613.2

Csd-1175
(4)
Abst. 8

Reprinted from
CROP SCIENCE, Volume 13
September-October, 1973, p. 535-539

High Lysine Mutant Gene (*hl*) that Improves Protein Quality and Biological Value of Grain Sorghum

Rameshwar Singh and John D. Axtell

High Lysine Mutant Gene (*hl*) that Improves Protein Quality and Biological Value of Grain Sorghum[1]

Rameshwar Singh and John D. Axtell[2]

ABSTRACT

Seeds from over 9,000 lines in the world sorghum [*Sorghum bicolor* (L.) Moench] collection were classified for endosperm phenotype to identify floury endosperm lines and evaluate each for potential increases in lysine concentration. Sixty-two floury endosperm lines were selected and analyzed for protein and lysine composition. Two floury lines of Ethiopian origin, IS 11167 and IS 11758, were exceptionally high in lysine at relatively high levels of protein.

The average whole grain lysine concentration of high lysine lines IS 11167 and IS 11758 was 3.34 and 3.13 (g/100g protein) at 15.7 and 17.2% protein, respectively. Both lines were also high in percent oil. Carbohydrate analyses of whole grain samples of the two high lysine lines were similar to that of normal sorghum grain except for a twofold increase in sucrose concentration.

The high lysine gene altered the amino acid pattern in *hl hl hl* endosperm tissue relative to normal endosperm checks. The major changes were increased lysine, arginine, aspartic acid, glycine, and tryptophan concentrations and decreased amounts of glutamic acid, proline, alanine, and leucine in the *hl hl hl* endosperm.

Inheritance studies suggest that the increased lysine concentration of each line is controlled by a single recessive gene, although it is not known whether the genes from both lines are allelic. The high lysine gene(s) present in IS 11167 and IS 11758 from Ethiopia is (are) herein designated as *hl*. The endosperm of kernels homozygous for the *hl* gene is partially dented.

The biological value of the high lysine lines was much higher than that of average sorghum lines. In a 28-day isonitrogenous feeding experiment the weight gain of weanling rats was three times higher on an IS 11758 ration and twice as high on an IS 11167 ration as weight gains on rations prepared from normal sorghum lines. When fed rations without any dilution except the usual 2% vitamin and 4% mineral supplementation, rats gained 94 g on high lysine sorghum (IS 11758) and 28.5 g on our current best nutritional quality sorghum line (IS 2319), versus 91.5 g on opaque-2 corn (*Zea mays* L.) and 30.2 g on normal corn in a 28-day feeding trial. Feed efficiency ratios for this trial were 3.0 for high lysine sorghum, 6.8 for IS 2319, 3.4 for opaque-2 corn, and 7.4 for normal corn.

Additional index words: Protein quality, Carbohydrate, PER, FER, Biological value, Cereal quality, Grain composition.

CONSIDERABLE progress has been made towards genetic improvement of plant protein quality in cereals. Opaque-2 and floury-2 mutant strains of maize (*Zea mays* L.), as well as high lysine mutant lines of barley (*Hordeum vulgare* L.), have been thoroughly investigated. This report describes a gene (or genes) that increases the relative amount of lysine in sorghum [*Sorghum bicolor* (L.) Moench]. The discovery of these mutant genes has opened new horizons for upgrading the nutritional quality of sorghum grain.

The importance of nutritional improvement of grain sorghum can be emphasized by the fact that it is the fourth most important cereal crop in the world. However, the nutritional quality of normal sorghum protein is not very good. The occurrence of several human diseases has been associated with the poor nutritional quality of sorghum. The presence of relatively high concentrations of leucine and/or imbalance in the leucine:isoleucine ratio in sorghum has been suggested as a possible factor in the development of pellagra in populations subsisting principally on this crop (Gopalan and Srikantia, 1960). Lysine has been re-

[1] Journal Paper No. 5176, Purdue University Agricultural Experiment Station. Part of a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree. Research was supported by the United States Agency for International Development through Contract 1175 and by a Rockefeller Foundation Graduate Scholarship. Received July 9, 1973.

[2] Former Rockefeller Scholar and Graduate Student, Department of Agronomy, Purdue University (now Junior Research Officer, Sorghum and Millets, G. B. Pant University of Agriculture and Technology, Pantnagar, Nainital, U.P., India) and Associate Professor of Genetics, Department of Agronomy, Purdue University, Lafayette, Indiana 47907.

ported as the first limiting amino acid in sorghum (Shelton et al., 1951).

Howe, Jansen, and Gilfillan (1965) compared the proteins of rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), corn, millet (*Panicum* spp.), sorghum, barley, rye (*Secale cereale* L.) and oats (*Avena sativa* L.) as such, and with lysine and threonine added, in the nutrition of albino rats. Casein at 9.05% protein was used as a check. Four-week weight gains of rats and protein efficiency ratios (PER) of different diets showed that millet and sorghum were the lowest in nutritional quality. Corn protein was more than twice as high as sorghum for weight gain and PER ratios. The casein diet was about twice as good as corn and four times better than sorghum at similar levels of protein. Trible (1971) found that the digestibility of sorghum protein was significantly lower than that of corn or wheat.

Published information concerning genetic improvement of protein quality is not presently available for sorghum. Opaque-2 was the first mutant gene found to change the normal amino acid composition of maize endosperm proteins (Mertz, Bates, and Nelson, 1964). An increase in lysine content of opaque-2 kernels was caused by a reduction in the proportion of alcohol soluble proteins or zein, along with an increase in the proportion of albumin, globulin, and glutelin protein fractions in endosperm tissue. The opaque-2 gene, which is inherited as a simple recessive, affects only the amino acid composition of the endosperm with no effect on the embryo (Nelson, 1969). A second mutant gene affecting lysine content (floury-2) was reported in 1965 (Nelson, Mertz, and Bates, 1965).

Munck, Karlsson, and Hagberg (1969) reported a high lysine, high protein barley line called Hiproly. It was selected from the world barley collection using dye-binding capacity as a screening technique. A recessive gene was responsible for the approximately 20 to 30% increase in lysine concentration.

Doll (1970) found two barley mutants with increased lysine content from ethylmethane sulfonate-treated material. Recently Ingversen et al. (1972) identified an ethyleneimine-induced high lysine mutant (Risö 1508) in barley. Lysine content of Risö mutant 1508 grain was as much as 51% higher than the parental variety. The increase was due to a decrease of the lysine deficient prolamin fraction and an increase in the lysine-rich albumin and globulin fraction at a constant level of glutelin.

Munck (1972) has described various environmental factors that affect the ultimate nutritional quality of barley seed. He reported that the lysine-rich fraction (albumins and globulins) is synthesized at a faster rate during early stages of seed formation, whereas lysine-poor storage proteins (such as prolamins) dominate later stages of protein synthesis. Glutelins, which are intermediate in lysine content, increase linearly with time during seed development. Thus, factors such as seed size and seed maturation can affect the overall amino acid composition of the grain.

The objectives of this study were 1) to identify lines with a floury endosperm phenotype from the world sorghum collection and evaluate samples of each line for protein and lysine composition, 2) to determine the mechanism of inheritance of improved lysine content of selected high lysine lines, and 3) to ascertain the biological value of lines with superior protein quality using weanling rat feeding experiments.

MATERIALS AND METHODS

Over 9,000 lines from the world sorghum collection were screened for the floury endosperm phenotype by examination of longitudinal sections of 15 to 20 kernels from each line. Defatted whole kernel samples of each floury endosperm line were analyzed for nitrogen by the micro-Kjeldahl procedure and converted to percent protein by multiplying nitrogen values by a factor of 6.25. Protein content is expressed as percent of dry sample. Total carbohydrate was calculated by adding values for reducing sugars, sucrose, water-soluble polysaccharides (WSP), and starch. The procedure described by Shannon (1968) was used for the determination of reducing sugars, sucrose, and WSP. Amylose and starch were determined by the procedure of Shuman and Plunkett (1961). Amino acid analysis was obtained by ion-exchange resin chromatography using a Beckman 120C amino acid analyzer. Methionine and cystine were determined as methionine sulfone and cysteic acid using the method described by Moore (1963), and tryptophan was determined by the method of Slump and Schreuder (1969). Endosperm tissue for amino acid analysis was obtained by hand dissection of whole kernels without removal of the pericarp. Percent oil was determined by nuclear magnetic resonance at the University of Illinois, Urbana, Illinois.

PP3R (ms_3), a Purdue random mating sorghum population, was used as the "normal" parent in the inheritance studies.

Two rat feeding experiments were performed to evaluate the biological value of the two high lysine, floury endosperm lines. In Experiment 1, male weanling Wistar rats with similar initial weights were used to evaluate six different isonitrogenous diets. Rats were arranged in groups of six to make their average weight close to the overall mean weight and then placed in individual wire-mesh cages. Experimental diets were fed to each rat for 28 days. Individual weight gains and feed consumption were measured on days 7, 14, 21, and 28. Special feed cups minimized the feed spillover. Rats were offered food and water *ad libitum* with water changed on alternate days. The feed efficiency ratio (grams of feed consumed per gram of body weight gain) and protein efficiency ratio (grams of weight gain per gram of protein consumed) were calculated for each rat.

Grain samples used for this feeding trial were grown in Puerto Rico during the 1972-73 winter season. Two high lysine, floury endosperm lines were used in this experiment along with three normal sorghum lines as checks. All feeds were brought to approximately 10.0% protein by the addition of corn starch. Crude protein in these rations ranged from 10.0 to 11.2% with a mean of 10.5%. A casein diet was also fed for comparison. Grain samples were ground twice in a coffee grinder. Each 100 g of basal diet consisted of varying amounts of ground sorghum grain and corn starch plus 4 g of mineral mixture (Hawk-Oser salt mixture number three, Nutritional Biochemicals, Inc., Cleveland, Ohio) and 2 g of vitamin mixture (Vitamin supplement, General Biochemicals, Chagrin Falls, Ohio). Protein and lysine were determined for both whole grain samples and prepared diets.

Experiment 2 was planned to compare the biological value of one high lysine with two normal sorghum lines, normal corn, and opaque-2 corn at their inherent grain protein levels with 4% mineral and 2% vitamin supplementation. A sorghum line with high nutritional quality, IS 2319, and another high protein (14%) line, IS 1484, were used as normal sorghum check lines. A casein diet at 17% protein was also used as a check. Other experimental conditions were similar to those in Experiment 1.

The experimental design of both Experiments 1 and 2 was a randomized complete block design with six replications. Data obtained were analyzed statistically by methods given by Cochran and Cox (1957).

RESULTS AND DISCUSSION

Sixty-two floury endosperm lines were identified from the world sorghum collection on the basis of endosperm phenotype. Floury grains have a soft, chalky white endosperm when longitudinal sections are examined. It is important to use fully mature ker-

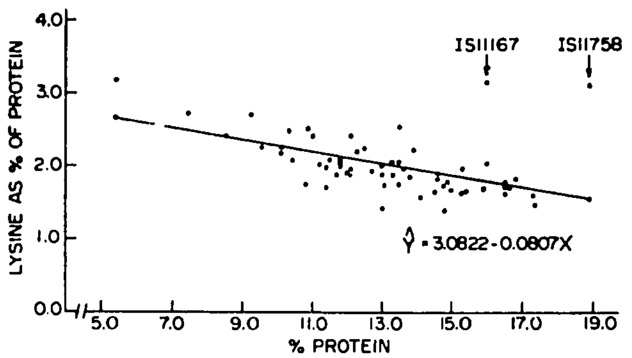

Fig. 1. Regression of lysine (single analyses) on percent protein for 62 floury endosperm lines selected from the world sorghum collection.

nels for this classification, because seed of normally vitreous genotypes may also appear floury when immature. The protein and lysine concentrations of whole grain samples from single analysis of each of these selected lines are shown in Fig. 1. Two floury lines, IS 11167 and IS 11758, of Ethiopian origin were markedly different from the other lines. Most of the floury endosperm sorghum lines, however, had normal amounts of protein and lysine. This parallels the situation in corn, where many genes express floury/opaque endosperm phenotypes but only opaque-2, opaque-7, and floury-2 have a pronounced effect on lysine content (Nelson et al. 1965; McWhirter, 1971).

The chemical composition and seed characteristics of whole grain samples of IS 11167 and IS 11758 in comparison with averages of normal sorghum lines are presented in Table 1. The lysine concentration (percent of sample) of both high lysine lines was approximately double that of the average for sorghum. Tannin (expressed as catechin equivalents) in both Ethiopian high lysine lines was low, and fell below the level known to interfere with protein availability in monogastric animals (Oswalt, 1973). The high percent oil and increased percent germ of the high lysine grain may be in part a consequence of the dented endosperm phenotype. Even though the endosperm of IS 11167 and IS 11758 was partially dented, the 100-seed weight of both lines was nearly equivalent to the average of 31 lines and hybrids of normal sorghum. Several endosperm mutants with shrunken kernel phenotypes have been studied in maize and all are characterized by a variable but substantial reduction of starch content in endosperm tissue, with a concomitant accumulation of total sugars and/or water soluble polysaccharides (Creech, 1968; Barbosa, 1971). The data (Table 1) demonstrate that the starch concentration of whole grain samples of both high lysine lines was nearly equivalent to that of the normal check line. There was no change in the relative amounts of reducing sugars or water soluble polysaccharides in the high lysine lines, but there was a twofold increase in sucrose concentration. An increase in sucrose of similar magnitude has also been reported for opaque-2 corn (Barbosa, 1971). The dented endosperm phenotype associated with the high lysine characteristic in the original Ethiopian sorghum lines, and also in F_1 panicles segregating for the *hl* gene, may be due to a pleiotropic effect of the *hl* gene itself or, alternatively, it may be a consequence of linked modifier genes that alter the normal plump configuration of the sorghum grain. The complete absence of vitreous starch in *hl hl hl* endosperm tissue may influence the normal conformation of the grain.

Table 1. Chemical composition and seed characteristics of whole grain samples of high lysine and normal sorghum lines.

Character	High lysine lines*		Normal sorghum
	IS 11167	IS 11758	
Protein composition			
Protein, %	15.70	17.20	12.70†
Lysine, g/100 g protein	3.33	3.13	2.05†
Lysine, % of sample	0.52	0.54	0.26†
Protein per seed, mg	4.38	4.21	3.53†
Lysine per seed, mg	0.15	0.13	0.07†
Chemical composition			
Catechin equivalent value	0.34	0.37	0.38‡
Oil, %	5.81	6.61	3.32†
Seed characteristics			
Percent germ	14.60	16.30	10.10§
Seed weight, g/100 seeds	2.78	2.45	2.75†
Carbohydrate composition			
Reducing sugars, % of sample	0.38	0.32	0.34¶
Sucrose, % of sample	3.08	2.61	1.03¶
Total sugars, % of sample	3.46	2.93	1.34¶
WSP, % of sample	0.91	1.01	1.11¶
Starch, % of sample	58.90	57.80	60.80¶
Amylose, % of starch	25.00	26.20	25.00¶
Total carbohydrates, % of sample	63.27	61.74	63.25¶

* Protein composition data are based on the averages of six analyses per line. Chemical composition, seed characteristics and carbohydrate composition data are based on single analysis. † Average of 31 genotypes over six locations and two years (Schaffert, 1972). ‡ Average of 300 low tannin lines in the world sorghum collection (Axtell et al., 1973). § Average of four lines (Jambunathan and Mertz, 1973). ¶ Values based on one normal line (IS 8313).

Table 2. Segregation ratios of F_2 kernels from crosses between normal (PP3Rms_3) and high lysine sorghum lines.

F_1 cross		Number of F_2 kernels				
Female	Male	Vitreous	Floury	Total	X^2*	Prob.
PP3Rms_3 ×	IS 11167	3,593	1,167	4,760	0.592	0.25-0.50
PP3Rms_3 ×	IS 11758	2,376	815	3,191	0.497	0.25-0.50

* Based on 3:1 expected ratio.

Phenotypic classification of kernels was carried out on F_2 seeds derived from crosses between genetic male sterile "normal" plants (PP3Rms_3) and each high lysine sorghum line. Normal (low lysine) kernels were plump with a vitreous endosperm while high lysine kernels were floury with a partially dented endosperm. All F_1 seeds obtained from ms_3 × IS 11167 and ms_3 × IS 11758 crosses had a vitreous endosperm. Chi-square analysis of F_2 segregation ratios (Table 2) indicated a good fit, 3 vitreous:1 floury ratio, for progeny of both crosses. The results of this study suggest that the high lysine character is inherited as a simple recessive.

Data on protein and lysine concentration of endosperm tissue from vitreous and floury F_2 kernels borne on F_1 panicles (Table 3) provide critical evidence of

Table 3. Protein and lysine concentration of defatted endosperm tissue of segregating F_2 vitreous and floury kernel classes derived from crosses between normal (PP3Rms_3) and high lysine sorghum lines.

Cross	Panicle number	F_2 seed class	% protein	Lysine (g/100 g protein)	Lysine (% of sample)
PP3Rms_3 × IS 11167	1	Vitreous	9.8	1.63	0.165
	1	Floury	10.1	2.63	0.286
	2	Vitreous	10.6	1.37	0.145
	2	Floury	11.2	2.24	0.251
Mean		Vitreous	10.2	1.52	0.155
		Floury	10.6	2.53	0.268
PP3Rms_3 × IS11758	1	Vitreous	12.2	1.10	0.134
	1	Floury	14.8	2.29	0.389
	2	Vitreous	10.4	1.36	0.141
	2	Floury	11.9	2.78	0.331
Mean		Vitreous	11.3	1.23	0.137
		Floury	13.3	2.54	0.360

Table 4. Amino acid (duplicate runs, g/100 g protein) and protein content of defatted endosperm tissue from segregating F_2 vitreous and floury seed classes derived from crosses between normal (PP3R*ms*$_3$) and high lysine sorghum lines.

Amino acid	PP3R*ms*$_3$ × IS 11167 Vitreous	PP3R*ms*$_3$ × IS 11167 Floury	PP3R*ms*$_3$ × IS 11758 Vitreous	PP3R*ms*$_3$ × IS 11758 Floury
Lysine	1.20	2.18	1.30	2.59
Histidine	1.99	1.91	1.94	2.04
Arginine	2.55	3.66	2.77	4.44
Aspartic acid	5.56	7.49	6.14	7.24
Threonine	2.56	3.01	2.75	3.11
Serine	3.74	4.09	3.84	3.99
Glutamic acid	26.68	23.98	27.99	20.82
Proline	7.92	6.91	8.35	6.39
Glycine	2.17	2.97	2.37	3.40
Alanine	10.15	9.65	10.50	8.68
Cystine	2.07	1.52	1.83	1.76
Valine	4.35	4.98	4.71	4.76
Methionine	1.60	1.52	2.11	1.93
Isoleucine	3.90	4.16	4.05	3.99
Leucine	15.72	14.33	15.93	12.62
Tyrosine	4.28	4.32	4.54	4.24
Phenylalanine	5.43	5.45	5.52	5.24
Tryptophan	0.92	1.21	0.93	1.74
Percent protein	9.75	10.75	9.69	12.06
Leucine/Isoleucine	4.03	3.44	3.93	3.16

the effect of the allele on lysine, because genetic background differences should be randomized between normal and mutant classes. The average lysine concentration (expressed as percent of protein) of the endosperm of vitreous kernels from both crosses was 1.38% compared to 2.53% for the endosperm of floury kernels, which represents an average increase in lysine of 83%. The increases are more pronounced when lysine is expressed as percent of dry sample because of the consistently higher percent protein of floury endosperm tissue. The protein and lysine concentrations of embryo tissue from segregating F_2 normal and floury mutant kernels was 24.1 vs 24.4% protein and 5.11 vs 5.60% lysine (expressed as percent of protein), respectively. Therefore, the primary effect of the *hl* allele on protein and lysine concentrations of sorghum kernels was confined to endosperm tissue.

The amino acid compositions of defatted endosperm tissue of grain from vitreous and floury classes from segregating F_2 seeds are presented in Table 4. Lysine, arginine, aspartic acid, glycine, and tryptophan were consistently higher in the floury endosperm class. Glutamic acid, proline, alanine, and leucine were lower in the floury endosperm class. The shift in amino acid pattern in the floury endosperm class was comparable to that reported for the opaque-2 mutant in corn (Nelson, 1969).

The data suggest that the increased amount of lysine in IS 11167 and IS 11758 is controlled by a single recessive gene. Because the high lysine genes present in both Ethiopian lines were similar in their effect on protein and lysine, the gene symbol *hl* is herein assigned to both pending completion of allelism tests.

Table 5. Biological values of isonitrogenous (10% protein) diets prepared with high lysine and normal sorghum lines in a 28-day rat feeding experiment.

Source	Composition of grain: % protein	Composition of grain: Lysine (g/100 g protein)	Composition of feed: % protein	Composition of feed: Lysine g/100 g protein	Composition of feed: Lysine % of sample	Weight gain (g)	FER	PER
High lysine lines								
IS 11167	16.6	3.36	10.1	2.81	0.284	34.5	5.6	1.78
IS 11758	18.0	3.38	10.0	3.15	0.315	48.8	4.9	2.06
Normal lines								
IS 2319	12.7	2.30	10.9	2.25	0.245	25.3	7.5	1.24
IS 2520	13.3	1.85	11.2	1.76	0.197	10.3	13.3	0.61
IS 1269	14.8	2.10	10.7	2.01	0.215	14.0	13.3	0.74
Mean of normal lines	13.6	2.08	10.9	2.01	0.219	16.5	11.3	0.86
Casein	91.0	8.00	13.3	7.36	0.979	85.8	3.5	2.20
C.V., %						31.0		
S.E. of individual line mean						4.6		
LSD 5% (between individual line means)						13.4		
LSD 1% (between individual line means)						18.2		

Biological Value

Experiment 1. Based on their improved amino acid composition, the high lysine sorghum lines should have a higher biological value than the average sorghum. Therefore, the biological value of two high lysine lines, three normal sorghum lines, and a casein-bearing diet were compared. IS 2319 has good nutritional quality and was used as the best check line in nutritional studies at Purdue (Oswalt, 1973). All grain rations were fed as isonitrogenous diets at approximately 10% protein except for the casein diet, which was fed at 13.3% protein.

Data on the chemical composition of whole grain samples and composition of the rations, as well as rat weight gain, feed efficiency ratio (FER), and protein efficiency ratio (PER), are given in Table 5. The average initial weight of weanling rats was 47.2 g. There was no significant difference in average initial weight between groups of rats placed on different diets. Weight gain on the IS 11758 diet was nearly double that of IS 2319 and three times higher than the average of normal sorghum lines. Gain in weight of rats on the IS 11167 rations was 71% of gain on the IS 11758 ration. However, it was significantly ($P < .01$) superior to the control sorghum lines, and was 36% better than IS 2319 in terms of rat weight gain. The PER values for both high lysine sorghum lines were higher than the average PER for normal sorghum, but were lower than that for casein.

Experiment 2. This experiment was designed to compare the biological value of a high lysine sorghum line and other grain diets when fed at their inherent grain protein levels. Because sorghum grain is consumed by large numbers of people, it is important to evaluate the nutritional value of the whole grain in a monogastric test animal. IS 11758 was compared with two normal sorghum lines (IS 2319 and IS 1484), opaque-2 corn, normal corn, and a casein diet. Opaque-2 corn was included in this trial as a cereal with good nutritional quality. Protein and lysine concentrations of the rations and the 28-day rat weight gain and FER values are presented in

Table 6. Biological value of 94% whole grain rations prepared from high lysine sorghum, normal sorghum, opaque-2 corn, and normal corn in a 28-day rat feeding experiment.

Source	Composition of grain: % protein	Composition of grain: Lysine (g/100 g protein)	Composition of feed: % protein	Composition of feed: Lysine g/100 g protein	Composition of feed: Lysine % of feed	Weight gain (g)	FER
High lysine line							
IS 11758	18.4	3.38	18.4	3.17	0.583	94.2	3.0
Normal sorghum							
IS 2319	12.7	2.30	12.6	2.23	0.281	28.5	6.8
IS 1484	14.0	1.93	12.6	2.01	0.253	19.2	8.5
Mean for normal sorghum	13.3	2.11	12.6	2.12	0.267	23.9	7.6
Opaque-2 corn	12.5	4.00	12.1	3.89	0.471	91.5	3.4
Normal corn	9.4	2.73	8.6	2.90	0.249	30.2	7.4
Casein	91.0	8.00	17.2	6.42	1.104	181.2	2.0
C.V., %						16.7	
S.E. of individual line mean						5.0	
LSD 5% (between individual line means)						14.7	
LSD 1% (between individual line means)						19.9	

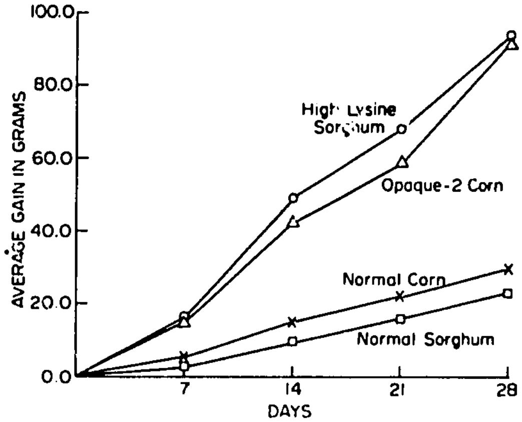

Fig. 2. Average weekly cumulative weight gains of weanling rats fed rations composed of 94% whole grain, 2% vitamins, and 4% minerals.

Table 6. Weight gains at 7-day intervals through the 28-day feeding trial are also given in Fig. 2.

The rat weight gains show that the biological value of IS 11758 was four times higher than the average of two normal sorghum lines and over three times higher than IS 2319 when fed at whole grain protein levels (Table 6, Fig. 2). When compared to the corn diets, the IS 11758 diet fed at 18.4% protein was equivalent to the opaque-2 corn diet containing 12.1% protein. The FER value of the high lysine sorghum ration indicated that less than half as much feed was required per unit gain than was needed on normal sorghum diets. These two experiments demonstrate unequivocally that the two Ethiopian high lysine sorghum lines are substantially superior in biological value to any sorghum line currently identified.

It should be emphasized that no information is available on the yield potential of IS 11167, IS 11758, or other *hl hl* lines or hybrids that may be derived from them in the future. The dented, floury endosperm phenotype may pose agronomic problems similar to those encountered in other cereals containing mutant genes for improved quality. The identification of a high lysine gene in IS 11167 and IS 11758, however, represents a significant step toward improvement of the nutritional quality of grain sorghum.

REFERENCES

Axtell, J. D., D. L. Oswalt, E. T. Mertz, R. C. Pickett, R. Jambunathan, and G. Srinivasan. 1973. Components of nutritional quality in grain sorghum. Int. Symp. Protein Quality Maize; Proc. (El Batan, Mexico). (In Press.)

Barbosa, H. M. 1971. Genes and gene combinations associated with protein, lysine, and carbohydrate content in the endosperm of maize (*Zea mays*, L.). Ph.D. Thesis. Purdue University, Lafayette, Indiana.

Cochran, W. G., and G. M. Cox. 1957. Experimental design. 2nd ed. John Wiley and Sons, Inc., New York.

Creech, R. G. 1968. Carbohydrate synthesis in maize. Advan. Agron. 20 275-322.

Doll, H. 1970. Variation in protein quantity and quality induced in barley by EMS treatment. Manuscript, Danish AEC Research, Riso, Dk-4000 Roskilde, Denmark.

Gopalan, C., and S. G. Srikantia. 1960. Leucine and pellagra. Lancet 1:954.

Howe, E. E., G. R. Jansen, and E. W. Gilfillan. 1965. Amino acid supplementation of cereal grains as related to the world food supply. Amer. J. Clin. Nutr. 16:315-320.

Ingversen, J., A. J. Anderson, H. Doll, and B. Koie. 1972. Selection and properties of high lysine barleys. *In* Use of nuclear techniques for the improvement of seed protein. IAEA, Vienna. (In Press.)

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

McWhirter, K. S. 1971. A floury endosperm, high lysine locus on chromosome 10. Maize Genet. Coop. Newsletter 45:184.

Mertz, E. T., L. S. Bates, and O. E. Nelson. 1964. Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science 145:279-280.

Moore, S. 1963. On the determination of cystine as cysteic acid. J. Biol. Chem. 238:235-237.

Munck, L. 1972. Barley seed proteins. Symp. seed proteins. The AVI Publishing Company, Inc. Westport, Connecticut. p. 144-164.

————, K. E. Karlson, and A. Hagberg. 1969. Comparing the strategy of breeding for protein quantity and quality in barley and broad bean. Int. Barley Genet. Symp., Proc. 1971. Washington State Univ. Press, Pullman, Washington. p. 544-548.

Nelson, O. E. 1969. The modification by mutation of protein quality in maize. Approaches breed. improved plant protein, Symp. IAEA, Vienna. p. 41-55.

————, E., E. T. Mertz, and L. S. Bates. 1965. Second mutant gene affecting the amino acid pattern of maize endosperm proteins. Science 150:1469-1470.

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

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

Shannon, J. C. 1968. A procedure for the extraction and fractionation of carbohydrates from immature *Zea mays* kernels. Purdue agr. exp. sta. Res. Bull. 842.

Shelton, M., J. R. Couch, F. Hole, J. H. Jones, R. E. Leighton, C. M. Lyman, and J. K. Briggs. 1951. Grain sorghum byproduct feeds for farm animals. Texas Agr. Exp. Sta. Bull. 743.

Shuman, A. C., and R. A. Plunkett. 1964. Determination of amylose content of corn starch. *In* Methods in carbohydrate chem. 4:174-178. Academic Press, New York.

Slump, P., and H. A. W. Schreuder. 1969. Determination of tryptophan in foods. Anal. Biochem. 27:182-186.

Trible, L. 1971. Grain sorghum in swine rations. 7th biennial grain sorghum res. util. conf. p. 54.