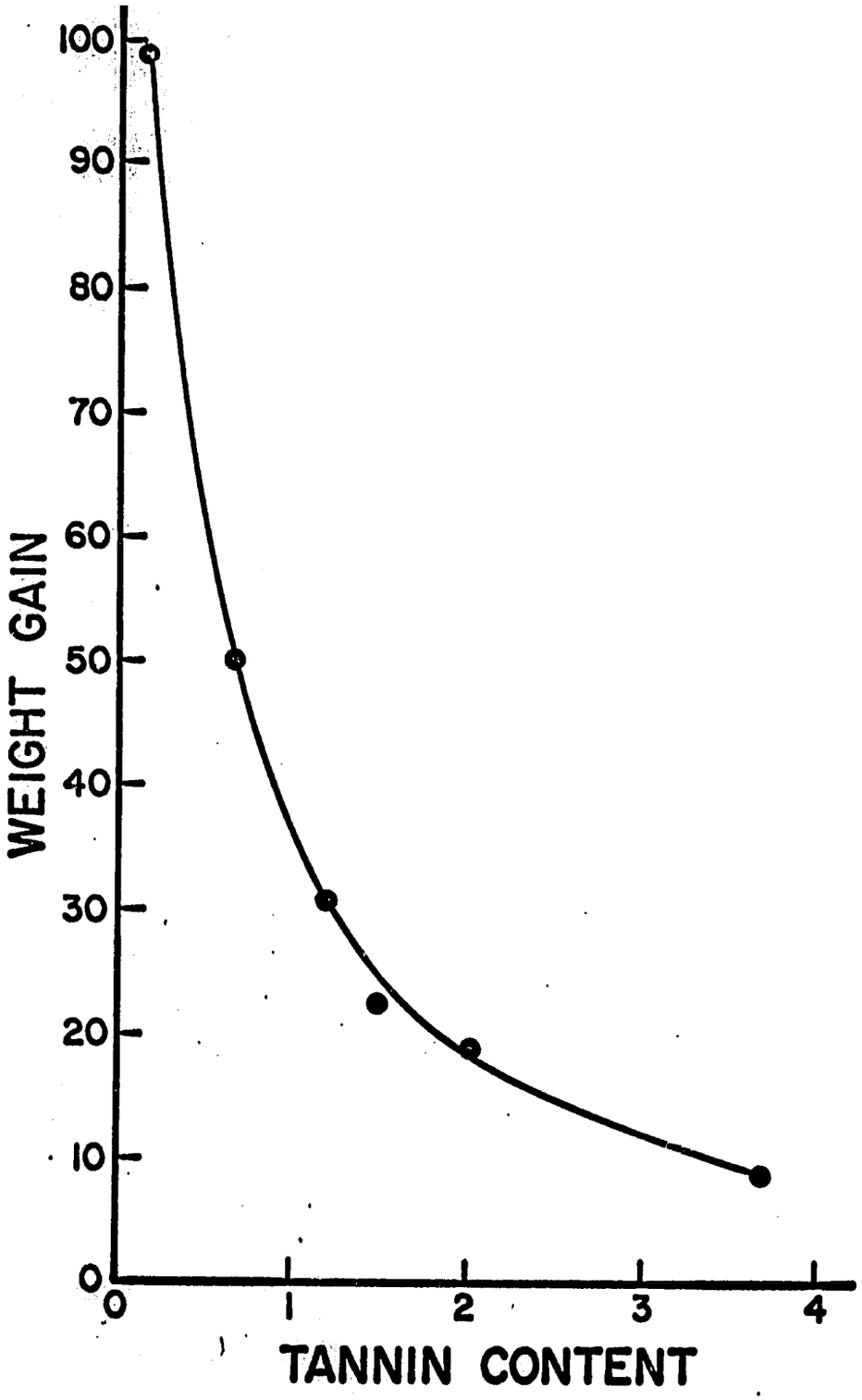

Figure 3. Effect of tannin content (expressed as catechin equivalents) on the biological value of sorghum grain in a 28-day weanling rat feeding experiment.

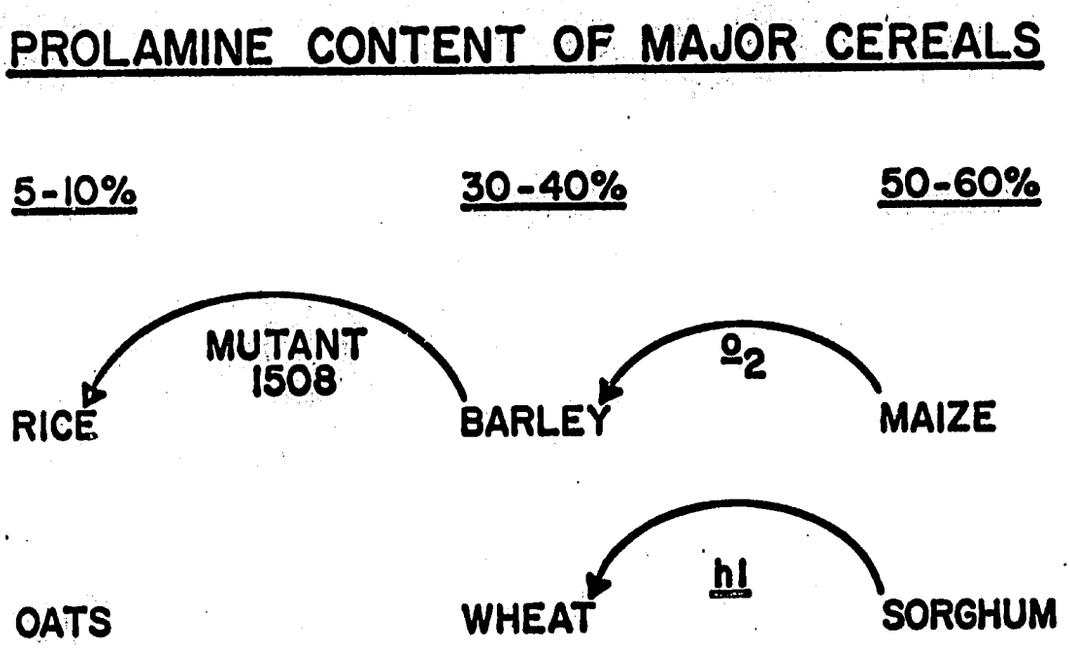

Figure 4. Effect of high lysine gene mutations in maize (o_2), sorghum (hl), and barley (1508) on prolamine content.