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PROTEIN QUANTITY, QUALITY, AND AVAILABILITY IN *SORGHUM BICOLOR* (L.) MOENCH GRAIN

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of

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by

Robert Eugene Schaffert

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of

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ABSTRACT

Schaffert, Robert Eugene. Ph.D., Purdue University, December 1972. Protein Quantity, Quality, and Availability in Sorghum bicolor (L.) Moench Grain. Major Professors: R. C. Pickett and J. D. Axtell.

Thirty-five genotypes were planted in six locations including Indiana, Nebraska, and Texas in each of two years for the purpose of estimating genotype-environment interactions for yield, protein, lysine, oil, and seed size. Significant genotype-year-location interactions existed for grain yield, percent protein, protein yield, percent lysine of sample, lysine yield, percent oil, seed weight, protein per seed, height and days to median flower. The relative small genotype-year and genotype-location interactions of nearly equal magnitude indicated that years and locations could be interchanged for percent protein, lysine as a percent of protein, lysine as a percent of the sample, percent oil, seed weight, and protein per seed without increasing the variance of a genotype mean. Correlations between genotypes averaged across environments indicated that percent protein could be increased by selecting for increased oil or protein per seed. Protein per seed was not correlated with yield but percent oil was negatively correlated with yield. The positive correlation between days to flower and percent lysine of protein and the negative correlation between days to flower and percent protein indicated that selection for these traits should be done within maturity groups.

The *in vitro* dry matter disappearance (IVDMD) and *in vitro* nitrogen loss (IVNL) techniques were used to study the effect of tannin on the nutritive value of sorghum grain. The IVDMD technique detected differences within high tannin varieties, within low tannin varieties and between high and low tannin varieties. The addition of small amounts of urea to the system decreased the experimental error nearly 75%. Significantly lower values for IVDMD and IVNL were observed for the high tannin varieties. The average difference of IVDMD between high and low tannin varieties with urea and one incubation was 14.1%, without urea and two incubations was 8.3%, and with urea and two incubations was 7.8%. Approximately 50% of the difference in IVDMD between high and low tannin varieties was accounted for by differences in tannin content and insoluble protein. It was assumed that the tannin bound to the protein to form an insoluble complex. The IVDMD technique can be an important tool of the plant breeder for selecting genotypes with superior nutritive value.

Four levels of protein supplementation, with low and high tannin sorghum grain each with a low and high level of protein in the grain were fed to weanling rats. Thirteen-day weight gain, feed efficiency ratio, protein efficiency ratio, and feed consumed were highly significant for tannin levels and levels of protein supplementation. Protein efficiency ratio and feed consumed were highly significantly different for levels of grain protein. The high tannin grain had poorer weight gains, reduced feed efficiency and lower protein efficiency. Feed consumption of the high tannin diets was greater or equal to the low tannin diets. Significant differences between high and low tannin genotypes for weight gain were observed at intermediate levels of protein supplementation

(5 and 10% soybean meal) but not at the high level (15%) of supplementation. The lack of antagonistic interactions with supplemental protein for weight gain, feed efficiency, protein efficiency and feed consumption indicated that protein from soybean meal can be supplemented without changing the relative performance of different sorghum genotypes.

SECTION I

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

SECTION I

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

Introduction

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

Materials and Methods

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

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

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

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

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

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

[1] Shuman Chemical Laboratory, Battle Ground, Indiana.

[2] University of Illinois, Champaign, Illinois.

were determined by making the appropriate calculations.

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

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

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

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

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

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

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

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

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

Results and Discussion

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

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

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

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

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

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

* Normal fertility.

\+ High fertility.

space for genotypes, locations and years.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

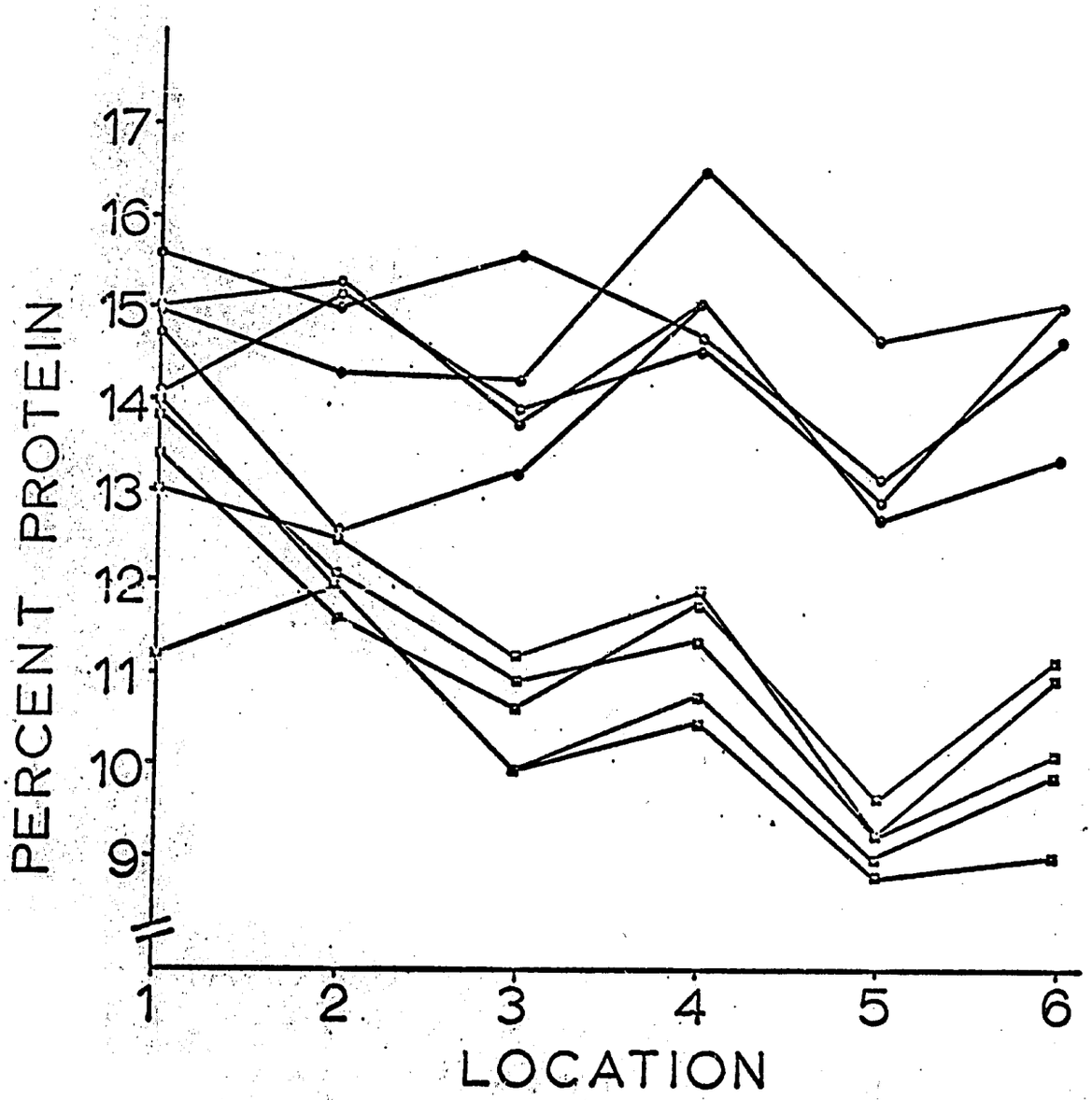
selection for percent protein via percent oil would tend to lower grain yield whereas selection for greater protein content per seed would tend to increase percent protein without reducing grain yield. Both seed weight and oil can be measured without destroying the seed.

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

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

Conclusions

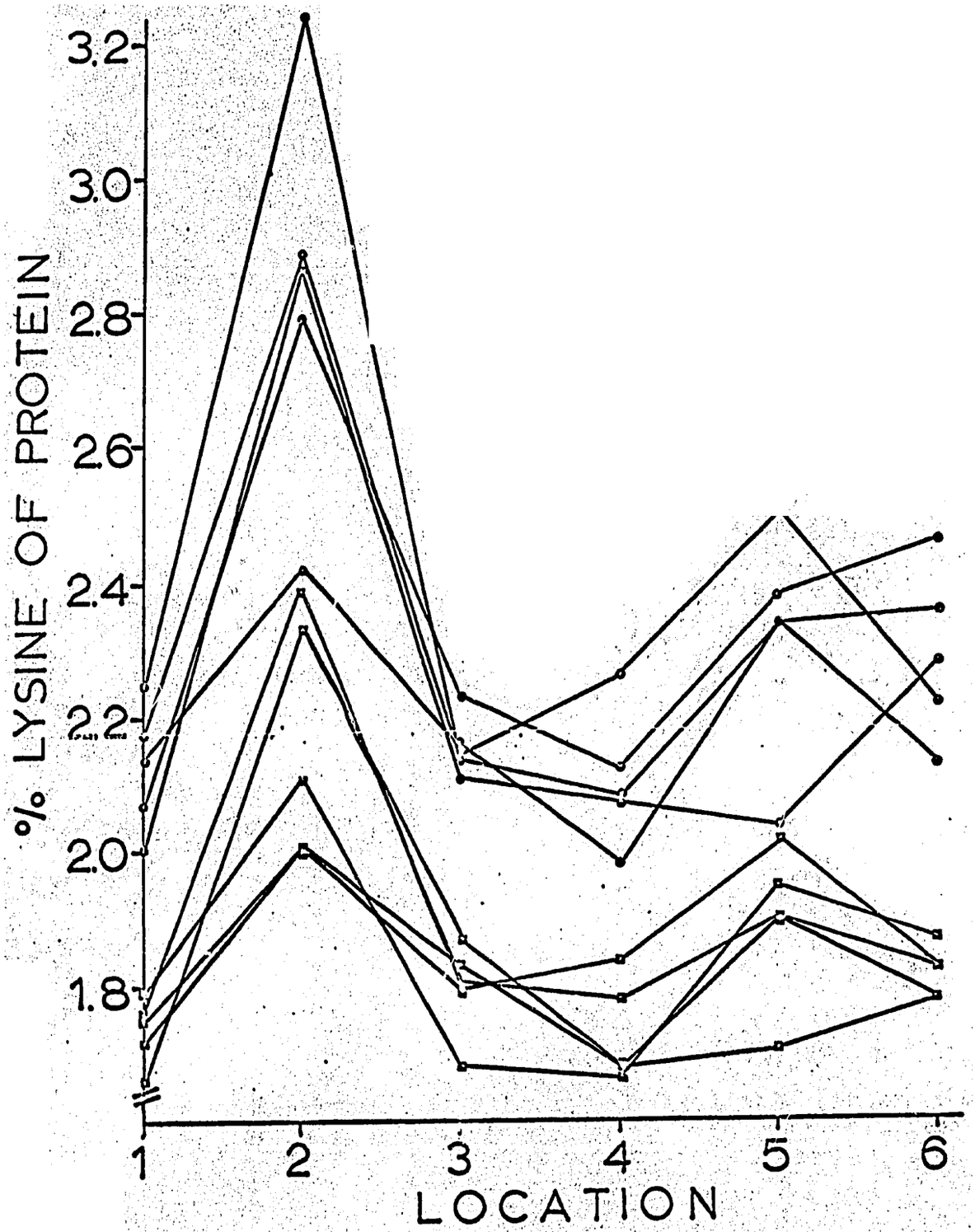
Significant second order interactions existed for all the variables studied in this investigation except lysine as a percent of protein. In general, the first order interactions were generally of similar magnitude and smaller than the second order interaction and experimental error. This allows the plant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



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

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

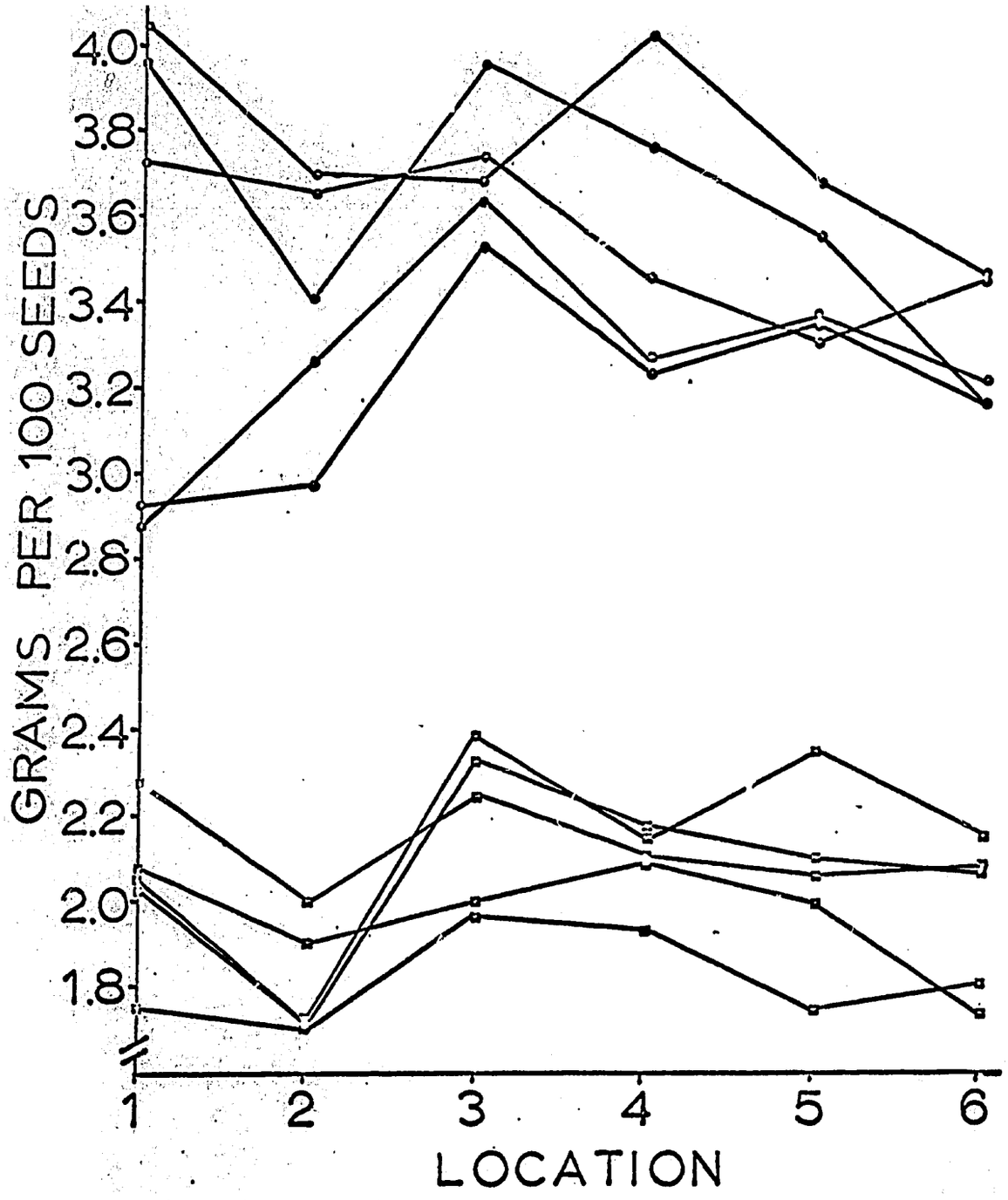
Figure 1.1. Genotype-location interaction of the five highest and five lowest genotypes for percent protein.



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

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

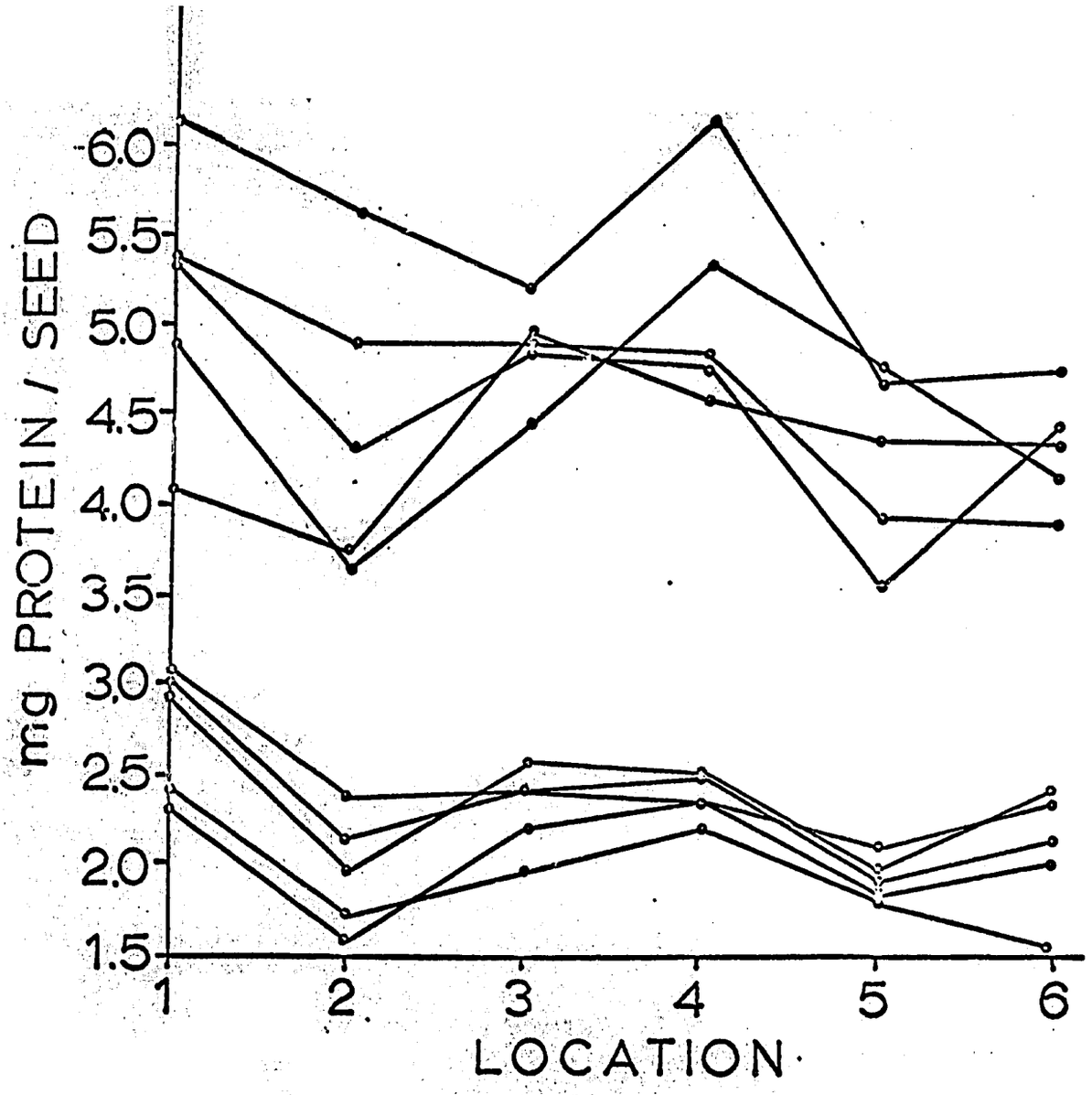
Figure 1.2. Genotype-location interaction of the five highest and five lowest genotypes for percent lysine of protein.



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

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

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



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

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

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

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

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

List of References

Anderson, V. L. 1970. Restriction erros for linear models (an aid to develop models for designed experiments). Biometrics 26:255-268.

Anderson, R. L. and T. A. Bandcroft. 1952. Statistical theory in research. McGraw-Hill Book Company, Inc., New York.

Burr, I. W. and L. A. Foster. 1972. A test for equality of variances. Mimeograph Series No. 282. Department of Statistics, Purdue University, Lafayette, Indiana.

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, Lafayette, Indiana.

Collins, F. C. and R. C. Pickett. 1972. Combining ability for yield, protein, and lysine in an incomplete diallel of Sorghum bicolor (L.) Moench. Crop Sci. 12:5-6.

Comstock, R. E. and R. H. Moll. 1963. Genotype-environment interactions. Statistical genetics and plant breeding. National Academy of Science-National Research Council, Washington, D. C.

Liang, G. H. L. and T. L. Walter. 1966. Genotype × environment interactions from yield tests and their application to sorghum breeding programs. Can. J. Genet. Cytol. 8:306-311.

Matzinger, D. F. 1963. Experimental estimates of genetic parameters and their applications in self fertilized plants. Statistical genetics and plant breeding. National Academy of Sciences-National Research Council, Washington, D. C.

Ostle, B. 1963. Statistics in research. Iowa State University Press. Ames, Iowa pp. 310-312.

Shutz, W. M. and R. L. Bernard. 1967. Genotype × environment interactions in the regional testing of soybean strains. Crop Sci. 7: 125-130.

SECTION II

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

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Introduction

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

Review of Literature

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

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

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

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

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

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

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

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

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

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

Materials and Methods

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

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

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

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

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

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

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

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

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

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

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

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

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

Results and Discussion

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

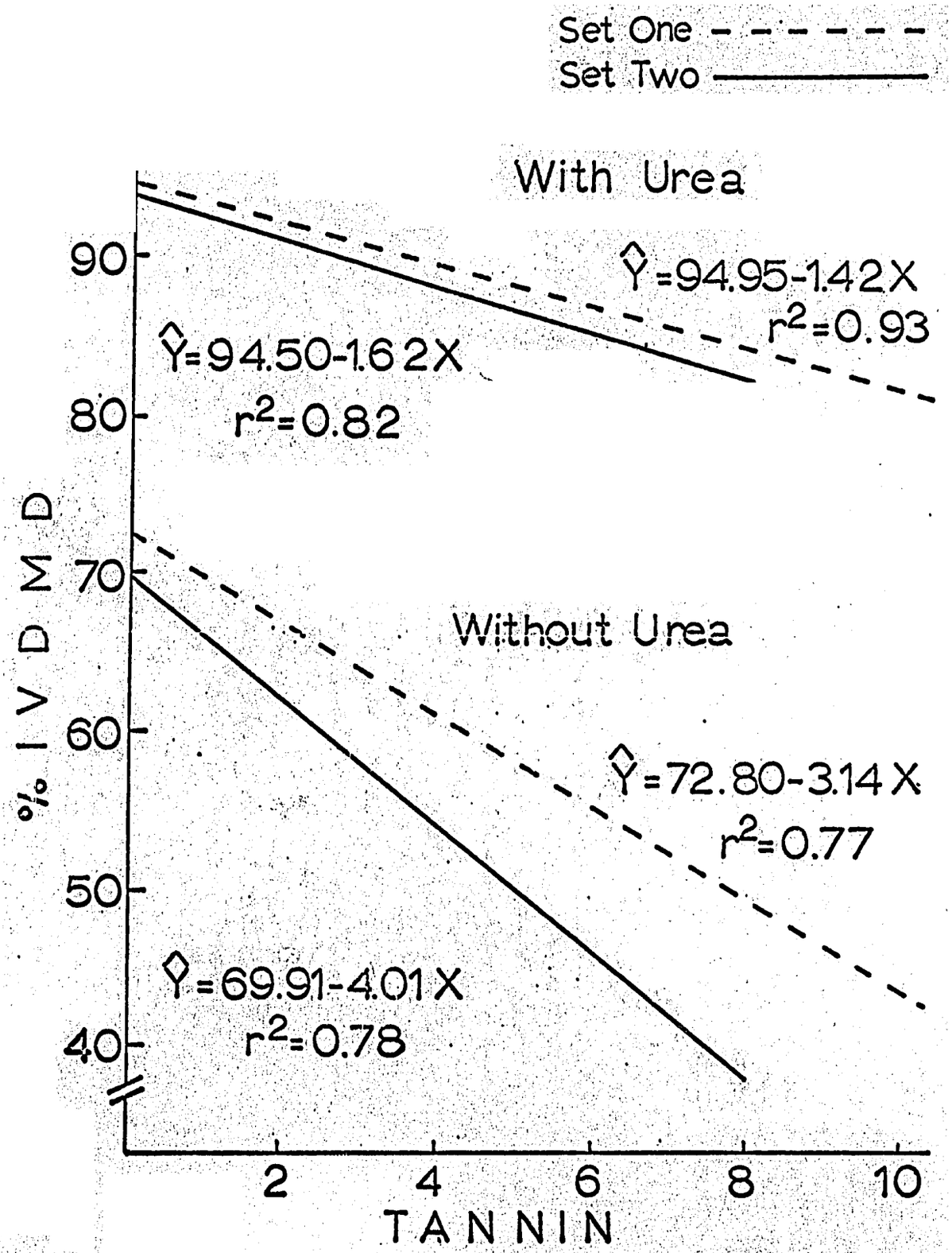

Figure 2.1. Effect of supplemental nitrogen on the relationship between tannin and IVDMD.

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

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

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

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

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

* Significant at 5% level of probability.

** Significant at 1% level of probability.

\+ Lines versus hybrids.

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

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

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

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

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

* Significant at 5% level.

** Significant at 1% level.

\+ Hybrids versus lines.

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

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

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

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

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

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

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

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

Conclusions

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

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

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

List of References

A.O.A.C. 1960. Methods of analysis. 9th ed. Association of Official Agriculture Chemists. Washington, D. C.

Burns, R. E. 1963. Methods of tannin analysis for forage crop evaluation. Tech. Bull. N. S. 32. Georgia Agr. Expt. Sta., Athens, Ga.

Burns, R. E. 1971. Method of estimation of tannin in grain sorghum. Agron. J. 63:511-512.

Chang, S. I. and H. L. Fuller. 1964. Effect of tannin content of grain sorghums on their feeding value for growing chicks. Poultry Sci. 43:30-36.

Connor, J. K., I. S. Hurwood, H. W. Burton and D. E. Fuelling. 1969. Some nutritional aspects of feeding sorghum grain of high tannin content to growing chickens. Australian J. of Expt. Agr. and Animal Husb. 9:497-502.

Damron, B. L., G. M. Prine and R. H. Harms. 1968. Evaluation of various bird-resistant and non-resistant varieties of grain sorghum for use in broiler diets. Poultry Sci. 47:1648-1650.

Driedger, A. and E. E. Hatfield. 1972. Influence of tannins on the nutritive value of soybean meal for ruminants. J. of Animal Sci. 34:465-468.

Fuller, H. L., D. K. Potter, and A. R. Brown. 1966. The feeding value of grain sorghums in relationship to their tannin content. Tech. Bull. N. S. 176. Georgia Agr. Expt. Sta., Athens, Ga.

Glick, Z. and M. A. Joslyn. 1970a. Food intake depression and other metabolic effects of tannic acid in the rat. J. Nutrition 100: 509-515.

Glick, Z. and M. A. Joslyn. 1970b. Effect of tannic acid and related compounds on the absorption and utilization of protein in rats. J. Nutrition 100:516-520.

Halloran, H. R. and A. B. Maunder. 1971. Nutritional evaluations with "Bird resistant" and yellow endosperm sorghums. Poultry Sci. 50:1582.

Handler, P. and R. D. Baker. 1944. Toxicity of orally administered tannic acid. Science 99:393-394.

McGinty, D. D. 1968. Factors affecting the digestibility of varieties of sorghum grain by cattle. Ph.D. Thesis. Texas A & M University, College Station, Texas.

Nawar, I. A., H. E. Clark, R. C. Pickett and D. M. Hogsted. 1970. Protein quality of selected lines of Sorghum vulgare for the growing rat. Nutrition Reports International 1:75-81.

Oswalt, D. L. 1972. Personal communication.

Ringrose, R. C. and C. L. Morgan. 1940. The nutritive value of Lespedeza for poultry feeding. South Carolina Agr. Exp. Sta. Ann. Rept. 53:91.

Rostango, H. S. 1972. Nutritive evaluation of sorghum grains in chicks. Ph.D. Thesis. Purdue University, West Lafayette, Indiana.

Schmid, A. R., G. C. Martin and L. S. Roth. 1969. Effects of N supplementation on in vitro digestibility of corn, sorghum and alfalfa. Agron. J. 57:213-216.

Stallcup, O. T. and G. C. Davis. 1962. Nutritive value of AKS 614 grain sorghum. Ark. Farm Research. 11:5.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Company, Inc., New York.

Stephenson, E. L., J. O. York and D. B. Bragg. 1967. A comparison of the nutritive value of different milo hybrids for chicks. Poultry Sci. 46:1323.

Stephenson, E. L., J. O. York and D. B. Bragg. 1968. Comparative feeding values of brown and yellow grain sorghum. Feedstuffs 40(20):112 and 114.

Strumeyer, D. H. and M. J. Malin. 1969. Identification of amylose inhibitor from seeds of Leoti sorghum. Biochem. Biophys. Acta 184:643-645.

Tilley, J. M. A. and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Brit. Grassl. Soc. 18:104-111.

SECTION III

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

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Introduction

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

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

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

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

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

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

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

Materials and Methods

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

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

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

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

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

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

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

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

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

Component of Ration	Low Tannin								High Tannin								Weanling Rat Requirement†
	IS2549				IS0062				IS6992				IS1210				
	% Soybean Meal				% Soybean Meal				% Soybean Meal				% Soybean Meal				
	0	5	10	15	0	5	10	15	0	5	10	15	0	5	10	15	
Protein	8.08	10.21	13.10	17.48	10.53	13.39	15.78	18.71	7.46	10.30	12.55	15.50	8.46	11.50	14.30	17.30	16.0
Arginine	0.29	0.55	0.77	0.98	0.43	0.65	0.86	1.08	0.35	0.57	0.78	1.00	0.34	0.56	0.78	0.99	0.2
Histidine	0.16	0.23	0.30	0.38	0.19	0.26	0.33	0.40	0.16	0.24	0.31	0.38	0.20	0.27	0.34	0.41	0.4
Isoleucine	0.33	0.46	0.58	0.71	0.39	0.51	0.64	0.76	0.29	0.41	0.53	0.66	0.33	0.45	0.57	0.70	0.5
Leucine	1.16	1.37	1.57	1.78	1.43	1.63	1.84	2.05	0.94	1.15	1.36	1.57	1.16	1.37	1.57	1.78	0.9
Lysine	0.19	0.36	0.52	0.69	0.24	0.40	0.57	0.73	0.18	0.34	0.50	0.67	0.17	0.33	0.50	0.66	1.0
Methionine plus Cystine**	0.14	0.21	0.28	0.35	0.24	0.31	0.38	0.49	0.22	0.28	0.32	0.42	0.26	0.33	0.40	0.46	0.6
Phenylalanine plus Tyrosine	0.76	1.02	1.28	1.53	0.95	1.21	1.47	1.72	0.64	0.90	1.16	1.42	0.83	1.09	1.35	1.61	0.9
Threonine	0.35	0.45	0.55	0.66	0.33	0.43	0.53	0.64	0.23	0.33	0.43	0.53	0.25	0.35	0.45	0.56	0.5
Valine	0.43	0.56	0.70	0.83	0.55	0.68	0.81	0.94	0.36	0.50	0.63	0.76	0.41	0.54	0.67	0.81	0.4

* Tryptophane was not measured due to breakdown during hydrolysis.

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

† Mertz (1969).

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

Results and Discussion

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

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

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

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

* Significant at the 0.05 level of probability.

** Significant at the 0.01 level of probability.

+ Runs were confounded with T × G × S.

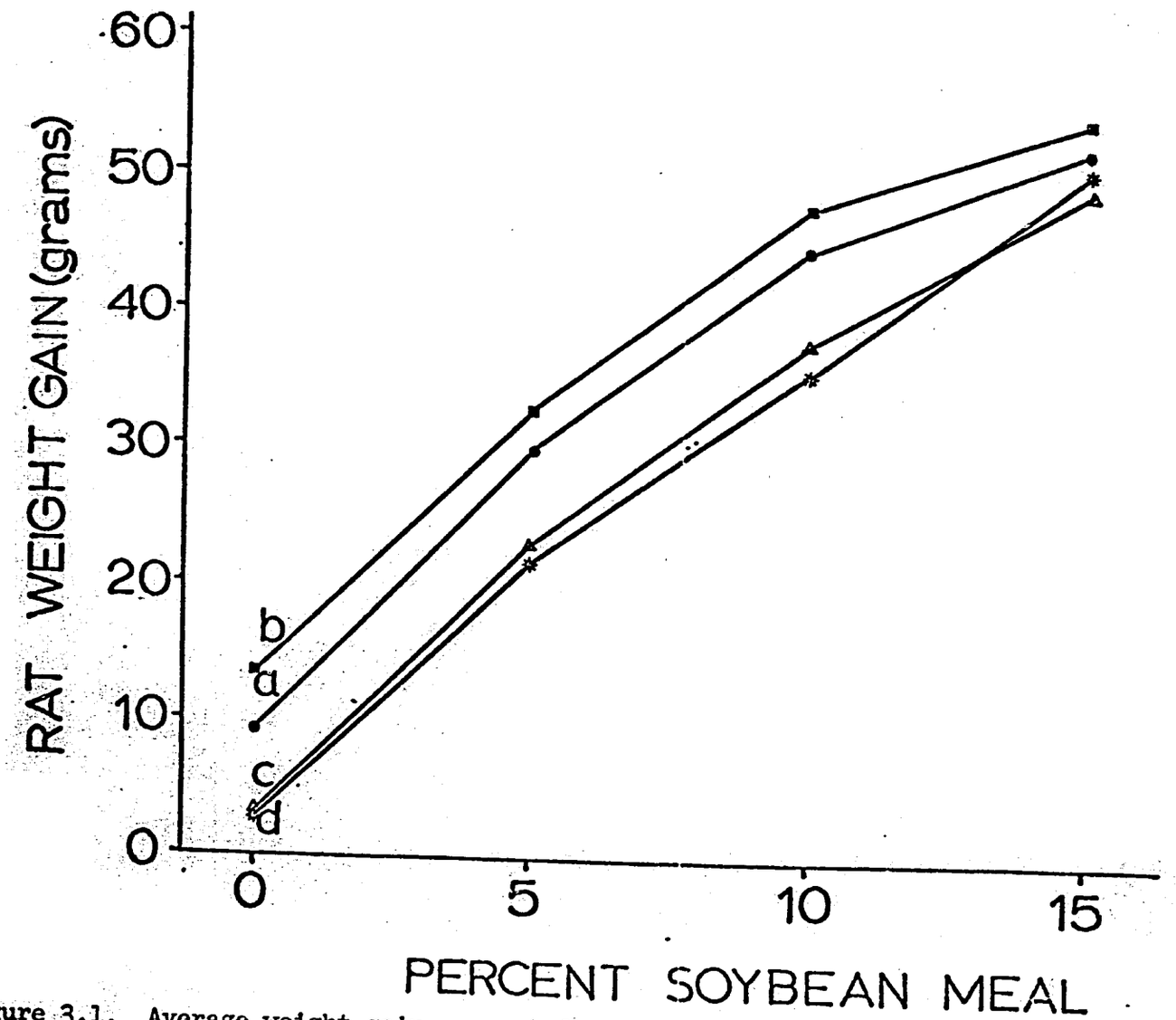

Figure 3.1. Average weight gain per rat for 13 days. a = IS2549 (low tannin, low protein), b = IS0062 (low tannin, high protein), c = IS6992 (high tannin, low protein), and d = IS1210 (high tannin, high protein).

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

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

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

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

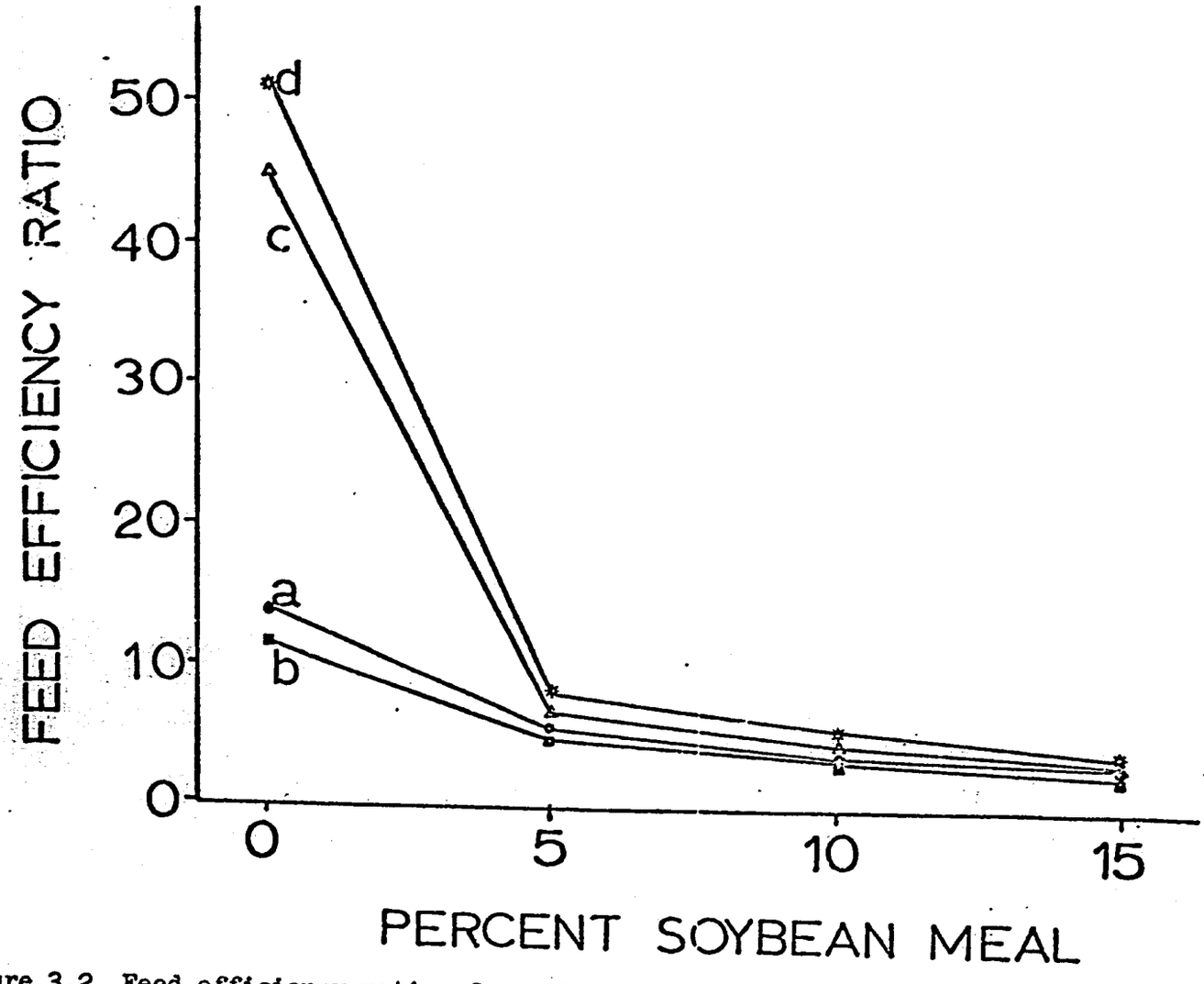

Figure 3.2 Feed efficiency ratios for rats fed 13 days. a = IS2549 (low tannin, low protein), b = IS0062 (low tannin, high protein), c = IS6992 (high tannin, low protein), and d = IS1210 (high tannin, high protein).

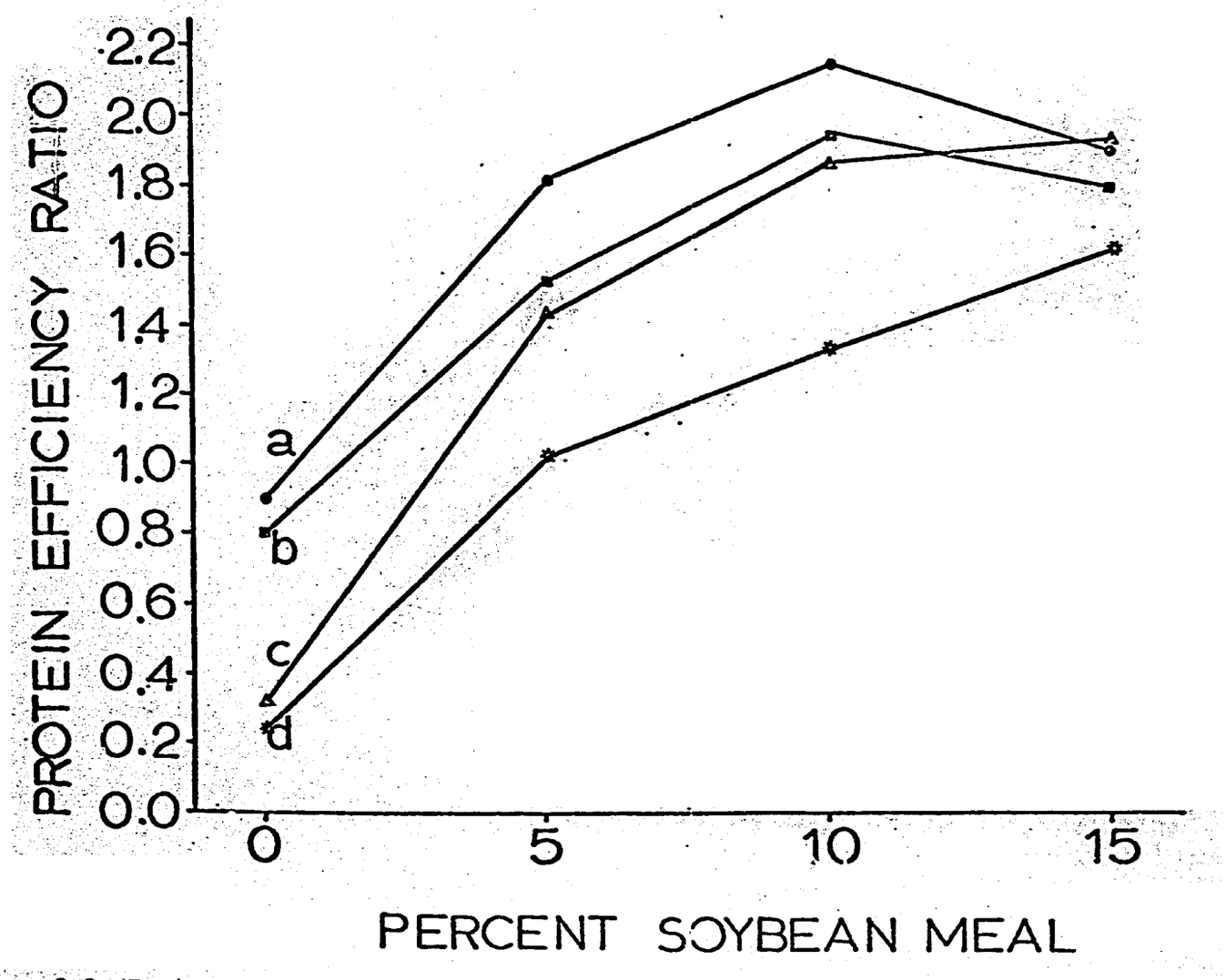

Figure 3.3 Protein efficiency ratios for rats fed 13 days. a = IS2549 (low tannin, low protein), b = IS0062 (low tannin, high protein), c = IS6992 (high tannin, low protein), and d = IS1210 (high tannin, high protein).

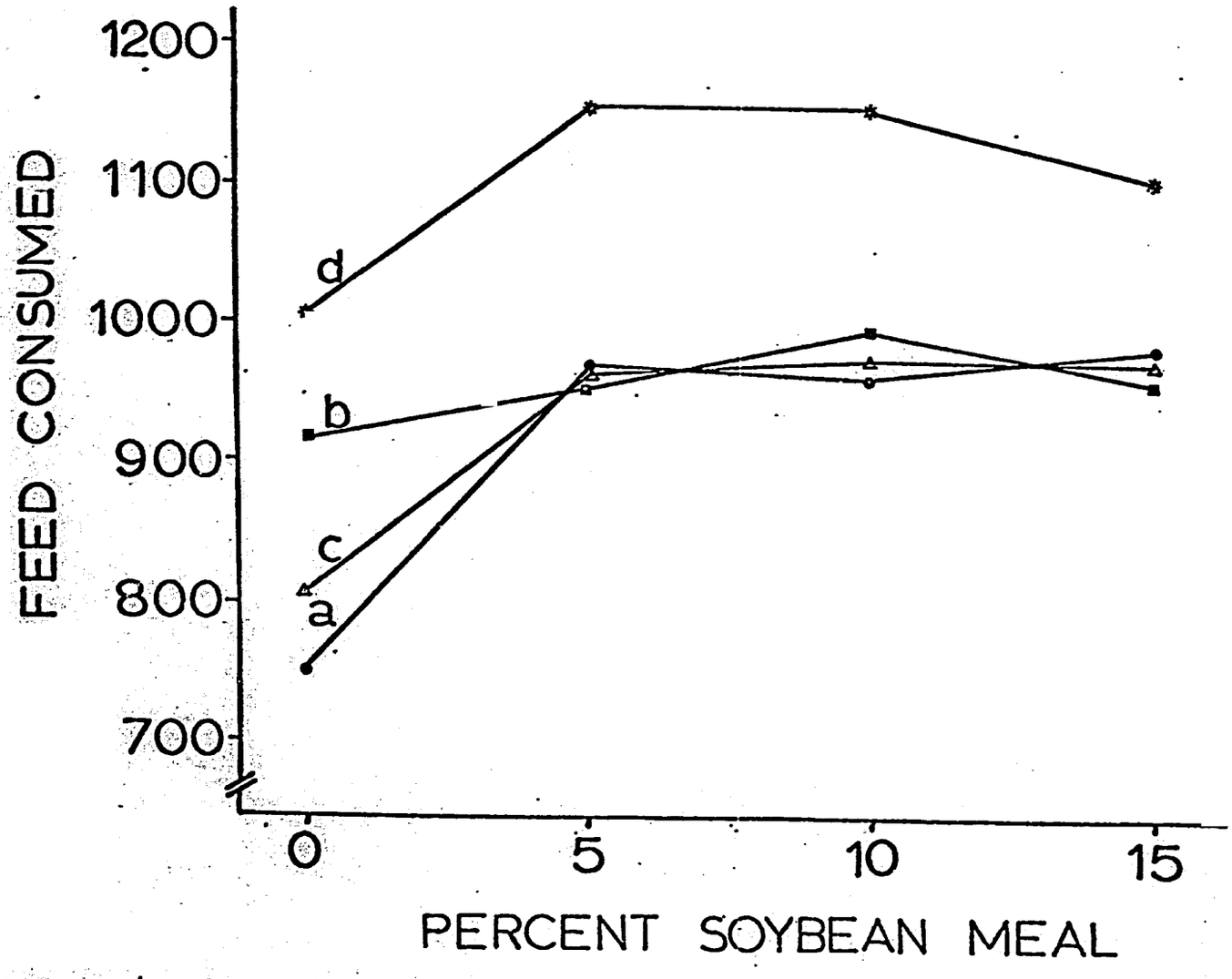

Figure 3.4. Grams of feed consumed by six rats in 13 days. a = IS2549 (low tannin, low protein), b = IS0062 (low tannin, high protein), c = IS6992 (high tannin, low protein), and d = IS1210 (high tannin, high protein).

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

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

Conclusions

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

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

List of References

Chang, S. I. and H. L. Fuller. 1964. Effect of tannin content of grain sorghum on their feeding value for growing chicks. Poultry Sci. 43:30-36.

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

Connor, J. K., I. S. Harwood, H. W. Burton and D. E. Fuelling. 1969. Some nutritional aspects of feeding sorghum grain of high tannin content to growing chickens. Australian J. of Expt. Agr. and Animal Husb. 9:497-502.

Glick, Z. and M. A. Joslyn. 1970a. Food intake depression and other metabolic effects of tannic acid in the rat. J. Nutrition. 100: 509-515.

Glick, Z. and M. A. Joslyn. 1970b. Effect of tannic acid and related compounds on the absorption and utilization of protein in rats. J. Nutrition 100:516-520.

McGinty, P. D. 1968. Factors affecting the digestibility of varieties of sorghum grain by cattle. Ph.D. Thesis. Texas A & M University, College Station, Texas.

Mertz, E. T. 1969. Amino acid and protein requirements of fish. Fish in Research, Academic Press Inc., New York.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures in statistics. McGraw Hill Book Co., Inc., New York.

Tamir, M. and E. Alumot. 1969. Inhibition of digestive enzymes by condensed tannins from green and ripe carobs. J. Sci. Fd. Agric. 20:199-202.

Tamir, M. and E. Alumot. 1970. Carob tannins-growth depression and levels of insoluble nitrogen in the digestive tract of rats. J. Nutrition 100:573-580.

Vohra, P., F. H. Kratzer and M. A. Joslyn. 1966. The growth depressing and toxic effects of tannin to chicks. Poultry Sci. 45:135-141.

VITA

Robert Eugene Schaffert was born on October 10, 1944 in Aurora, Nebraska, the fourth son of Christine and Rudolf F. Schaffert. He was raised on a grain and livestock farm near Aurora, Nebraska. He attended grade schools in Hamilton County, Nebraska and was graduated from Aurora High School in 1962.

From 1962 to 1967 he attended the University of Nebraska. In January of 1966 he received his Bachelor of Science degree in Honors Agriculture. At that time he began his graduate program under the direction of Dr. C. O. Gardner in the area of corn breeding and genetics. In August of 1967 he received the Master of Science degree from the University of Nebraska.

In 1967 he began working on his Ph.D. degree at Purdue University where he was employed as a graduate instructor. Between 1967 and 1972 he taught courses in introductory agronomy and genetics and conducted research in the area of nutritional quality of sorghum under the direction of Dr. R. C. Pickett and Dr. J. D. Axtell. In August of 1972 he completed the requirements for the Ph.D. degree.

He is a member of Alpha Zeta, Gamma Sigma Delta, Sigma Xi, American Society of Agronomy, Crop Science Society of America, American Genetic Association and the American Association for the Advancement of Science.