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INHERITANCE AND IMPROVEMENT OF PROTEIN QUALITY AND CONTENT IN SORGHUM BICOLOR (L.) MOENCH

January 1, 1971 - December 31, 1971

Report No. 8

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B. Present Data from Studies in Progress.

1. Comparison of Protein Data from Distributed Sets of Breeding Source Material With 1970 Indiana Protein Test Results.

Sets of seed have been distributed to overseas cooperators at their request. Grain samples have been returned at our request for analysis. The cooperators have furnished data regarding flowering dates, heights, insect damage, bird damage, susceptibility to disease and a yield rating. The lines were rated with a "1" if their yield was very high down to a "9" if their yield was very low with a rating of "5" being average for the area. These values are all based on one-row plots so part of the variability would be thus explained.

The 41 lines most frequently returned for analysis are included in this summary. Generally, the average and the range of protein percent was higher overseas where the yield of grain was generally at a lower level than in the United States.

Table 1. A Comparison of Protein Percent in Sorghum Grain in Overseas Tests with Results from Indiana Tests.

I.S. Line	Number of Locations	USA Locations[2]		Overseas Locations[1]		Overseas Locations[1]		
		Percent Protein (Mean)	Percent Protein (Range)	Percent Protein (Mean)	Percent Protein (Range)	Number of Locations	Mean Yield Score[3]	Yield Score Range[3]
57	5	13.7	11.8–15.6	13.5	11.6–15.6	3	5	1–9
62	4	13.1	11.6–13.9	14.8	12.0–17.8	3	4	3–7
73	4	13.1	11.6–13.9	16.6	13.5–18.5	4	6	1–9
75	5	13.7	11.9–15.3	16.9	10.6–16.1	3	4	1–9
113	3	13.3		14.0	9.6–19.2	3	2	1–3
185	4	12.6	10.9–13.2	15.2	12.1–18.6	3	2	1–5
418	4			13.4	11.0–14.6	4	5	3–9
508	4	11.3	8.4–18.3	13.7	9.8–17.1	3	5	3–9
514	5	16.6	15.0–21.9	15.6	13.6–17.1	3	6	1–9
682	3	10.2	8.2–18.2	12.8	11.8–14.3	5	5	3–9
831	5	11.0		13.3	11.5–16.3	5	3	1–7
887	5	15.7	12.3–23.9	16.6	13.5–19.7	3	9	9–9
1025	5	14.1		13.0	10.4–14.5	3	6	3–9
1210	4	15.5	11.1–23.9	14.7	12.8–15.9	4	5	3–9
2319	5	13.0	9.6–20.0	13.7	11.7–17.3	3	6	3–9
2401	5	10.0	9.4–10.6	12.5	10.9–14.2	4	4	3–9
2586	5	14.2	10.8–19.4	13.0	12.1–13.9	3	3	1–9
2630	4	14.0	11.0–17.0	14.0	11.2–18.4	4	6	1–9

Table 1. Continued

I.S. Line	Number of Locations	USA Locations[2] Percent Protein (Mean)	USA Locations[2] Percent Protein (Range)	Overseas Locations[1] Percent Protein (Mean)	Overseas Locations[1] Percent Protein (Range)	Overseas Locations[1] Number of Locations	Overseas Locations[1] Mean Yield Score[3]	Overseas Locations[1] Yield Score Range[3]
2728	4	13.6		12.8	11.9–13.6	3	2	1–3
2805	4	9.0	7.5–10.9	11.3	9.6–12.8	4	5	1–9
3486	4	12.4		14.3	9.8–17.8	2	4	1–7
3528	3	11.3	11.0–11.9	12.0	9.5–13.9	2	5	1–9
3542	4	12.8	10.6–14.8	13.7	10.4–16.4	4	6	1–9
3552	4	13.0	12.3–13.6	13.5	10.4–15.4	4	5	3–9
3922	4	12.3	9.8–16.8	13.6	10.4–16.2	3	6	1–9
3982	5	10.6	9.0–12.9	14.5	11.0–17.8	3	6	1–9
4328	4	13.8	12.7–14.9	14.6	10.9–16.8	2	1	1
5437	3	11.8	11.1–13.2	11.5	10.3–12.3	2	9	9–9
6039	4	9.5	8.8–10.8	12.5	10.1–13.8	3	4	3–7
6901	4	15.7	12.9–22.1	13.0	8.9–15.6	2	7	5–9
6903	3	10.4	9.2–14.2	12.7	10.2–16.1	2	9	9–9
6992	3	11.1	9.6–11.8	12.6	11.8–13.6	2	3	1–5
7093	2	12.8	11.8–13.6	11.2	10.0–12.4	1	9	9
8093	3	11.4	10.9–12.2	13.0	11.8–14.3	1	9	9
8231	4	12.4	11.8–13.0	12.4	10.5–13.6	3	4	1–9
8260	2	8.0	7.2– 9.0	13.6	13.4–13.7	3	7	5–9

Table 1. Continued

I.S. Line	Number of Locations	USA Locations[2] Percent Protein (Mean)	USA Locations[2] Percent Protein (Range)	Overseas Locations[1] Percent Protein (Mean)	Overseas Locations[1] Percent Protein (Range)	Overseas Locations[1] Number of Locations	Overseas Locations[1] Mean Yield Score[3]	Overseas Locations[1] Yield Socre Range[3]
8309	2	12.8	10.6–18.5	13.8	10.4–17.1	1	9	9
8322	3	14.9	11.1–17.3	13.1	11.6–16.2	2	8	7–9
8353	2	14.9	13.0–17.5	13.7	13.1–14.4	2	5	1–9
8361	3	14.7	11.7–25.1	11.1	10.4–11.9	3	4	3–5
8377	3	16.6		16.2	15.6–16.8	1	9	9

[1] Data around the world including Recife, Brasil; Nigeria, Vale do Curu, Brasil; Korea and Vicosa, Brasil.

[2] Results of the study conducted in 1970 and earlier years in the U.S.

[3] Yield score 1 = high yield, 3 = above average, 5 = average, 7 = below average, 9 = lowest.

2. Comparison of Protein Breeding Source Data from 1970 Overseas and Local Tests.

Selected sets of potentially useful diverse sources of breeding materials were sent to cooperators requesting seed in 1969 and 1970. Samples returned by our request were analyzed for protein. Over 41 lines were returned from more than one location and from these 10 were selected which gave the highest protein yield as pounds protein per acre in Indiana. To demonstrate the performance pattern that is being identified from this phase of our program of helping sorghum quality improvement in other countries, it will be noted from the data that lines hold up well in some cases and in others the range in protein varies considerably. The higher protein percent values are associated with the poor yield ratings. The information being received from the cooperators is useful to us for selection purposes and as shared with others will enable them to select lines for their breeding programs with higher dependability in yield and protein production.

	U.S.A. Locations			Overseas Locations			Yield Ratings*	
I.S.	No.	Protein Average Percent	Range	No.	Protein Average Percent	Range	Ave.	Range
0185	4	15.2	12.2-18.6	3	12.6	10.9-13.2	2	1-5
2401	5	12.5	10.9-14.2	4	10.0	9.4-10.6	4	3-9
2586	5	13.0	12.1-13.9	3	14.2	10.8-19.4	3	1-9
4328	4	14.6	10.9-16.8	2	13.8	12.7-14.9	2	1-3
8231	4	12.4	10.5-13.6	3	12.4	11.8-13.0	4	1-9
8353	2	13.7	13.1-14.4	2	14.9	13.0-17.5	5	1-9
0057	5	13.5	11.6-15.6	3	13.7	11.8-15.6	5	1-9
0062	4	14.8	12.0-17.8	3	13.1	11.6-13.9	4	3-7
0075	5	16.9	10.6-16.1	3	13.7	11.9-15.3	4	1-9
6039	4	12.5	10.1-13.8	3	9.5	8.8-10.8	4	3-7

* Yield rates 1 = very high, 9 = very low.

3. Summary of Experiments Conducted with Chicks.

Selected grain samples were supplied to the Purdue University Animal Science Department for chick feeding experiments directed by Drs. John Rogler and William Featherston as a Ph. D. Animal Science funded research project of their graduate student Horacio Rostagno of Argentina. The influences of amino acid composition and polyphenol levels on baby chick blood serum content, growth rates and the response of older chicks left on a high polyphenol sorghum grain level were studied. This work is not entirely completed, but the thesis is to be completed in 1972. The first experiment (Table 1) involved eight grain samples selected for high and low levels of protein, lysine and polyphenol levels in a 2x2x2 factorial design. The analysis of the chick blood serum indicated a significantly higher level of most amino acids in the blood serum from chicks fed the grain with lowest polyphenol content at all levels of protein and lysine. The second experiment involved feeding day-old chicks a ration based on 73.25 percent of the selected grains, 16.50 percent soybean oil meal and 10.25 percent of a basal oil, mineral and vitamin supplement. The *in vitro* dry matter disappearance results for all of these samples were relatively high. The weight gains for these rations which had a protein content of approximately 15.2 percent or more are shown in Table 2. These results indicated that these supplemented grain samples gave comparable feed efficiency results to corn.

Fourteen-day old chicks were fed various selected samples of grain sorghum supplemented with a mineral and vitamin mixture (Table 3). Chromic oxide was used as a marker while the chicks were fed for two days. The excreta were collected and analyzed and the indigenous amino acids were determined by feeding a protein free diet. The availability of the amino acids and the apparent amino acid digestibility were determined. Large differences in availability of the amino acids were found among rations. Those high in catechin equivalent values had considerably less available amino acids and when tannic acid was added to RS610 to bring the level of catechin equivalent up to the level found in BR64, the apparent amino acid digestibility was lowered. To further attempt to identify the influence of catechin equivalent levels on the chick response, day-old chicks were fed selected rations of low, high and adjusted catechin equivalent levels for three weeks (Table 4). The grain was fed alone and with a protein supplement of soybean oil meal up to 15 percent protein. The decrease in weight resulting when tannic acid was added to RS610, NK300 and corn to the level of BR64 indicates that an adverse response in growth rate may be expected from the so-called bird resistant or at least the varieties high in catechin equivalent levels. This experiment was continued to test the hypothesis that the chicks will become accustomed to the ration and increase their growth rate as they become older and the results in Table 5 did not show a favorable adjustment to the high polyphenol level in BR64.

An additional experiment was designed to supplement individual amino acid levels into a uniform ration using glutamic acid to make all isonitrogenous (Table 6). The sorghum samples were autoclaved at 250°F with 15 pounds per square inch pressure for 30 minutes as another treatment to determine the influence of heat on the sorghum growth response with samples containing high and low catechin equivalent levels. The increase in weight gain when RS610 was supplemented with individual amino acids to the level of corn indicates the necessity for balance of amino acid considerations. The response of BR64 is especially interesting as the adverse influence of its high catechin equivalent level was seemingly overcome by the supplementation of amino acids to the level of corn. This response is being studied further and if the exact influence can be identified supplementation may be advisable in order to improve a diet consisting of grain containing high levels of polyphenols. The identification of the specific amino acid(s) involved will guide in the selection of items to be entered into breeding programs for development of a more desirable amino acid balance in new hybrids and lines.

Table 1. Milograms of AA/ml of Baby Chick Blood Plasma.

Identification	025149 HHH 1	025154 HHL 2	025035 HLH 3	025075 HLL 4	025042 LHH 5	925074 LHL 6	025134 LLH 7	025187 LLL 8	RS610 9	AA ref 10	AA ref 11
IVDMD %	90	48	71	40	91	56	90	59			
Catechin Equivalent	.7775	12.74	.3938	11.54	.472	9.72	.426	5.4	.69		
Aspartic	4.7	4.7	5.2	5.1	4.7	4.6	4.8	3.4	6.4	5.5	14.2
Glutamic	36.1	24.6	41.1	28.1	47.7	27.1	32.6	23.5	34.6	42.5	83.7
Proline	29.4	15.4	37.7	18.9	38.7	17.9	29.1	14.0	32.7	18.5	16.4
Glycine	43.6	38.9	49.1	43.7	46.9	40.5	45.6	37.2	46.7	47.4	103.3
Alanine	56.0	37.4	53.3	39.8	47.0	40.8	48.5	38.7	55.7	87.3	133.9
Valine	18.9	16.1	18.5	16.2	17.0	16.9	16.3	13.5	17.6	21.7	39.7
Cystine	17.5	10.1	22.2	10.9	21.6	9.9	15.9	9.6	16.9	21.1	29.3
Methionine	5.5	5.6	4.3	4.3	6.0	4.9	6.7	4.5	4.6	8.8	15.1
Isoleucine	8.1	6.5	7.5	8.4	7.2	5.4	6.7	5.5	7.4	5.3	10.1
Leucine	26.6	11.3	31.5	11.1	26.5	9.7	26.2	15.4	31.5	16.2	22.9
Tyrosine	32.3	16.7	42.4	13.4	42.1	13.7	30.8	17.1	44.4	15.2	16.7
Phenylalanine	21.8	15.4	24.2	13.6	22.6	13.9	21.7	15.2	23.3	11.1	14.8
Lysine	41.6	44.8	48.6	36.7	6.5	63.1	52.6	34.4	49.9	67.3	73.5
Histidine	15.9	13.9	17.7	10.9	17.7	8.4	16.4	16.9	14.7	16.1	21.3
Arginine	14.8	20.8	14.1	16.9	16.8	12.9	20.4	15.9	13.3	20.5	72.6
Protein	13.0	11.8	2.3	13.0	10.05	10.07	8.88	9.4			
Lysine	2.38	2.64	1.90	1.83	2.39	2.50	2.07	2.19			

Three 12 day-old chicks fed every 30 minutes for 6 hours (300 mg).

Table 2. Amino Acid Supplemented and Heat Treated Iso-Nitrogenous Growth Responses of First and Second Week Baby Chicks.

	Corn	RS610	RS610 to Corn	Corn to RS610	BR64	BR64 to Corn	Auto-Claved RS610	Auto-Claved BR64	Dig. RS610 to Corn	Dig. BR64 to RS610
Soybean oil meal	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5
Basal oil	10.25	10.25	10.25	10.25	10.25	10.25	10.25	10.25	10.25	10.25
Corn	70.00			70.00						
RS610		70.00	70.00				70.00		70.00	
BR64					70.00	70.00		70.00		70.00
Cerelose	1.29	2.44	2.69	1.33	.45	.74	2.44	.45	2.77	1.18
Glutamic	1.96	.81	.20	1.86	2.80	2.08	.81	2.80		.99
Protein %	14.8	15.5	15.5	14.8	14.3	14.3	15.5	14.3	15.5	14.3
Threonine	.245	.259		+.014	.273					.013
Glycine	.259	.224	+.035		.224	+.035			.049	.064
Valine	.322	.343		+.021	.315	+.007			+.003	.162
Cystine	.126	.070	+.056		.077	+.049			+.067	.014
Methionine	.119	.070	+.049		.077	+.042			+.057	.021
Isoleucine	.238	.266		+.028	.252				.248	.129
Leucine	.903	.868	+.035		.805	+.098			+.063	.468
Phenylalanine	.336	.336			.308	+.028			+.005	.162
Lysine	.210	.154	+.056		.154	+.056			+.064	
Histidine	.196	.147	+.049		.154	+.042			+.065	.011
Arginine	.343	.259	+.084		.266	+.077			+.110	.038

+ amount of individual amino acid added.

Table 3. The Comparison of Availability and Apparent Digestibility of Amino Acids by Chicks.

Treatment	AA Availability					Apparent AA Digestibility						
	RS610 1	NK300 2	BR804 3	BR64 4[a]	BR64 4[b]	RS610 1	NK300 2	BR804 3	BR64 4	RS610 5	RS610 6	RS610 7
Tannic Acid Equiv. in Diet	.33	.59	1.00	1.41	1.41	.33	.59	1.10	1.41	.59	1.10*	1.41*
Aspartic	82.7	47.3	27.5	46.9	60.4	75.4	38.4	13.8	29.7	75.9	71.4	65.8
Glutamic	84.9	52.9	31.7	31.9	37.1	81.2	49.4	25.6	22.5	83.1	78.9	75.9
Proline	80.5	52.5	38.4	34.6	37.1	76.1	48.0	26.3	19.0	75.7	69.8	70.1
Alanine	86.1	55.4	20.8	40.7	42.0	82.6	52.2	16.0	29.2	83.1	78.2	75.4
Valine	79.1*	14.1*		29.3	36.7	70.7	6.5		12.9	70.0	62.8	58.1
Cystine	77.1*	54.9	57.0	48.3	50.2	69.2	47.0	33.6	27.6	67.8	53.5	53.9
Methionine	69.3	42.4	45.0	11.8	32.4	57.3	30.8	33.5		60.2	53.7	
Isoleucine	81.1	59.5	30.7	24.8	35.4	74.5	55.0	19.3	15.0*	76.3	70.0	65.4
Leucine	86.1	56.3	36.6	32.6	35.8	83.0	53.6	28.5	22.1	83.8	79.9	76.3
Tyrosine	82.7	59.1	38.0	29.9	41.5	76.9	54.3	27.6	13.3	79.9	71.8	67.7
Phenylalanine	83.9	53.6	33.9	32.5	40.3	78.8	48.8	25.8	18.4	81.4	75.8	70.8
Lysine	72.3	35.6	46.6	56.7	75.7	58.8	23.1	33.0	27.0	65.9	59.7	42.3
Histidine	70.7	43.6	55.0	46.0	59.1	65.4	38.2	37.8	25.6	61.3	50.3	49.1
Arginine	80.2	46.6	52.6	45.7	63.2	71.8	35.2	39.0	26.9	72.7	64.9	60.2
Average of all Amino Acids	79.8	48.2		36.6	46.3	73.0	41.5	27.7	20.9	74.1	67.1	62.6

* Tannic acid added to diet to this level.
[a] Corrected for tannin by substraction of 7-1 + PFD.
[b] Corrected for tannic acid using PFD + 1.41 percent tannic acid.

Table 4. Chick Growth Response to Levels of Catechin Equivalent From 0-21 Days.

Treatment	3 Weeks Weight Gain (g)	3 Weeks Feed Consumption (g)	Feed Efficiency Feed/Gain
Corn lb/lb	282.2	535.3	1.90
RS610 lb/lb	202.2	382.6	1.90
NK300 lb/lb	146.3	359.1	2.46
BR64 lb/lb	112.9	315.5	2.81
RS610 Iso-N to BR64	187.4	376.1	2.01
NK300 Iso-N to BR64	122.0	315.9	2.61
RS610 + Tannic Acids to BR64	149.0	326.4	2.20
NK300 + Tannic Acids to BR64	68.8	234.1	3.50
Corn + Tannic Acids to BR64	169.7	362.2	2.14

Table 5. Response of Change in Diet on Average Rate of Growth of Chicks 0–35 Days.

1st 3 Weeks		4th and 5th Weeks								
			1st Week		2nd Week		Final			
Diet	Final Weight	Diet	Weight Gain (g)	Feed Consumed (g)	Weight Gain (g)	Feed Consumed (g)	Weight Gain (g)	Feed Consumed (g)	Feed Efficiency Feed/Gain	Leg Score
Corn	319.2	BR64	51.3	264.4	44.6	228.3	95.9	492.8	5.20	1.17
Corn	324.4	RS610	102.3	312.0	118.5	301.0	220.7	613.0	2.81	1.53
BR64	148.1	BR64	46.7	161.3	41.5	163.8	88.2	325.1	3.71	1.83
BR64	156.5	RS610	72.1	156.6	89.2	216.9	161.4	373.6	2.31	1.63

Table 6. Weight Gained on Iso-N and Supplemented Diets.

	1 Week	2 Weeks	3 Weeks		
	Weight Gain (g)	Weight Gain (g)	Weight Gain (g)	Total Feed Consumed (g)	Feed Efficiency Gain/Feed
Corn	96.7	186.2	291.1	525.8	1.81
RS610	84.3	145.9	225.4	424.7	1.89
RS610 to Corn	92.0	170.9	265.2	468.5	1.77
Corn to RS610	91.1	177.4	273.6	495.5	1.81
BR64	76.1	109.4	103.2	296.3	2.89
BR64 to Corn	92.5	169.3	225.7	501.1	2.22
Autoclaved RS610	88.6	160.0	234.4	463.7	1.98
Autoclaved BR64	78.9	113.2	115.4	326.3	2.83
Available RS610 to Corn	91.1	170.2	261.9	464.7	1.78
Available BR64 to RS610	85.3	132.0	143.5	371.8	2.60

4. The Use of Classification by Race as a Selection Basis for Protein Inheritance Studies.

Samples selected for complete amino acid analysis were classified into the races as suggested by Drs. Harlan and de Wet of the University of Illinois in order to study the differences which might be attributed to races. There are wide variations of percent protein in each class (Table 1), but there are significant differences among the classes as sampled and reported here. The higher averages of the Bicolor, Guinea and Durra selection and their hybrids are indicative of their generally lower yield in other trials and the generally observed increased percent protein when yield decreases. Table 2 shows the relationships of selected amino acids contents as percent of protein in the races. No significant difference is indicated among the races for levels of threonine and cystine, but there would appear to be significant differences for other amino acids among the races. This finding will be useful in attempting to isolate genetic sources for development of new hybrids and lines containing given levels of desirable amino acid compositions. The frequency of samples identified in each of the classes indicates the need to select more widely from the races with lower frequencies and the need to continue to classify a larger sample of the total collection to identify any significant class differences. Currently yields of protein are being summarized by races in order to take into consideration yield of grain with percent protein and amino acid levels.

Table 1. Protein Content of 418 Grain Sorghum Samples Classified by Races*.

	Race	Number of Lines	Percent Protein Average	Percent Protein Range
1	Bicolor	41	13.65	10.04 - 18.41
2	Guinea	19	13.28	10.37 - 16.87
3	Caudatum	48	11.90	7.95 - 15.50
4	Kafir	24	11.98	9.00 - 16.81
5	Durra	23	13.18	8.63 - 16.81
6	G B	7	12.74	11.16 - 13.75
7	C B	33	12.39	8.88 - 18.17
8	K B	35	12.34	9.13 - 18.19
9	D B	52	13.52	9.94 - 19.56
10	G C	73	12.06	8.75 - 14.19
11	G K	6	12.72	10.89 - 14.89
12	G D	31	13.06	10.56 - 15.17
13	K C	5	12.70	11.56 - 13.36
14	D C	7	12.76	10.00 - 20.00
15	K D	15	13.34	10.06 - 16.54
		419		
			Average 12.78	
			C.V. 14.56	
			L.S.D.(.05) 0.98	

* Suggested races according to Drs. Harlan and de Wet of the University of Illinois.

Table 2. Analysis of Protein and Individual Amino Acids by Race of 419 Different Lines (average values for the number of lines indicated in each race).

	Races*	No. of lines	Percent Protein	Lysine	Threo-nine	Cystine	Iso-leucine	Leucine	Isoleucine/ Leucine Ratio
1	Bicolor	41	13.65	2.03	3.22	0.86	4.07	14.65	.278
2	Guinea	19	13.28	2.00	3.34	0.88	4.06	14.41	.282
3	Caudatum	48	11.90	2.29	3.20	0.85	3.99	13.49	.296
4	Kafir	24	11.98	2.14	3.26	0.93	4.06	14.10	.288
5	Durra	23	13.18	2.15	3.31	0.94	4.04	14.43	.280
6	G B	7	12.74	2.21	3.30	0.88	3.94	13.72	.287
7	C B	33	12.39	2.03	3.20	0.90	4.05	14.22	.283
8	K B	35	12.34	2.13	3.28	0.89	4.15	14.38	.289
9	D B	52	13.52	2.17	3.25	0.86	4.13	14.54	.284
10	G C	73	12.06	2.27	3.27	0.87	4.06	14.01	.290
11	G K	6	12.72	2.13	3.37	0.90	4.06	14.91	.272
12	G D	31	13.06	2.06	3.24	0.96	4.07	14.20	.287
13	K C	5	12.70	2.10	3.15	0.80	4.06	13.88	.293
14	D C	7	12.76	2.17	3.20	0.80	3.59	12.58	.285
15	K D	15	13.34	2.07	3.32	0.83	4.35	15.41	.282
Average			12.78	2.13	3.25	0.88	4.07	14.22	.285
CV Percent			14.6	16.40	7.50	23.20	8.60	9.30	
LSD (.05)			0.97	0.18	NS	NS	0.18	0.69	

* Races as described and proposed by Drs. J. R. Harlan and J. M. J. de Wet, University of Illinois. "A Simplified Classification of Cultivated Sorghum". In Press.

5. Microscopic Examinations and Protein and Starch Investigations. on Eight Selected Sorghum Lines.

Eight samples selected for high and low values of protein, lysine and IVDMD were sent to Dr. J. S. Wall for microscopic examinations of the grain structure. Mr. Henry Seckinger and Dr. Michael Wolf of the USDA Cereal Microscopic Investigations group conducted an extensive study of these grain samples which had been fed to chicks by the Animal Science Department group at Purdue University. Detailed histological studies of the grain structure did not reveal distinguishing characteristics related to apparent chick blood plasma amino acid contents and expected growth responses. Cell wall structure and pericarp of the different grain samples were not significantly different. Starch was digested from tissue sections and protein matrix structure and protein bodies were examined. The protein containing the deposits of kafirin were fairly uniform and large (2-3u) in all samples except the high lysine variety, 025154. The high lysine variety exhibited a wider variation in protein body sizes which were smaller (0.25-1u). The only characteristic of the kernel that correlated well with digestibility was the occurence of pigment. (Table 1).

Table 1. Composition of Protein and Starch in Sorghum Samples from Purdue University.

Sample Identity	% IVDMD	% Protein	% Lysine	Catechin Equiv.(%)	% Protein in Hexane Extracted Endosperm	% Salt Soluble Protein	% Alcohol Soluble Protein	% Protein in Residue	Total % Recovery	% Amylose	% Starch
025035	71	12.3	1.90	.39	12.47	3.11	14.69	70.00	87.80	30.2	73.8
025042	91	10.5	2.39	.47	10.28	3.94	13.59	74.89	92.42	26.9	76.2
025075	40	13.0	1.83	11.54	9.63	3.72	26.34	62.68	92.74	27.7	77.3
925074	56	10.7	2.50	9.72	7.79	4.92	13.07	65.48	83.47	25.7	81.1
025149	90	13.0	2.38	.78	12.21	4.85	14.73	65.09	84.67	25.7	74.6
025134	90	8.8	2.07	.43	9.35	4.09	10.23	69.32	83.64	29.1	74.3
025187	59	9.4	2.19	5.39	9.36	4.68	13.45	57.02	75.15	28.5	76.8
025154	48	11.8	2.64	12.74	10.77	2.92	14.05	72.80	89.77	24.0	77.9

6. Sorghum Silage Digestibility as Affected by Polyphenolic (Tannin) Content of Grain.

Sheep were fed silage made in plastic bags from the forage-type yield trials conducted in 1970 to determine the influence of high polyphenol content of grain and forage phenotypes on digestibility by a ruminant animal after being ensiled. The experiment was conducted by V. L. Lechtenberg of the Department of Agronomy, Purdue University in conjunction with forage research trials. Following a seven-day adjustment period, ad libitum intake was measured for 14 days. Feces were then collected for a seven-day period and the digestibility coefficients calculated (see Table 1). Each silage was supplemented with soybean oil meal, vitamins and minerals and fed to five yearling wethers. Thus, the data shown represents the average of five animals on each silage. The results as shown in Table 2 indicate that ensiling of sorghums containing high and low levels of polyphenols (tannins) can result in equal rates of performance when fed to sheep.

Table 1. Summary of Sorghum Silage Study, Purdue University, 1971.

Variety	Animal no.	Intake (% of b.w.)	(b.w. 3/4)	Intake (g/kg b.w.3/4)	Digest-ibility
FS24	8	2.33	21.7	65.0	63.8
	10	2.69	23.8	77.3	70.2
	13	2.36	23.3	67.4	69.6
	18	2.47	26.0	73.1	67.2
	7	2.20	28.3	67.2	62.4
	Ave.	2.41			66.6
BR64	5	2.35	22.9	66.8	69.3
	16	2.57	22.5	72.7	71.9
	17	2.51	23.9	72.3	69.1
	20	2.70	26.0	80.0	60.4
	21	2.76	24.1	79.8	62.5
	Ave.	2.58			66.6
Rx955017	1	2.31	28.4	70.5	69.4
	11	2.57	25.2	75.3	62.1
	14	2.50	23.6	71.6	75.7
	4	2.31	26.7	69.2	71.6
	9	2.68	24.9	78.3	70.6
	Ave.	2.47			69.8
Rx955025	3	2.11	22.9	59.8	75.8
	6	2.64	27.0	79.2	63.1
	9	2.33	26.4	69.2	65.5
	12	2.33	26.4	69.2	68.6
	15	1.84	25.7	54.4	75.3
	Ave.	2.25			69.7

Table 2. Comparison of Intake and Digestibility of Four Selected Ensiled Samples of Grain Sorghum, Purdue University, 1971.

Silage Variety	Height (cm)	% dry matter	Level of Polyphenols in Grain	Intake Percent of Body Weight	Digest-ibility
FS24	260	32		2.41	66.6
BR64	150	48	high	2.58	66.6
Rx955017	255	34		2.47	69.8
Rx955025	350	41		2.25	69.7
Average				2.43	68.2
C.V. (%)				8.99	7.08
L.S.D. (.05)				8.5	3.06

7. The Status of Protein Evaluations on the World Sorghum Collection.

Through the assistance of Dr. J. R. Harlan of the University of Illinois the project received the most complete and accurate set of origin identification known. This information on the first 12,250 I.S. numbers has been computerized and sent to those requesting such information. This information has been extremely useful to insure the selection of materials with a wide range for identification of protein quality and quantity studies.

This project has made one or more nitrogen determinations on 7,107 of the first 10,000 I.S. entries. In addition, one or more complete amino acid determinations have been made on 693 of the 7,107 lines and to date over 70 lines have been evaluated by rat feeding trials this year. The rat feeding trial entries were selected on various test results including polyphenol (tannin) content, in vitro dry matter disappearance percents and different environmental performance results. In addition to the I.S. collection items the project has investigated a similar volume of entries consisting of additional new lines, breeding nursery acquisitions, new hybrids and segregating materials. Thus, the total volume of the present inventory of sorghum source materials exceeds 24,000 plus one or more hybrids per line in most cases. The processing of data to identify the potentially most useful lines, hybrids and populations is progressing toward a selection of several hundred representative entries to enable a more intensive concentration on a reduced number basis.

8. Interaction of Vertical Planting Systems with Environmental Factors Within the Canopy - 1970. (Analyzed in 1971 by Dr. Raymond Bula, USDA, Purdue University).

The major meteorological parameters of eight different sorghum canopies were found to be similar during the 1970 growing season. There were some indications that the canopies consisting of single or double alternate rows of short and medium height plants and single alternate rows of short, medium and tall plants had a more favorable environment for dry matter accumulation or photosynthesis than canopies consisting of all tall or all short plants. The canopy consisting of double rows, short, medium and tall plants was intermediate to the all medium height and all tall height plant canopies. The meteorological characteristics of the canopy derived from a uniform seed mixture of the three height plants were almost identical to those of the canopy consisting of all tall plants.

Air temperatures, measured at 75 cm above the soil surface and averaging over the nine week measuring period, ranged from 20.4°C to 20.7°C. The lower average temperatures were noted in those canopies consisting of small height plants or alternate small and medium height plants. Average soil temperatures, generally followed the pattern described for the air temperatures. Average carbon dioxide concentrations were drastically reduced to a very low level of 75 cm above the soil surface and were lowest in the canopy consisting of medium height plants and highest in the canopy consisting of all short height plants. Water vapor pressure deficits of 75 cm above

the soil surface were less in the canopies consisting of all tall, alternate short, medium and tall, and the mixture of all three heights t. in the canopies consisting of short, medium, or short and medium height plants. As photosynthetic activity began in early morning the CO_2 level was very rapidly reduced in these trials.

Incoming solar radiation, measured at 75 cm above the soil surface, did not have any consistent relationship with type of plant canopy. This very likely reflects inadequate sampling of this parameter rather than a lack of relationships.

A similar study was conducted in the 1971 crop season utilizing the project's seed inventory and at essentially no expense to this project.

9. Fractionation of Sorghum Proteins.

Though there have been a number of reports regarding the composition of amino acids in grain sorghum, relatively little is known about the proteins of this important grain. Fractionation of sorghum proteins has been carried out by others using the classical Osborne-Mendel procedures. This method appears to be unsatisfactory in selectively extracting different classes of proteins. In these studies, Drs. Mertz and Jambunathan of the Purdue University Department of Biochemistry employed the procedure of Landry and Moureaux for the fractionation of sorghum. (J. Landry and T. Moureaux Bull. Soc. Chim. Biol 52 1021 (1970)). This procedure solubilizes most of the nitrogen and yields five different soluble fractions. In addition to studying the characteristics of soluble proteins of sorghum, we also wanted to see whether two nutritionally different sorghum samples would yield different patterns of protein distribution.

Four samples of sorghum were chosen for this study. They were all inbred lines and grown at Purdue Agronomy Farm during the years 1969 and 1970. The World Germplasm Collection numbers of the four samples with the Purdue number in parentheses were as follows: IS 0062 (925006), IS 2283 (925074), IS 3982 (025042) and IS 6992 (025154). The samples were ground in a burr mill and were mixed with vitamins and minerals in the proportion of 95% ground sorghum, 4% minerals and 1% vitamin mix. This diet was fed to six weanling rats

for a period of four weeks and the weight gain was noted. The samples IS 3982 and IS 6992 were each fed to three rats for a period of one week due to the low supply of these two samples. However, these rats were previously fed a cereal diet for a period of one week to make them accustomed to the cereal diet. Rats which were fed the sorghum samples 0062 and 3982 gained an average of 8.7 gm and 6 gm respecitvely per week, and those on 2283 and 6992 lost 0.2 gm and 9 gm respectively. Thus among the four samples, two were nutritionally good sorghums and two were nutritionally poor sorghums.

The samples were ground in a Wiley mill, defatted with n-butanol and were made to a fine powder using a microball mill. Two gram portions of each sample were taken for protein fractionation studies. The procedure consists of extracting the protein with 20 ml of the following solvents successively for the number of minutes shown in parentheses: fraction I - 0.5 M NaCl (60), Same (30), Same (30), H_2O (15) same (15); fraction II - 70% isopropanol (v/v) (30), same (30), same (30); fraction III - 70% isopropanol and 0.6% 2 mercaptoethanol (v/v) (30), same (30); fraction IV - Borate buffer with NaCl (μ0.5, pH 10) with 0.6% 2 mercaptoethanol (v/v) (Borate mixture) (60), same (30), same (15); fraction V - Borate mixture with 0.5% sodium lauryl sulfate (w/v), (60), same (30), same (15). The sodium chloride extractions (fraction I) were performed at $4^{o}C$, the remainder at room temperature (25°).

Nitrogen was determined on the samples and the extracts by the microKjeldahl method and the protein was calculated by multiplying the nitrogen values by a factor of 6.25. Lysine, which is the most limiting amino acid in sorghum samples, was determined on the sample and the extracts by using a Beckman-Spinco automatic amino acid analyzer.

Table 1 shows the distribution of nitrogen in the five fractions of the kernel. A comparison of the nitrogen distribution between the two nutritionally good samples and poor samples show that in samples 0062 and 3982 fraction I had about 16% of the total nitrogen while in samples 2283 and 6992 the same fraction contained only about 4% of the total nitrogen. The distribution of nitrogen in fraction V was, however, opposite to that seen in fraction I. While the samples 0062 and 3982 had about 26% of nitrogen in fraction V, the samples 2283 and 6992 had 47% and 59% of nitrogen respectively in fraction V. Nitrogen distribution in fractions II, III and IV did not reveal the marked difference exhibited in fractions I and V.

Lysine distribution in the five fractions is shown in Table 2. The lysine distribution is concentrated between fractions I and V and to some extent in fraction IV also. While the lysine concentration in the nutritionally good samples show equal distribution between fractions I and V, fraction V alone of the nutritionally poor sample contains more than 75% of the total soluble lysine, the rest being distributed mainly in fraction IV.

Another observation which was made during the extraction of proteins was that while the solutions of fraction IV and V of the nutritionally good samples 0062 and 3982 were almost colorless, the same extractions of samples 2283 and 6992 were very dark in color. The results obtained thus far reveal that the percentage of nitrogen distributed in fraction I may have a direct relation with the nutritional quality of that particular sorghum. This can be further checked by determining the lysine content of the fraction I which is high (about 6%) in a nutritionally good quality sorghum and low (less than 2%) in a nutritionally poor sorghum sample. It is possible that there may be a factor which may bind to the saline soluble protein in sample 2283 and 6992 thus preventing them from solublizing in that solvent. The inhibiting substance may have been released by the combined action of high pH mercaptoethanol and sodium lauryl sulfate in fraction V giving rise to a high nitrogen (lysine) containing fraction V. Since the fraction IV and V were highly colored in these two samples, pigment may play a role as one of the factors. We are pursuing our studies in this direction.

Another interesting observation was also made with the sample 2283. This sample was ground and mixed with vitamins, minerals as described earlier. Another diet was prepared similarly but was made to 5% in total lysine by adding pure lysine monohydrochloride to the mixture. Both these diets were fed to six weanling rats for a period of one week. These rats were previously fed a standard sorghum diet for one week.

At the end of one week the rats were weighed. Those which were fed sorghum sample 2283 alone lost an average of 2.8 gm in one week while those on sample 2283 supplemented with lysine lost an average of 3.1 gm in one week. This experiment strongly indicates the possibility of some factor being present in the sample 2283 which prevents the lysine from becoming available to the rats.

Similar experiments with another good sorghum sample produced a four-fold increase in the weight gain of rats fed a lysine supplemented sorghum diet as compared to those fed the unsupplemented diet. At this stage we are not sure whether the pigment and/or some other factor(s) is responsible for this phenomenon. Subfractionation of fraction IV and V proteins may reveal some information regarding the association of pigments with the proteins.

Table 1. Nitrogen Distribution in Sorghum Kernels, Purdue University, 1971.

(Purdue)	IS 0062 925006	IS 2283 925074	IS 3982 025042-1	IS 6992 025154
Fraction I	16.8	3.7	15.5	4.4
Fraction II	13.4	1.9	14.7	8.4
Fraction III	21.6	21.1	17.3	10.9
Fraction IV	4.9	13.6	7.0	10.4
Fraction V	26.0	47.1	26.7	58.9
Insoluble Nitrogen	12.3 (87.7)	12.6 (87.4)	18.8 (81.2)	7.0 (93.0)
Average weight gain per week in rat feeding experiments	8.7*	-0.2*	6.0**	-9.0**
Polyphenols (tannin) (as Catechin equivalents)	0.52	6.88	0.47	4.62

* 4 weeks duration

** 1 week duration

Table 2. Lysine Distribution in Sorghum Fractions, Purdue University, 1971.

	(Purdue) IS 0062 925006	IS 2283 925074	IS 3982 025042-1	IS 6992 025154
Fraction I	46.8*(6.5)	0.8(0.5)	45.5(6.6)	1.9(0.7)
Fraction II	0.8(0.1)	0.1(0.1)	3.5(0.5)	2.8(0.6)
Fraction III	0.5(0.1)	1.1(0.1)	2.0(0.3)	0.8(0.1)
Fraction IV	4.2(2.0)	22.7(4.0)	6.8(2.2)	15.3(2.5)
Fraction V	47.7(4.3)	75.3(3.9)	42.2(3.6)	79.3(2.3)

* Percent of the total soluble lysine of the kernel. Values in parentheses are the grams of lysine per 100 grams of protein in the fraction. The whole kernels contained the following percent of lysine and protein respectively; No. 925006: 2.75, 11.15; No. 925074: 2.88, 7.69; No. 025042-1: 3.04, 9.42; and No. 025154: 2.74, 10.04.

10. Evaluation of Nutritional Quality of Cooked Sorghum (Chapaties).

One of the ways in which sorghum is consumed by people in India is in the form of chapaties (unleavened bread). The present experiment was designed to test whether cooking the sorghum (as done in India) changes the nutritional quality of the sorghum. Four samples were chosen for this study, one of them being 925074 (I.S. 2283) to see whether cooking would improve the quality by destroying growth inhibitors. All the samples were ground in a burr mill and half of the ground sample was saved for cooking and the other half was mixed with vitamins and minerals to make a proportion of 95% ground sorghum, 4% minerals and 1% vitamins. The other half portion was mixed with enough water to make a stiff dough by kneading well. Small portions of this were rolled out into discs and cooked on a hot plate. They were then placed on direct flame for a short period. The cooked sorghum was dried in a vacuum oven overnight and was ground using a burr mill. Vitamins and minerals were added to make a 95% sorghum, 4% mineral and 1% vitamin diet.

Both the cooked and uncooked sorghum samples were fed to 6 rats for a period of 4 weeks. It is obvious from the results shown in Table 1 that the cooking considerably reduces the nutritional quality of the sorghum including that of sample no. 925074.

Table 1. Evaluation of Nutritional Quality of Cooked Sorghum (Chapaties). (Data by Jambunathan)

	917025		925074		0819		954062	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
Average weight gain at the end of:								
First week	4.3*	1.7*	-4.5*	-4.8*	4.7	-1.8	3.4	3.0
Second week	8.7	3.7	-3.0*	-5.8*	10.5	-0.2	6.3	5.2
Third week	14.7	7.8	-0.8	-6.3	15.8	1.5	9.8	7.5
Fourth week	20.2	10.5	-0.8	-8.5	21.8	2.7	12.1	8.3
Average feed consumed at the end of fourth week:	210.2	160.5	139.8*	115.8*	195.5	137.2	162.2	150.7
Protein %	9.44	9.87	7.19	7.62	10.25	10.69	8.69	9.13
Lysine %	2.86	2.54	3.22	3.01	2.66	2.24	2.72	2.21

*Not significant at the 5% level. Rest of the values differ significantly at the 5% level for the same feed (cooked vs. uncooked).

11. Short Term Rat Feeding Trials vs. Long Term Trials.

The results obtained with short term feeding experiments (three rats, one week) have been compared in Table 1 with the results obtained by feeding the same sample to six rats for a period of four weeks. It appears that the short term feeding results could be advantageously utilized when there is a low supply of sample and the results agree well with the results obtained by using six rats for a period of four weeks.

The object of these experiments was to determine the minimum amount of sorghum required for a meaningful rat assay of biological value. The rats were fed a standard sorghum diet (0819) for one week and were then divided into groups of three having the same total weight gain.

Approximate feed needed to conduct the experiments for three rats = 150 grams.

Approximate feed needed to conduct a standard two week experiment for six rats = 600 grams.

Approximate feed needed to conduct a standard 4 week experiment for six rats = 1350 grams.

Table 1. Results of Short Term Feeding Experiments.

Exp. I *	925006	925018	954116	0819	954054	954131-2	925060	925074
Average weight gain at the end of four weeks (6 rats)	34.81	20.27	16.93	15.47	15.23	12.51	11.80	-0.58
	925006	0819	954116	925018	954053	954131-2	925060	925074
Average weight gain at the end of one week (3 rats)	6.5	5.8	5.0	4.7	4.0	3.3	[illegible]	-4.3

Exp. II*	925005	0819	029040	925226	BR64		
Average weight gain (4 weeks, 6 rats)	33.4	24.8	23.9	19.7	3.7		
	925005	0819 (new lot)	925226 (methanol HCl treated)	925226	029040	925226 (vanillin-HCl treated)	BR64
Average weight gain (1 week, 3 rats)	6.7	6.7	4.3	4.0	3.3	-0.7	-0.2

* The underlined samples are not significantly different from each other at the 5% level.

12. The Relationship of Forage Protein at Several Stages of Plant Development with Grain Protein in High and Low Grain Protein Varieties of Sorghum bicolor (L.) Moench.

Several low grain protein sorghum lines were observed to have a lighter green plant color than the high protein lines in the sorghum nursery in 1970. The low protein lines appeared to be nitrogen deficient but the high protein lines in adjacent rows appeared healthy. At that time it was postulated that there may be a relationship between the amount of nitrogen in the forage at an early stage of development and grain of sorghum.

Two high grain protein lines (IS 8301 and IS 0354) and two low grain protein lines (IS 2549 and IS 2805) and the Redlan hybrid of each were grown in 1971 by Kofoid and Schaffert. These entries were planted in a randomized complete block design in replicated plots at the Purdue Agronomy Farm[1] and Pinney-Purdue Agricultural Center[2]. The plots consisted of four rows spaced 30 inches apart by 20 feet in length. Twenty-eight days after planting and at two week intervals five competitive plants were harvested at the soil surface from each plot. The plants were cut into small pieces and dried to constant weight in a 140°F dryer. The panicle and forage were kept separate. The dry plant samples were ground and a micro-Kjeldahl analysis for percent nitrogen was conducted. Crude protein

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[1] 500 pounds of N and enough P_2O_5 and K_2O were added to bring the level of each to 500 available nutrients.

[2] [illegible] of N, 60 pounds of P_2O_5 and 60 pounds of K_2O were applied [illegible] planting.

was calculated by multiplying the percent nitrogen by 6.25. Grain and forage yields were determined at the final harvest by harvesting ten feet of a center row of each plot.

The forage protein and the total yields and protein percentages are reported in Table 1. The percent protein of the forage significantly decreased as expected for each period of harvesting, but the forage protein level of the high grain protein entries was significantly higher (see Tables 2 and 3), than the forage protein of the low grain protein lines. The hybrids were intermediate in both cases and not significantly different. The protein values of the hybrids of the two low protein lines generally increased over their male parent and the protein value of the hybrid of the two high protein lines decreased from their male parent for both forage and grain. This is most likely due to the fact that the Redlan female parent is intermediate for grain protein. The response of their hybrid indicates that the inheritance of the forage protein is not dominant and probably quantitative. The analysis of variance of these data in Table 2 and 3 showed a significant difference for forage protein between the four inbred lines and all eight entries. The hybrids were not significantly different. The four lines and four hybrids (Tables 4 and 5) were significantly different at the final harvest for percent grain protein and protein yield but not for grain yield. There were significant differences for forage protein, forage yield and forage protein yield among the eight entries but there was no significant difference among them for total

plant crude protein. The fact that the grain yields were not significantly different but that percent protein and protein yield were significant indicates that protein differences do occur in the same yield level and that improvement can be made by selection.

The correlations (Table 6) between forage protein and grain protein increase each harvest until the fifth or sixth harvest and then level off. A large increase in the correlation is observed just after anthesis as the panicle starts to fill.

These differences and correlations represent a narrow base of material and will be investigated across a wider array of material in 1972. If this positive relationship between forage protein and grain protein is present in a wide array of materials, tremendous advancements may be made in selection for protein improvement in grain sorghum. More efficient progress could be made in a random mating population if plants with superior protein genes could be identified before anthesis and the inferior plants removed.

Table 1. Average Forage Crude Protein at Two-Week Intervals of Two High and Two Low Grain Protein Lines and Their Redlan Hybrids From Two Replications in Each of Two Locations.[1]

	Low Grain Protein %		High Grain Protein %	
	Line Average	Hybrid Average	Line Average	Hybrid Average
Harvesting Stage:				
4 weeks growth	25.3	25.1	26.3	25.3
+ 6 weeks	17.9	17.7	20.2	18.0
+ 8 weeks	14.0	13.6	15.2	13.7
+ 10 weeks	11.1	12.2	11.6	11.4
+ 12 weeks	8.3	10.3	11.2	9.8
+ 14 weeks	6.6	7.5	10.3	7.6
+ 16 weeks	6.4	6.1	8.5	6.6
Mature	5.9	6.5	7.3	6.7
Grain Yield[2] (lbs/acre)	6356	5211	5821	7147
Grain Protein[2] (%)	9.7	10.3	12.1	10.1
Grain Protein Yield (lbs/A)	616.5	536.7	704.3	721.8
Forage Yield (lbs/acre)	11,598	11,526	8,154	9,966
Forage Protein (%)	5.9	6.5	7.3	6.7
Forage Protein Yield (lbs/acre)	684.1	740.0	595.2	666.2
Total Protein Yield (lbs/acre)	1300.6	1276.7	1299.5	1388.0

[1]Pinney-Purdue Agricultural Center (northern Indiana) and Purdue University Agronomy Farm (high fertility plots).

[2]Yields and protein percentages are at 100 percent dry matter.

Table 2. Analysis of Variance for Forage Crude Protein of the Four Sorghum Lines and the Four Hybrids Analyzed Separately.

Source of Variation	df	Mean Square		F-ratio	
		Lines	Hybrids	Lines	Hybrids
Locations (L)	1	4.08	0.01	0.87	0.02
Reps/L	2	0.35	4.27	-	-
Varieties (V)	3	40.26	1.08	8.56*	1.97
L x V	3	2.06	0.64	0.44	1.17
Error a	6	4.70	0.55	-	-
Harvests (H)	7	705.34	672.16	372.17*	1010.76**
L x H	7	13.58	6.47	7.17**	9.73**
V x H	21	3.74	1.42	1.97*	2.13**
L x V x H	21	1.56	3.06	0.82	4.60**
Error b	56	1.89	0.66	-	-

* F is significant at p = 0.05.
** F is significant at p = 0.01.

Table 3. Analysis of Variance for Forage Crude Protein of the Four Sorghum Lines and Their Hybrids.

Source of Variation	df	Mean Square	F-ratio
Locations (L)	1	2.27	0.88
Reps/L	2	1.16	
Varieties (V)	7	20.09	7.32**
L x V	7	1.42	0.51
Error a	14	2.74	
Harvests (H)	7	1373.99	852.30**
L x H	7	16.98	10.55**
V x H	49	2.71	1.68*
L x V x H	49	2.42	1.50*
Error b	112	1.61	

Table 4. Analysis of Variance for Grain Crude Protein and Yield of Four Sorghum Lines and Their Four Redlan Hybrids.

Source of Variation	df	Mean Square		
		Percent Protein	Yield (1000 lbs/acre)	Protein Yield (lbs/acre)
Locations (L)	1	1.033	2.08	0.24
Reps/L	2	1.471	6.06	9.89
Varieties (V)	7	5.028**	5.82	7.10[F]
L x V	7	1.896**	4.47	4.17
Error	14	0.426	2.71	3.29

[F] Significant at p = 0.10.
* Significant at p = 0.05.
** Significant at p = 0.01.

Table 5. Analysis of Variance for Forage Crude Protein and Yield of Four Sorghum Lines and Their Four Redlan Hybrids.

Source of Variation	df	Mean Square			
		% Protein	Yield (1000 lbs/A)	Protein Yield (lbs/A)	Total Plant Crude Protein Yield (lbs/A)
Locations (L)	1	0.548	20.27*	8.88*	6.50
Reps/L	2		9.95	4.95	21.52
Varieties (V)	7	3.061**	14.94*	4.38[F]	2.33
L x V	7	3.345**	5.21	2.88	7.29
Error	14	0.376	4.09	1.86	5.64

[F] Significant at p = 0.10.
* Significant at p = 0.05.
** Significant at p = 0.01.

Table 6. Simple Correlations Between Percent Grain Crude Protein and Percent Forage Crude Protein for each of the Eight Harvests.

Harvest	4 lines and 4 hybrids	4 lines
1	r = 0.15	r = 0.15
2	r = 0.26	r = 0.27
3	r = 0.37	r = 0.37
4	r = 0.28	r = 0.41
5	r = 0.66	r = 0.80
6	r = 0.60	r = 0.81
7	r = 0.52	r = 0.77
8	r = 0.50	r = 0.55

13. Protein and Amino Acid Differences Between Embryo and Endosperm Portions of Sorghum bicolor (L.) Moench Grain.

Complete amino acid determinations were run on the embryo and endosperm portions of entries from the world collection. Protein and oil data for these 46 entries were included in the 1970 annual report. The analysis of variance and means of amino acids that varied between the embryo and endosperm are in Table 1. In some cases the value for the whole seed is not intermediate between the embryo and endosperm but is within the limits of experimental error and the differences observed between embryo and endosperm size.

Note that lysine and protein x lysine increase significantly in the embryo while isoleucine, leucine, phenylalanine and tyrosine decrease significantly in the embryo. Isoleucine and leucine do not decrease in the embryo at the same rate and the isoleucine/leucine ratio increases significantly. Differences of other amino acids between the embryo and endosperm were either not significant or inconsistent across different investigations. Additional studies are being conducted to investigate these amino acids.

The correlations between oil, embryo, endosperm, protein and lysine are in Table 2. The fact that oil and embryo are not significantly correlated indicates that the separations were probably not complete. The most important points are that percent protein and percent lysine are significantly correlated with percent oil. This

evidence indicates that improvement in whole seed protein should be achieved by selecting for oil. Additional correlations are recorded in Table 3.

One item of interest is the significant correlation between oil and endosperm lysine of 0.457 and the significant correlations between embryo lysine and endosperm lysine. If these positive relationships prove to be real, a significant gain in lysine ought to be realized by selecting for oil determined by NMR.

Table 1. Means and Analysis of Variance of Protein and Selected Amino Acids for Embryo, Endosperm and Whole Seed of 46 Sorghum Entries.

Source of Variation	df	Percent Protein	Lysine	Protein x Lysine	Isoleucine	Leucine	Isoleucine/ Leucine	Phenyl-alanine	Tyrosine
Entry	45	9.18**	1.87**	743**	0.21**	1.33*	0.0009	0.65	0.22
Treatment	2	953.64**	92.06**	57518**	24.73**	1304.41**	0.4064**	40.25**	53.61**
Error	90	1.26	.76	410	0.11	0.84	0.0008	0.62	0.17
Means									
Whole Seed		12.4	2.22	27.26	4.24	15.90	0.266	5.17	4.76
Endosperm		11.5	1.2[illegible]	14.07	4.17	16.41	0.254	5.50	4.75
Embryo		19.8	4.01	80.84	2.94	6.94	0.423	3.74	2.89

* Significant at p = 0.05.
** Significant at p = 0.01.

Table 2. Correlations Between Oil, Embryo, Endosperm, Protein and Lysine for 46 Sorghum Entries.

	Percent Oil	Percent Embryo	Percent Endosperm	Whole Seed Protein	Whole Seed Lysine	Embryo Protein	Embryo Lysine	Endosperm Protein	Endosperm Lysine
Percent Oil	1.000	0.123	-0.456**	0.446**	0.339*	0.139	0.575**	0.365*	0.457**
Percent Embryo		1.000	0.042	0.116	0.059	0.058	0.175	0.100	0.142
Percent Endosperm			1.000	-0.243	0.066	-0.165	-0.098	-0.225	0.003
Whole Seed Protein				1.000	-0.291	0.512**	0.273	0.932**	0.138
Whole Seed Lysine					1.000	-0.104	0.442**	-0.325*	-.611**
Embryo Protein						1.000	0.389**	0.626**	0.255
Embryo Lysine							1.000	0.223	0.769**
Endosperm Protein								1.000	0.058
Endosperm Lysine									1.000

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

Table 3. Select Correlations Between Whole Seed Protein, Lysine and Leucine.

Dependent Variable	Independent Variable	Correlation (r)
Whole Seed Protein	Percent oil	.446**
Whole Seed Lysine	Percent oil	.339*
Whole Seed Leucine	Percent oil	.078
Whole Seed Protein	Percent embryo	.116
Whole Seee Lysine	Percent embryo	.059
Whole Seed Leucine	Percent embryo	.035
Whole Seed Protein	Percent endosperm	.243
Whole Seed Lysine	Percent endosperm	.066
Whole Seed Leucine	Percent endosperm	.075
Whole Seed Protein	Protein in embryo	.512**
Whole Seed Lysine	Lysine in embryo	.442**
Whole Seed Leucine	Leucine in embryo	.022
Whole Seed Leucine	Leucine in endosperm	.339*

* Significant at p = 0.05 level.
** Significant at p = 0.01 level.

14. Data Concerning Twenty Select Lines for International Protein Yield Trials and Evaluations in 1972.

Sets of potential breeding materials (500 lines) selected for protein yield potential, diversity and potential adaptation were distributed to overseas cooperators based on their requests. In addition these 500 lines were entered simultaneously in protein yield trials at two or three Indiana locations for two or more years. The Indiana tests contained 10 to 15 commercial hybrid checks representing the various height and maturity groups. Production of lines was then compared to the average commercial check production and removed if the line results were significantly lower than the hybrid check average. The data returned by overseas cooperators were utilized to evaluate yield potential and line adaptation (rating of 1 = good to 9 = poor) to further identify the lines showing a wide range of adaptation with highest protein yield potential. The 1971 yield trials contained 84 lines and 16 commercial checks in replicated trials at three Indiana locations.

The average for lines and hybrids and the ranges in grain yield, percent protein and kilogram protein per hectare were as follows:

	84 lines (range)	16 hybrids (range)	ave. of 100 entries (lines + hybrids)	lines selected (range)
grain (kg/ha)	4240 - 10,420	7410 - 11,930	7545	7720 - 10,420
percent protein	8.6 - 13.1	9.5 - 10.9	10.5	9.3 - 11.3
protein (kg/ha)	470 - 998	769 - 1201	785	730 - 998

The 954... represent numbers which have been selected from the materials received from the Texas Sorghum Conversion Program. The 932... and 956... are Purdue University numbers for items originating from materials not yet having been given I.S. numbers and the other entries are I.S. number selections as indicated.

The grain produced in 1970 was fed to rats after chemical evaluations. Table 1 includes data on all twenty lines selected for this replicated trial. The data includes the average two-week weight gain of six rats fed the sorghum samples plus a mineral-vitamin mix (5% of the ration). The variation in levels of polyphenols (tannins) from line to line can be compared to the rat growth response. Generally the lines high in polyphenols and low in protein gave the poorest rat weight gains. A range of rat growth responses is included in this selection to represent some of the responses we have found regarding the range in bird resistance, weather resistance and other traits seemingly associated with polyphenol (tannins) contents in order to evaluate these factors in a wider more controlled range of environments.

From these data twenty lines have been selected as relatively stable in a wide range of environments for protein yield as compared to the 16 commercial hybrids used as checks. They are entered with four checks into protein yield trials at one or more nitrogen levels with over 15 cooperators who have accepted the responsibility for such tests. The cooperators are located in Brasil, Peru, Colombia, Argentina, Puerto Rico, India, Ethiopia, Nigeria, Uganda, Indonesia,

Korea, Thailand, Philippines and West Pakistan. Data received from these cooperators will indicate the usefulness of our selection procedures and the stability of protein potential of this array of high producing lines. In addition these same lines will be screened for disease and insect resistances. (See attached map.)

Table 1. Data for the Selected Lines Entered into Cooperative Yield, Disease and Insect Resistance Trials.

298 entry no.	Identification	Overseas: no. of locations	Overseas: yield rating range	Overseas: yield rating average	Indiana - '70 grain[1]: polyphenol[2]	Indiana - '70 grain[1]: 2-week rat gain (g)	Indiana - '70 grain[1]: % protein in rat sample	3 locations - Indiana average'71: days to bloom	3 locations - Indiana average'71: ht. cm.	3 locations - Indiana average'71: grain kg/ha	3 locations - Indiana average'71: % protein	3 locations - Indiana average'71: kg protein/hectare
1	954114	7	3-9	7	0.23	7.17	10.0	83	126	8285	11.3	935
2	954255	7	1-9	5	3.35	6.79	10.2	81	124	7720	10.9	846
3	954063	8	1-9	5	0.23	9.00	8.6	80	136	8285	10.2	859
4	932127	8	1-9	6	0.98	1.93	9.3	79	146	9350	9.6	897
7	IS7822	7	1-9	6	1.79	-0.18	7.0	79	157	8160	10.0	812
8	954062	7	1-5	2	0.21	5.27	9.0	80	166	9040	10.3	931
9	932296	7	3-9	4	0.62	1.73	8.7	81	178	8850	9.5	844
10	IS8361	3	3-5	4	0.56			85	178	9038	10.7	964
11	932075	7	5-9	7	0.44	3.52	8.8	85	182	9290	10.2	909
12	956036				1.32	4.42	8.3	84	183	9670	9.9	954
13	954130	9	1-9	5	0.18	3.48	9.9	84	186	8220	10.2	839
14	954206	7	3-9	6	0.21	8.27	8.5	85	189	9540	9.5	906
15	954164	7	1-9	4	0.29	5.87	10.6	86	193	8975	11.0	961
18	954104	6	1-9	5	0.28	2.97	8.3	87	231	8220	9.8	806
19	932065	7	1-9	6	3.22	5.40	9.1	89	263	8660	9.7	847
20	IS9198	7	1-5	3	7.60	-5.92	7.7	97	267	8160	8.9	730
21	IS7579	6	3-9	7	0.27	7.90	8.6	92	267	10420	9.3	958
22	IS9569	7	1-9	5	3.16	5.75	9.4	90	269	8910	9.9	885
23	932027	6	1-9	5	2.85	6.73	8.8	90	269	9540	10.3	948
24	IS4225	6	3-6	4	2.46	5.82	8.5	80	291	8660	11.0	998
Average of 16 commercial hybrid checks								76	161	9400	10.3	922
Average of 84 lines and 16 hybrids in this trial								83	181	7545	10.5	785

[1]Selected lines were entered in yield trials for more than two years in Indiana.

[2]Expressed as catechin equivalents.

PURDUE INTERNATIONAL SORGHUM PROJECT

PROTEIN YIELD TRIALS - 1972

KEY COOPERATORS

15. The Effect of Bagging and Flag Leaf Removal on Grain Quality of Sorghum.

In 1969 Collins (1) reported a significant change in yield, 100 seed weight, percent protein and protein yield due to flag leaf removal and bagging the panicle. When small plots of sorghum are grown for observation and seed increase, panicles of some of the plants are covered with a bag to insure self-pollination. Frequently in the process of bagging the panicle the flag leaf is removed. Grain from the study conducted in 1967 by Collins were analyzed for amino acid distribution. The genotypes used in this study were RS610, IS2319, IS0508, Redlan x IS2319 and Redlan x IS0508. The treatments were:

Treatment Number	Treatment
1	Control
2	Flag leaf removed only
3	Head bagged only
4	Flag leaf removed and head bagged

The experiment was grown in 1967 at the Purdue University Agronomy Farm. The design was a randomized complete block replicated four times. Each plot consisted of two rows 38 inches apart and 15 feet long. The study was grown on a Chalmers silty clay loam soil of high fertility. Two hundred pounds per acre of elemental nitrogen was applied prior to planting. Plants were thinned to similar stands of approximately 100,000 plants per acre.

Grain yield was determined by harvesting 10 feet from each row of the plot. Percentage protein was calculated by multiplying 6.25 by the proportion of nitrogen determined by the micro-Kjeldahl analysis. Seed weight was determined from the weight of 100 seeds. Amino acid

percentages were determined from ion-exchange column chromatography and expressed as a percent of protein. Statistical analyses involved analysis of variance for each character.

The analysis of variance in Table 1 shows significant treatment effects for 100 seed weight, yield, percent protein and percent methionine. The treatment means in Table 1 show that: 100 seed weight was increased by bagging, yield was decreased by all treatments and the percent protein was increased by all treatments. This significant change indicates that all protein values will be biased upward by any of the three treatments that might be used when selfing the plant to keep the seed pure and should not be used for chemical samples when comparing with non-treated samples. Since the interaction is not significant in any case, comparisons could be made where all samples were treated alike. Methionine was the only amino acid significantly affected by the treatments. The general trend was for the percent of the amino acids tested to increase or all to decrease with the treatments.

This evidence and other evidence presented by Collins (1) relating to plant to plant variation within a homogeneous row has led to the following standard procedure for obtaining a chemical sample. Ten consecutively uniformly spaced non-bagged plants within a row that are bordered by comparable height rows should be harvested for the chemical analysis. Any deviation from this results in coefficients of variation (CV) that are unacceptable and biased chemical

values that may mask the true pattern of inheritance. The large CVs for the sulfur-bearing amino acids (Methionine and Cystine) are due to the possible loss of these amino acids in the hydrolysis prior to the amino acid analysis. A different procedure will be required for an accurate determination of these amino acids as these two will always be underestimated.

Literature Cited

1. Collins, F. C. 1969. Genetic and Environmental Control of Protein Quantity and Quality and Their Relationships with Certain Agronomic Characters in Sorghum bicolor (L.) Moench. Ph. D. Thesis. Purdue University.

Table 1. Analysis of Variance Mean Squares and Treatment[a] Means of Five Sorghum Varieties Grown in 1967 for 100 Seed Weight, Yield, Protein and Selected Amino Acids Expressed as a Percent of Protein.

Source of Variation	df	100 Seed Weight (g)	Yield (kg/plot)	Percent Protein	Lysine	PxL	Threonine	Methionine	Cystine
Varieties (V)	4	1.92**	20.3**	45.3**	0.40**	271.7**	0.15	0.20*	0.17**
Treatments (T)	3	.20*	6.1**	2.2**	0.01	6.7	0.08	0.15*	0.01
Blocks	3	.25*	1.2*	0.1	0.04	10.9	0.17	0.09	0.12**
V x T	12	.02	0.3	0.6	0.03	4.2	0.12	0.05	0.02
Error	57	.067	0.37	0.43	0.02	6.5	0.13	0.056	0.027
Treatment		Means							
1		2.73	4.19	11.7	1.97	23.0	3.14	0.97	0.83
2		2.70	4.02	11.6	1.94	22.6	3.12	1.16	0.87
3		2.88	3.50	12.2	1.95	23.3	3.02	1.14	0.86
4		2.90	2.96	12.3	1.96	24.0	3.17	1.13	0.87
CV %		9.28	16.5	5.48	7.53	10.94	11.63	21.5	19.3

[a] Treatment 1 = control, Treatment 2 = flag leaf removed, Treatment 3 = head bagged, Treatment 4 = flag leaf removed and head bagged.
* Significant at p = 0.05.
** Significant at p = 0.01.

Table 1. Continued

Source of Variation	df	Methionine + Cystine	Isoleucine	Leucine	Isoleucine/ Leucine	Phenyl-alanine	Tyrosine	Valine
Varieties (V)	4	0.22	0.11	2.34**	0.0008**	0.90	0.10	0.08
Treatments (T)	3	0.23	0.18	0.74	0.0003	0.67	0.07	0.08
Blocks	3	0.38	0.07	0.62	0.0001	1.23	0.01	0.12
V x T	12	0.11	0.17	0.71	0.0002	0.52	0.04	0.13
Error	57	0.12	0.12	0.34	0.0003	0.61	0.046	0.079
	Treatment				Means			
	1	1.80	3.85	13.6	0.28	4.71	4.18	4.96
	2	2.03	3.93	13.6	0.28	4.76	4.11	4.97
	3	2.00	3.81	13.9	0.28	4.94	4.23	5.03
	4	2.00	3.93	14.0	0.28	5.12	4.24	5.10
	CV %	17.1	8.8	4.2	6.1	16.0	5.1	5.6

[a] Treatment 1 = control, Treatment 2 = flag leaf removed, Treatment 3 = head bagged, Treatment 4= flag leaf removed and head bagged.
* Significant at p = 0.05.
** Significant at p = 0.01.

16. The Effect of Sampling from Different Panicle Branches on Protein, Lysine, Oil and 100 Seed Weight in Sorghum bicolor (L.) Moench.

Screening of the world collection and inheritance studies of protein quantity and quality at Purdue University have often resulted in coefficients of variation that are much too large to detect significant differences in the range of variability that exists in sorghum. Many steps have been taken to reduce the coefficients of variability. These include sorting the genotypes into height and maturity groups, increasing plot size to a minimum of three thirty-inch rows, harvesting a minimum of 10 competitive plants for chemical analysis, adequate weed control, high soil fertility and using a randomized complete block design in replicated trials. These practices have reduced the coefficients of variation appreciability.

In some screening experiments for chemical determinations by Schaffert a few panicle branches have been taken from several panicles in order to reduce the amount of labor and space required for each chemical sample. Koenig and Maranville[1] reported a significant reduction of protein from the top to the bottom of the panicle. The following experiment was conducted to determine the effect of panicle branch position on protein quantity and quality.

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[1]R. F. Koenig and J. W. Maranville. 1970. Variations of Protein Content Within a Line of Sorghum. Annual Report 4. Research and Physiology of Yield and Management of Sorghum in Relation to Genetic Improvement. Cooperative Research by the University of Nebraska; Crop Research Division, ARS, USDA, and The Rockefeller Foundation

Four entries grown at two locations in Indiana in replicated yield trials were investigated. The panicles from ten competitive plants in each plot were partitioned into three nearly equal portions (top, middle and bottom) and threshed. The protein was calculated by multiplying the percent nitrogen of a defatted ground grain sample by 6.25. The percent lysine of protein was determined from ion exchange column chromatography. The percent oil was determined at the University of Illinois using the NMR (nuclear magnetic resonance spectroscopy) technique. The 100 seed weight was determined by weighing 100 whole random grains.

The average yield of these four entries was 6,631 pounds per acre calculated at 14.0 percent moisture in 1970. The treatment means for the five variables are shown in Table 1. The analysis of variance is shown in Table 2. The percent crude protein significantly decreased from the top one-third of the panicle to the bottom one-third of the panicle by an average of 1.1 percent. The percent lysine of protein did not vary significantly from the top to the bottom of the panicle. The percent lysine of the sample [(% protein x % lysine) /100] decreased significantly from the top to the bottom of the panicle by 0.021 percent. The percent oil increased significantly from the top to the bottom of the panicle by a value of 0.20 percent. One hundred seed weight did not vary significantly, but the trend was a reduction in seed weight from the top to the bottom of the panicle.

These results indicate that extreme care must be exercised when collecting panicle branches for chemical analysis of the grain. Panicle branches should be selected from the center of the panicle to obtain consistent results that represent the entire panicle.

Table 1. Average Values for Sorghum Grain for the Top, Middle and Bottom of the Panicle for Protein (P), Lysine as a Percent of Protein (L), P x L, Percent Oil and 100 Seed Weight Expressed in Grams.

Portion of Panicle	Protein	Lysine	PxL	Oil	100 Seed Weight
Top	12.32	1.98	24.3	2.9	2.08
Middle	11.70	2.00	23.3	3.0	2.08
Bottom	11.27	1.98	22.2	3.1	2.01
C.V.	5.23	8.29	7.72	2.78	10.79

Table 2. Analysis of Variance Mean Squares for the Five Variables Listed in Table 1 Above.

Source of Variation	df	Protein	Lysine	PxL	Oil	100 Seed Weight
Location (L)	1	9.10**	0.64**	7.5	0.088**	0.413**
Rep L	2	0.70	0.01	6.9	0.007	0.024
Entries (E)	3	7.50**	0.18**	53.5**	0.256**	0.441**
L x E	3	1.23*	0.05	3.7	0.022	0.011
Treatment (T)	2	4.45**	0.01	17.3*	0.066**	0.023
L x T[a]	2	0.36	0.05	2.2	0.007	0.036
E x T[a]	6	0.29	0.02	0.8	0.012	0.008
L x E x T[a]	6	0.22	0.01	1.6	0.005	.008
Error	22	0.38	0.027	3.2	0.007	0.049
Total	47					

*F - Significant at p = 0.05 assuming a fixed model.
**F - significant at p = 0.01 assuming a fixed model

[a]These sources of variation pooled with error do not change significance in any of the tests.

17. The Use of Opaque Seed Characteristics to Date.

Previous years' data have shown a normal distribution pattern of protein quantity and quality in the lines and their hybrids. No opaques have shown significant superiority to date. Selections have been continued with fifteen lines being held in an evaluation and inheritance study. The data for 1971 is still being processed and chemical samples being studied.

18. The Influence of a High Minimum Night Temperature on Floral Bud Initiation.

In 1970 eighty lines that normally flower in the temperate regions were planted by Oswalt in the field and in a greenhouse at Lafayette, Indiana. The temperature in the greenhouse was maintained at a minimum of 90°F night and day. Approximately one-third flowered and one-third did not flower. During the 1971 growing season twenty lines that flowered and twenty that did not flower with their Redlan hybrids were planted under the same greenhouse temperature conditions and in the field. In the greenhouse all lines repeated the performance of the previous year and their Redlan hybrid performed uniformly as their parental lines. All lines and their Redlan hybrids flowered in the field. Hand emasculations were attempted among selected lines for a continuation of this study.

19. A Study on Improving Protein Quality of Sorghum bicolor (L.) Moench with Diverse Lines.

Sorghum grain serves as a human food in some parts of the world. In recent years sorghum grain has been used extensively in the livestock industry in this country. With increased utilization of acreage for the cultivation of sorghum, farmers are interested in improving the yield and the quality of this grain.

Lowe (8) and Collins (4) reported an inverse relationship between yield and percent protein in sorghum. Kambal and Webster (7), Malm (9), Beil and Atkins (2) and Abifarin (1) observed that general combining ability (GCA) and specific combining ability (SCA) were important sources of variations for yield and other traits in sorghum. Niehaus and Pickett (10) and Malm (9) found that heterosis in sorghum was greatly influenced by diverse lines.

Research studies in relation with inheritance of yield, protein and amino acid content have been limited. This paper presents a study of gene effects, heterosis, GCA and SCA of sorghum grain for yield, protein and amino acids.

Materials and Methods

Three male sterile inbred lines (Combine Kafir-60, Martin and Redlan) and 24 male fertile inbred lines were selected from the world sorghum collection and grown at Purdue University Agronomy Farm in 1969. Each male sterile line was crossed with each of the 24 male lines, as a result 24 F_1 hybrids for each male sterile line were

produced. The lines and hybrids were grown in 1970. The experiment was replicated four times as a randomized complete block design. The entire experiment was conducted under high fertility conditions established by the application of adequate quantities of nitrogen, phosphorus and potassium during the past several years. The relative importance of certain phenotypic characters with respect to grain yield were studied and reported by Gelaw (6) in 1971. Complete amino acid analysis was done for parental and hybrid grain samples. Only two replications chosen at random were used to obtain protein, lysine, threonine, methionine, cystine, isoleucine, leucine, phenylalanine, tyrosine and valine contents in the whole grain.

The analysis of variance for parental and F_1 hybrids were studied by Srinivasan for yield, protein and amino acid contents. Percent heterosis was calculated for each character utilizing the mid-parent means. In the combining ability analysis of variance, male and female effects were expressed as GCA while the interaction among male and female was expressed as SCA as suggested by Cockerham (3) and Comstock et al (5). Phenotypic correlation coefficients were calculated for all characters in parents and in hybrids.

Results and Discussion

The analysis of variance among parental lines and among hybrids are presented in Table 1. Significant differences were found for yield, protein, leucine, among the male parents and for yield, protein, phenylalanine and tyrosine among the female parents. The analysis of

variance showed significant genotypic differences in yield, protein, lysine and leucine for Combine Kafir-60 hybrids, for yield and protein for Martin hybrids, and for protein for Redlan hybrids. Apparently the response for phenylalanine and tyrosine from the female parents were not contributed to their hybrids.

The average performance of parents, hybrids and the percent heterosis over mid-parent mean are given in Table 2. Compared to mid-parental values, the F_1 hybrids showed heterosis for yield, for lysine and for threonine. The percent heterosis for yield, lysine and threonine were 10.74, 4.42 and 1.17 respectively. Hybrids showed a negative heterosis of 5.53 for percent protein. Heterosis for the other amino acids were negative in nature though not high. Inbred parental lines that were superior for certain characteristics did not necessarily produce superior hybrids.

The results of the combining ability analysis of variance are shown in Table 3. SCA was non significant for yield, protein and for all the amino acids studied. Yield, protein, lysine and leucine showed variation due to male GCA while only protein showed variation due to female GCA. Additive gene action may be important for yield and for protein in sorghum but the nature of the gene action of the other amino acid contents could not be predicted in this study.

Estimates of phenotypic correlations among the characters for parents and for F_1 hybrids are given in Table 4. Grain yield and

percent protein were negatively correlated ($r = -.414$ for parent and $r = -.254$ for F_1 hybrids). Although grain yield and lysine percent of protein were negatively correlated, the estimate was non-significant and near zero. The correlation coefficients among lysine with other amino acids were all positive except with leucine for F_1 hybrids. Isoleucine, leucine, phenylalanine, tyrosine and valine were all positively correlated among each other.

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Table 1. Analysis of Variance of F_1 Hybrids and Parents.

Character	CK-60 Hybrids	Martin Hybrids	Redlan Hybrids	Male Parent	Female Parent
Yield	*	*	NS	**	**
Protein	**	**	*	**	*
Lysine	*	NS	NS	NS	NS
Threonine	NS	NS	NS	NS	NS
Methionine	NS	NS	NS	NS	NS
Cystine	NS	NS	NS	NS	NS
Isoleucine	NS	NS	NS	NS	NS
Leucine	*	NS	NS	*	NS
Phenylalanine	NS	NS	NS	NS	*
Tyrosine	NS	NS	NS	NS	**
Valine	NS	NS	NS	NS	NS

* Significant at the .05 level.
** Significant at the .01 level.
NS Non-significant.

Table 2. Average Performance of Parents, F_1 Hybrids and Percent Heterosis.

Character	Male Parent	Female Parent	F_1 Hybrid	Heterosis (percent)
Protein (kg/ha)	664	691	710	4.79
Yield (kg/ha)	5988	6338	6825	10.74
Protein (%)	11.14	10.92	10.42	-5.53
Lysine*	1.93	1.69	1.89	4.42
Threonine	3.51	3.32	3.46	1.17
Methionine	1.07	0.73	0.90	0.00
Cystine	0.91	0.88	0.84	-6.67
Isoleucine	4.44	4.16	4.28	-0.47
Leucine	16.63	16.26	16.16	-1.76
Phenylalanine	5.61	5.30	5.42	-0.73
Tyrosine	4.99	4.85	4.82	-2.03
Valine	5.73	5.60	5.64	-0.53

* Lysine, threonine, methionine, cystine, isoleucine, leucine, phenylalanine, tyrosine and valine are expressed in percent of protein.

Table 3. General and Specific Combining Ability Analysis of Variance.

Character	General Combining Ability		Specific Combining Ability
	Male Effects	Female Effects	Male and Female Interactions
Yield (kg/ha)	**	NS	NS
Protein	**	**	NS
Lysine	*	NS	NS
Threonine	NS	NS	NS
Cystine	NS	NS	NS
Isoleucine	NS	NS	NS
Phenylalanine	NS	NS	NS
Tyrosine	NS	NS	NS
Valine	NS	NS	NS

* Significant at the .05 level.
** Significant at the .01 level.
NS Non-Significant.

Table 4. Phenotypic Correlation Coefficients Among 24 Parents and Among 72 F_1 Hybrids.

	Yield	Protein	Lysine	Threonine	Methionine	Cystine	Isoleucine	Leucine	Phenylalanine	Tyrosine	Valine
Yield	1.000[1]	-.414**	-.035	-.007	.015	.098	-.105	-.102	-.085	-.116	-.038
	1.000[2]	-.254*	-.050	-.082	-.052	.079	-.086	-.072	.037	-.036	-.074
Protein		1.000	-.364	-.314	.018	-.286	-.107	.046	.076	-.052	-.254
		1.000	0.477**	-.300*	.174	.263*	.004	.185	.015	.060	-.227
Lysine			1.000	.569**	.140	.241	.097	.310	.074	.037	.315
			1.000	.516**	.186	.258*	.141	-.175	.126	.139	.421**
Threonine				1.000	.311	.438	.498	.241	-.347	.579**	.590**
				1.000	.274*	.643**	.654**	.465**	.676**	.755**	.663**
Methionine					1.000	.305	.244	.157	.320	.286	.264
					1.000	-.009	.173	.082	.164	.182	.217
Cystine						1.000	.019	-.095	.025	.240	.120
						1.000	.312**	.157	.313**	.440**	.317**
Isoleucine							1.000	.869**	.813**	.138	.849**
							1.000	.882**	.840**	.823**	.824**
Leucine								1.000	.816**	.725**	.715**
								1.000	.836**	.806**	.691**
Phenylalanine									1.000	.802**	.667**
									1.000	.874**	.712**
Tyrosine										1.000	.675**
										1.000	.684**
Valine											1.000
											1.000

* Significant at the .05 level.
** Significant at the .01 level.
[1]Correlation coefficients among parents.
[2]Correlation coefficients among F_1 hybrids.

20. The Effect of Environments on Protein, Lysine, Oil and Yield in *Sorghum bicolor* (L.) Moench.

A genotype by environment investigation was initiated in 1970 by Schaffert to study the effects of environments on protein, lysine, oil and yield in addition to other agronomic factors. This study consisted of 10 diverse lines from the world collection, the hybrid of each line by Redlan, the hybrid of each line by Martin, Redlan and Martin B lines and three commercial checks. The 35 entries were grown in 1970 and 1971 at four locations in Indiana, at Lubbock, Texas, and at Mead, Nebraska. The entries were planted in two-row plots in a randomized complete block design with two replications at each location. The chemical analysis has been completed on the samples collected in 1970. The samples collected in 1971 are being analyzed for protein, lysine and oil.

The results from 1970 are presented in Table 1. A significant genotype by environment interaction for percent crude protein, protein yield, grain yield, percent lysine of sample and percent oil was observed. The interaction for lysine was not significant. Simple correlation for these traits (Table 2) correspond with values reported in the literature except for the correlation of o.06 for yield and percent protein. This is probably due to a moisture and nitrogen deficiency at the southern Indiana location and the response on the high fertility plots at the Purdue University Agronomy Farm. The second year's materials are being evaluated for protein, lysine,

oil and yield and will be reported in 1972. The size and nature of these year and location interactions will have a significant influence upon the way breeding material should be released to the LDCs, and the degree of difficulty of finding and identifying high protein lines.

Table 1. Analysis of Variance for 35 Varieties from Six Locations. (Mead, Nebraska - Lubbock, Texas - Purdue Agronomy Farm - Purdue Agronomy Farm High Fertility - Southern Indiana Purdue Agricultural Center and Pinney Purdue Agricultural Center in northern Indiana - 1970.)

	Percent Protein (P)	Percent Lysine of Protein (L)	PxL	Yield (Y)[a]	PxY	PxLxY	Oil
Locations (L)	**	**	**	**	**	**	**
Varieties (V)	**	**	**	**	**	**	**
L x V	*	NS	**	**	**	**	**

* Significant at p = 0.05.
** Significant at p = 0.01.
[a] Yield data not available for Nebraska location.

Table 2. Simple Correlation Values for the Variables in Table 1 Above.

	% P	% L	P x L	Yield	P x Y	P x L x Y	Oil
% P	1.00	-0.70	0.74	0.06	0.49	0.34	0.52
% L		1.00	-0.07	-0.24	-0.51	-0.23	-0.35
P x L			1.00	-0.10	0.24	0.31	[a]
Yield				1.00	0.89	0.91	-0.25
P x Y					1.00	0.94	[a]
P x L x Y						1.00	[a]
Oil							1.00

[a] Correlations not calculated.

21. Progress on Developing Populations and Segregating Materials of Improved Protein Quantity and Quality, Sorghum bicolor (L.) Moench Genotypes.

Two hundred eight restorer lines (R-lines) were crossed onto NP3R, a Nebraska population (restorer) selected for yield and segregating for the male sterile gene ms_3. One hundred five non-restorer lines (B-lines) were crossed onto NP2B, a Nebraska population (non-restorer) selected for yield and segregating for the male sterile gene ms_3. The F_1's were planted in Puerto Rico for selfing and the F_2's will be planted at Lafayette in 1972 to force recombination (Doggett, 1970). The B and R populations will be maintained in separate isolated blocks. The R and B line entries used to form these new populations were selected from high yielding lines with high protein and/or high lysine. These populations will offer a new source of superior genetic recombinations as well as a segregating source of potential high quality material for distribution to developing nations. The exact distribution procedure used will depend upon the final results from current genotype by environment interaction studies. More than likely selections will be made at Purdue for nutritional quality. Improved segregating materials will then be distributed for cooperator selection of high nutritional quality with appropriate insect and disease resistance in the area of intended use. If the resistances for the local disease and insect problems are not present, they can quickly be added to this random mating segregating population. Random mating segregating populations

maintained with a simple male sterile gene segregating for male sterility and male fertility have a tremendous potential for producing superior recombinants and for distribution to developing nations.

Crossing was carried out at the Purdue University Agronomy Farm in 1971 with three commercial cytoplasmic male steriles (Redlan-A, Martin-A and OK 24-A). Some crossing was conducted to continue studies initiated prior to 1971 and some crossing was conducted as a part of the five-year work cycle corresponding to cycle year two and cycle year three. Hand crosses using hot water emasculation and polyethylene bagging were made between high protein lines and between high temperature sensitive and nonsensitive lines.

Selections were made in 28 segregating bulk populations. The selections were based upon seed size, yield, seed color (polyphenols), height, and other desirable agronomic characteristics. Some of these 28 populations are in sterile cytoplasm and some are in normal cytoplasm. Recombination is not being forced in these populations. Selections range from F_3's to F_5's and many will be evaluated in one-row plots next year as well as being composited to maintain the population. Some selections will eventually be released to the 148 cooperators around the world.

Individual plant selections were made in 250 F_2 segregating populations involving 140 lines selected for highprotein and/or high lysine. These 250 F_2 segregating populations represent 12 sets of

four by four partial diallel crosses initiated in 1970 as a recurrent selection scheme. Each population is in normal cytoplasm as each original cross was made by hand emasculation. Seven hundred twenty-three random F_3 plants will be selfed in the winter of 1971-72 at Puerto Rico. The resulting F_4 lines will be used in the recurrent selection scheme described in the 1969 annual report. In addition about two thousand single F_3 family rows will be planted at the Purdue University Agronomy Farm in 1972 for additional selection.

One thousand five hundred sixty F_3 families selected at random from 54 F_2 families in normal cytoplasm that represent 62 lines selected for high oil and high protein and/or high lysine and crossed by hot water emasculation were evaluated in 1971. Five hundred fifty-two individual plant selections were sent to Puerto Rico for selfing and will be evaluated as F_4 lines in 1972. About 300 F_3 families were harvested for yield and chemical analysis. About 25 to 50 of these selected on the basis of yield and protein will be distributed to the 148 cooperators around the world as provided for in objective 7. This will be the first release of segregating material selected for nutritional quality. The response of the cooperators to this material will influence future efforts regarding the release of segregating material.

Some lines that are showing superior yield and nutritional quality performance are partial conversions (F_2, BC_1, BC_2) from the Texas conversion program selected by R. C. Pickett and Lee House

in 1968. This indicates that nutritionally superior lines ought to be derived from these segregating populations.

About 30 F_1's made by hot water emasculation and their parents were grown in replicated two-row plots in 1971. These were evaluated for yield and chemical analysis and studies will be completed to gain information relating to the inheritance of protein quantity and quality. These results will greatly aid in determining the amount of heterosis from exotic germ plasm. In addition the balance of amino acids in parents and hybrids will be studied on select material. An additional 120 F_1's were planted in one-row plots for evaluation at the Purdue University Agronomy Farm. A limited number of plants of these F_1's were selfed in Puerto Rico the previous winter and make up a part of the 250 F_2 populations mentioned elsewhere.

22. A Comparison of Three-Row and One-Row Plots for Line Evaluations and Selection.

Fifty lines were selected from the world collection one-row growout in Puerto Rico and in the Purdue nursery in 1970. They were planted in one-row plots in three replications and in three-row plots in three replications in 1971 to study the variation in selection that would result when various workers estimated phenotypic characteristics in one-row plots and three-row plots. The influence of border effects on yield, flowering date, height and percent protein will also be studied. The data completed to date is shown in Table 1 for the summary of the single row compared to the middle row of the three-row plots.

The selections of lines based on harvested yield data from one-row plots compared to the yield data from three row plots would have resulted in 30 percent error in relative rank in lines selected. The magnitude of the error variances and the coefficients of variability indicate that the three-row plots are required to show adequate sampling for yield which is also expected to influence the protein yield for which analysis is being completed.

Table 1. Comparison of One-Row with Three-Row Plots for Screening New Lines.

	one-row plot	middle row of three-row plots
Yield		
Ave. bu/A	105.2	101.8
Error Mean Square	734.56	409.79
Coefficient of Variability (%)	26.4	19.8
Days to Flower		
Ave. (days)	82.5	80.0
Error Mean Square	6.75	3.59
Coefficient of Variability (%)	3.15	2.35
Height		
Ave. (cm)	248	240
Error Mean Square	313.69	217.87
Coefficient of Variability (%)	7.13	6.14

23. 1971 Preliminary Rat Feeding Trials.

Sorghum samples with potentially useful quality protein were selected and fed to rats. Each sample was supplemented with a vitamin and mineral mixture (5% of ration) and fed to six weanling rats for four weeks. The two-week weights were taken and subsequent feeding trials were shortened to two-week feeding periods to reduce the total amount of grain required per trial. The data for the May set of samples are included in Table 1. This set of samples shows a variation in percent protein, percent lysine of protein, percent *in vitro* dry matter disappearance and catechin equivalents. The correlations of these factors are shown in Table 2.

The summary of the data from 70 samples fed to rats are presented in Table 3 where the samples were sorted into five groups according to crude protein estimations. The correlations of percent protein, catechin equivalents (Vanillin-Hydrochloric acid method, Bates-Smith and Lerner) and two-week gains are shown in Table 4. The selection of these samples shows a decrease in amounts of catechin equivalents as the percent protein increased. This was due to our selection of samples low in catechin equivalents which were high in crude protein rather than a complete array of catechin equivalent levels.

These data show the strong influence of the substances represented by catechin equivalent evaluation on the rate of rat gain and has resulted in our screening for the catechin equivalent level before

estimating a rat growth response based on crude protein or amino acid balance. The significant correlations of protein and weight gain ($r = +.594^{**}$) and catechin equivalent and weight gain ($r = -.554^{*}$) for the overall estimates indicate the need to raise the protein content of the total ration and to evaluate the actual influence of the substances represented by catechin equivalents in the grain.

Table 1. Sorghum Project Rat Feeding Studies.

Sorghum Lines	Feed Consumed[1] (g)	Total wt. gain 2 weeks (g)	Total wt. gain 4 weeks (g)	*in vitro* DMD[2] (%)	Catechin Equivalent (%)	Protein (%)	Lysine of Protein (%)	P x L	Protein x *in vitro* DMD
925006	226	11.81[3]	34.81	93.6	.52	12.3	2.70	.332	1273
925018	175	07.63	20.27	91.5	.44	14.3	1.98	.283	1199
IS2319	191	05.73	15.47	87.4	.51	11.8	2.53	.298	0970
954116	182	04.45	16.93	89.7	.38	11.9	2.34	.279	1067
954054	170	03.90	15.23	83.5	.53	10.4	2.46	.256	0818
925060	174	03.70	11.80	68.2	6.26	12.4	2.65	.329	0791
954131-2	156	02.90	12.51	90.3	.42	11.8	2.22	.263	0975
925074	156	-03.28	-00.58	66.4	6.88	09.0	2.68	.242	0671

[1] 95% grain sorghum, 5% vitamin and mineral supplement.
[2] DMD = dry matter disappearance.
[3] Weight gains connected by a line are not significantly different (.05 level).

Table 2. Nutritional Evaluation of Selected Sorghum Lines for Rats. (Correlations)

	Protein	Lysine	Catechin Equivalent	*In-vitro*	Protein x *in vitro*	2nd week gains	4th week gains	Feed Consumption
Protein	1.000	-.587	-.453	.562	.753*	.728*	.612	.361
Lysine		1.000	.548	-.539	-.410	-.148	-.076	.331
Catechin Equivalent			1.000	-.962**	-.726*	-.652	-.653	-.382
In vitro				1.000	.871**	.744*	.754*	.491
Protein x *In vitro*					1.000	.884**	.884**	.670*
2nd week Gains						1.000	.977**	.846**
4th week Gains							1.000	.873**
Feed Consumption								1.000

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

Table 3. Mean Values of Protein, Catechin Equivalent and Weight Gain in Rats.

Range of Protein (percent)	Average Protein (percent)	Catechin Equivalent	2nd week gains (g)	No. of Observations
7.0 - 8.9	8.20	2.29	1.16	20
9.0 - 9.9	9.44	1.69	2.06	15
10.0 - 10.9	10.39	1.39	5.63	11
11.0 - 11.9	11.62	0.41	6.95	14
12.0 - 13.9	13.15	0.99	6.64	10

Table 4. Correlations Among Protein, Catechin Equivalent and Weight Gain in Rats.

	Protein	Catechin Equivalent	2-week Gains	Protein+	No. of Observations
Protein	1.000	-.019	.379	7.0-8.9	20
	1.000	.003	.013	9.0-9.9	15
	1.000	.427	-.014	10.0-10.9	11
	1.000	.276	-.081	11.0-11.9	14
	1.000	-.331	.385	12.0-13.9	10
	1.000	-.294	.594**	overall	70
Catechin Equivalent		1.000	-.648**	7.0-8.9	20
		1.000	-.555*	9.0-9.9	15
		1.000	-.561	10.0-10.9	11
		1.000	-.203	11.0-11.9	14
		1.000	-.375	12.0-13.9	10
		1.000	-.554**	overall	70

* Significant at the .01 level.
** Significant at the .05 level.
+ Average protein percent in the feed (fed to rats).

24. The Role of Polyphenols in Nutritional Quality of Sorghum bicolor (L.) Moench Grain.

The following abstract appeared in "Agronomy Abstracts" of the 1971 annual meetings of the American Society of Agronomy and was the abstract of a paper given by Robert E. Schaffert in August 1971.

"Influence of Tannin Content and Urea on the In Vitro Digestibility of Sorghum bicolor L. Moench Grain. R. E. Schaffert, V. L. Lechtenberg, R. C. Pickett, D. L. Oswalt and C. L. Rhykerd, Purdue University.

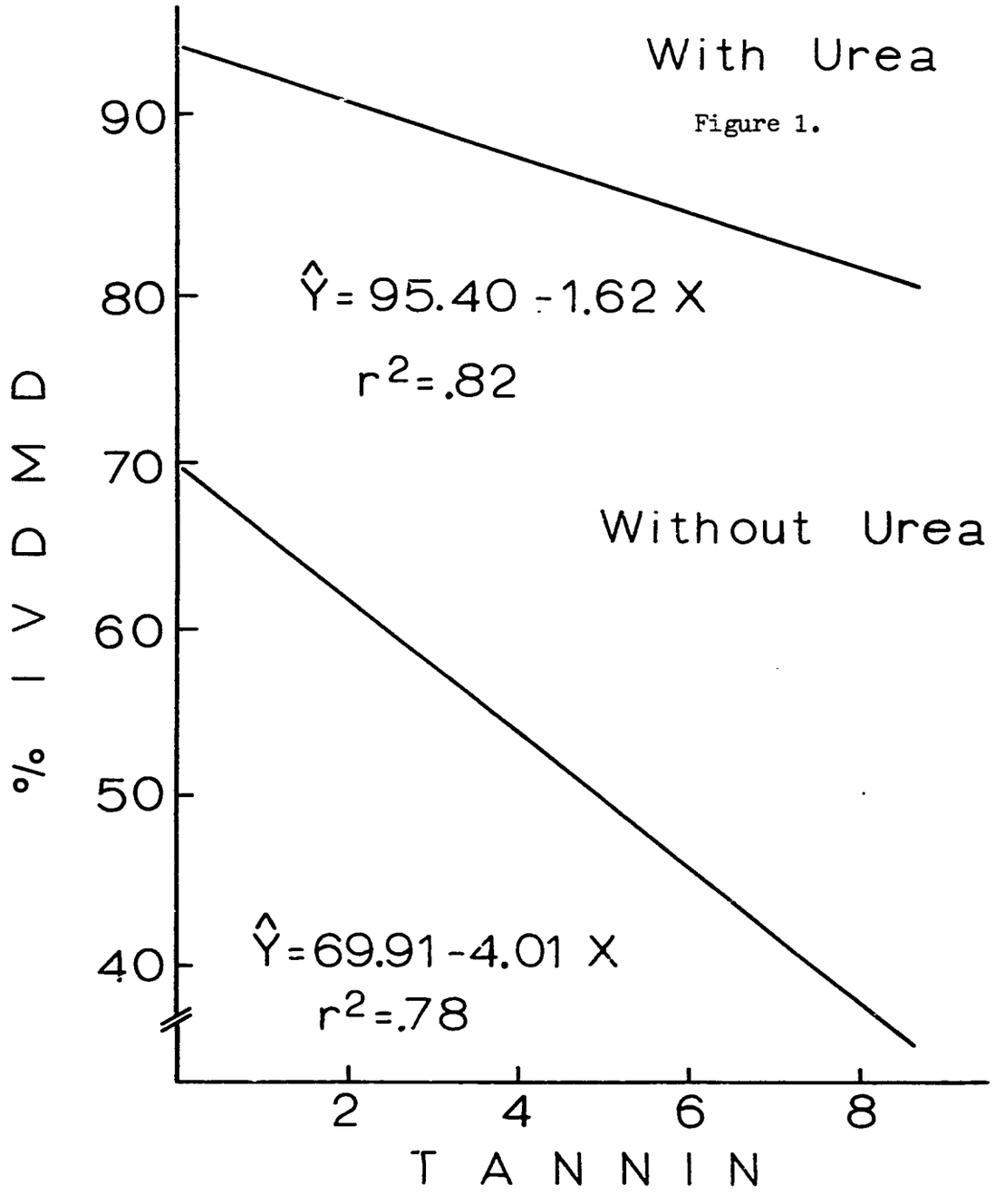
Two experiments were conducted to study the effects of urea on the relationship between tannin (catechin equivalents/100 g dry weight) and IVDMD of Sorghum bicolor L. Moench grain. In the first experiment 21 entries of lines and hybrids with tannin values ranging from 0.4 to 13.7 were studied. IVDMD was reduced 3.1 percentage units for each unit increase in tannin (r^2 = 0.77). IVDMD ranged from 35% to 80%. When urea was added to the in vitro system the IVDMD ranged from 78% to 95% and IVDMD was reduced 1.4 percentage units for each unit increase in tannin (r^2 = 0.93). In the second experiment one female line, 10 male lines from the world collection and their 10 hybrids were studied. The tannin values ranged from 0.2 to 7.5, and the IVDMD ranged from 43% to 81%. IVDMD was reduced 4.0 percentage units for each unit increase in tannin (r^2 = 0.78). When urea was added the digestibilities ranged from 78% to 95% and IVDMD was reduced 1.6 percentage units for each unit increase in tannin (r^2 = .081). These results suggest that tannin does not inhibit the microbial breakdown of grain sorghum directly but rather indirectly by reducing the amount of nitrogen available for bacterial growth."

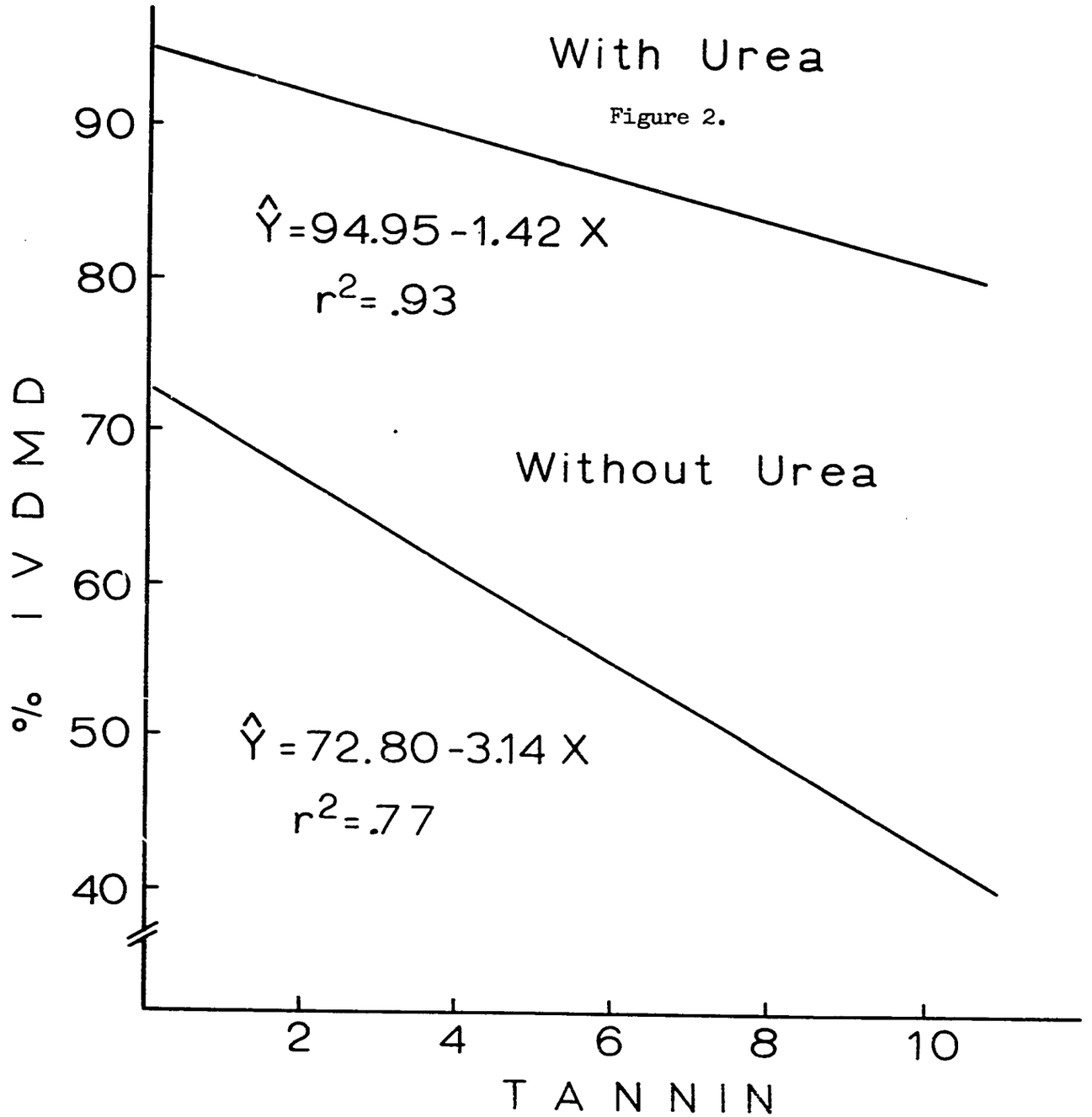
Figures 1 and 2 show these relationships graphically.

Entries selected from those used in the study described above were used to further investigate the role of polyphenols in the digestion of sorghum grain. The residue from IVDMD was collected and analyzed for nitrogen for both high and low tannin genotypes. A significantly larger amount of nitrogen was left in the residue of the high tannin entries. This indicates that the polyphenol somehow reduces the digestibility of the protein more than it reduces the digestibility of the carbohydrates.

This is in agreement with other investigations at Purdue University involving chick feed trials and rat feeding trials indicating that perhaps the polyphenol is complexing with the polypeptide forming a non-digestible complex. Preliminary results from protein fractionation studies also indicate that this could be the case.

Additional investigations are being planned to study the role of polyphenols in digestion.


With Urea
Figure 1.
$\hat{Y}$= 95.40 -1.62 X
r^2=.82
Without Urea
$\hat{Y}$=69.91-4.01 X
r^2=.78
% IVDMD
90
80
70
60
50
40
2
4
6
8
TANNIN


With Urea
Figure 2.
$\hat{Y}$=94.95-1.42 X
r^2= .93
Without Urea
$\hat{Y}$ = 72.80-3.14 X
r^2=.77
% IVDMD
90
80
70
60
50
40
2
4
6
8
10
TANNIN