EFFECT OF SUPPLEMENTAL PROTEIN ON THE NUTRITIVE VALUE OF HIGH AND LOW TANNIN *SORGHUM BICOLOR* (L.) MOENCH GRAIN FOR THE GROWING RAT[1]

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Summary

Grain sorghum diets with 0, 5, 10 and 15% soybean, seeds without hulls, solvent extracted, maximum 3% fiber (Ref. no. 5-04-612), (Soybean meal) supplementation using genotypes containing less than 1.0 or more than 4.0 catechin equivalents (tannins) were fed to weanling rats. Thirteen-day weight gains of 36 g and 28 g, feed consumption of 155 g and 170 g and feed efficiency ratios of 6 and 17 were highly significant for low and high tannin genotypes, respectively. Weight gains of 7, 27, 42 and 53 g, feed consumption of 145, 169, 168 and 166 g and feed efficiency ratios of 21, 6, 4 and 3 were obtained for the four levels of soybean meal supplementation, respectively. Average rat weight gains from the high tannin genotypes differed significantly from the low tannin rat gains for 0, 5 and 10% soybean meal supplemented diets, respectively, but the gains from the 15% soybean meal diets were not significantly different. Feed consumption of the high tannin diets was greater or equal to the low tannin diets. The lack of antagonistic interactions with supplemental protein for weight gain, feed efficiency and feed consumption indicated that protein from soybean meal can be supplemented without changing the relative performance of different sorghum genotypes.

Introduction

Tannins from many sources have been recognized for a long 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 (Tamir and Alumot, 1970).

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 Conner *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

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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. R. E. Schaffert, V. L. Lechtenberg, R. C. Pickett and C. L. Rhykerd (*unpublished data*) 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 solubility and probably 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 with 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

Ninety-six male weanling rats ranging in weight from 45 to 65 g were divided into groups containing six rats averaging 56 g per group. Each rat was individually placed into a separate cage. 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. Feed efficiency ratios (FER) were calculated as grams of feed consumed per gram of gain.

Each 100 g of the basal diet consisted of 79 g of grain sorghum, 4 g of mineral mix[5] 2 g of vitamin mix,[6] and 15 g of corn starch. The starch was replaced with 5, 10 and 15 g of soybean meal (53.25% protein, Ref. no. 5-04-612, Harris, Asplund and Crampton, 1968) to give four levels (0, 5, 10 and 15 g) of protein supplementation. Four different grain sorghum genotypes were used. India Sorghum (IS) 2549 (low tannin, low protein), IS 0062 (low tannin, high protein), IS 6992 (high tannin, low protein), and IS 1210 (high tannin, high protein).

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 1.

Due to a limitation in the number of rat feeding units only eight of the 16 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 pseudofactorial (2x2x2x2) and runs were confounded with the third order interaction of the pseudofactorial, actually the second order interaction of the 2x2x4 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 0 and 5% of the ration, and soybean meal as 0 and 10% 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 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).

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

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

TABLE 1. PROTEIN AND ESSENTIAL AMINO ACID REQUIREMENTS OF THE RAT AND PROTEIN AND ESSENTIAL AMINO ACIDS PROVIDED BY EACH RATION EXPRESSED AS A PERCENT OF DRY FEED[a]

Component of ration	Low tannin							
	IS 2549				IS 0062			
	% soybean meal				% soybean meal			
	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
Arginine	0.29	0.55	0.77	0.98	0.43	0.65	0.86	1.08
Histidine	0.16	0.23	0.30	0.38	0.19	0.26	0.33	0.40
Isoleucine	0.33	0.46	0.58	0.71	0.39	0.51	0.64	0.76
Leucine	1.16	1.37	1.57	1.78	1.43	1.63	1.84	2.05
Lysine	0.19	0.36	0.52	0.69	0.24	0.40	0.57	0.73
Methionine plus cystine[b]	0.14	0.21	0.28	0.35	0.24	0.31	0.38	0.49
Phenylalanine plus tyrosine	0.76	1.02	1.28	1.53	0.95	1.21	1.47	1.72
Threonine	0.35	0.45	0.55	0.66	0.33	0.43	0.53	0.64
Valine	0.43	0.56	0.70	0.83	0.55	0.68	0.81	0.92

Component of ration	High tannin								Weanling rat requirement[c]
	IS 6992				IS 1210				
	% soybean meal				% soybean meal				
	0	5	10	15	0	5	10	15	
Protein	7.46	10.30	12.55	15.50	8.46	11.50	14.30	17.30	16.0
Arginine	0.35	0.57	0.78	1.00	0.34	0.56	0.78	0.99	0.2
Histidine	0.16	0.24	0.31	0.38	0.20	0.27	0.34	0.41	0.4
Isoleucine	0.29	0.41	0.53	0.66	0.33	0.45	0.57	0.70	0.5
Leucine	0.94	1.15	1.36	1.57	1.16	1.37	1.57	1.78	0.9
Lysine	0.18	0.34	0.50	0.67	0.17	0.33	0.50	0.66	1.0
Methionine plus cystine[b]	0.22	0.28	0.32	0.42	0.26	0.33	0.40	0.46	0.6
Phenylalanine plus tyrosine	0.64	0.90	1.16	1.42	0.83	1.09	1.35	1.61	0.9
Threonine	0.23	0.33	0.43	0.53	0.25	0.35	0.45	0.56	0.5
Valine	0.36	0.50	0.63	0.76	0.41	0.54	0.67	0.81	0.4

[a]Tryptophan was not measured due to breakdown during hydrolysis.
[b]Methionine plus cystine were underestimated in the amino acid analysis due to some loss during hydrolysis.
[c]Mertz (1969).

Results and Discussion

The analyses of variance for weight gain, FER, and feed consumed are presented in table 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 1. The low tannin genotypes had significantly greater gains at the 0, 5 and 10% 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 or was above the rat requirement at the 15% supplementation level. However, the data in table 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 0, 5, or 10% 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 varia-

TABLE 2. ANALYSIS OF VARIANCES FOR WEIGHT GAIN, FEED EFFICIENCY RATIO (FER), AND FEED CONSUMED ON THE AVERAGE OF SIX RATS FOR 13 DAYS

Source of variation	df	Mean squares		
		Weight gain	Feed consumed	FER
Replications	1	99.9*	382.9*	13.6
Tannin (T)	1	419.0**	625.7**	929.9**
Grain protein (G)	1	15.0	855.9**	1.0
Soybean protein (S)	3	3332.3**	386.2**	1586.0**
T x G	1	16.2	446.2*	2.5
T x S	3	17.7	7.6	696.7**
G x S	3	0.8	61.9	4.0
T x G x S	2	2.3	19.7	0.2
Runs[a]	2	79.7*	61.9	48.3
Error	14	26.1	77.6	52.3
Pooled error		19.2	60.8	37.4
Standard error		4.4	7.8	6.1

*Significant at the 0.05 level of probability.
**Significant at the 0.01 level of probability.
[a]Runs were confounded with T x G x S.

tion of weight gain for each group of six rats were reduced between 33 and 77% by the addition of 5% soybean meal indicating that the relative variability was reduced.

The differences in feed efficiency between the high and low tannin genotypes shown in figure 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 shown by the difference in FER for the low and high tannin genotypes between the 0 and 5% 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. Similar effects may have caused the differences observed in this experiment.

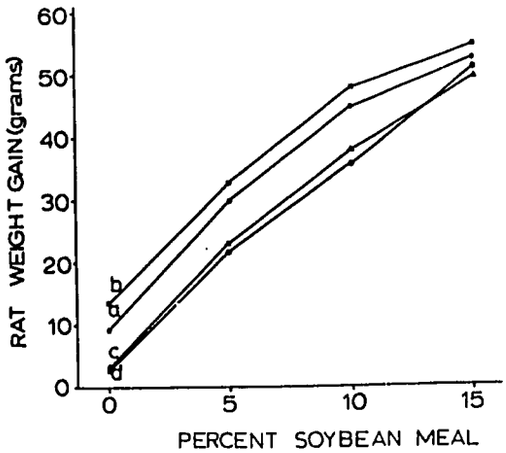

Figure 1. Average weight gain per rat for 13 days. a = IS 2549 (low tannin, low protein), b = IS 0062 (low tannin, high protein), c = IS 6992 (high tannin, low protein), and d = IS 1210 (high tannin, high protein).

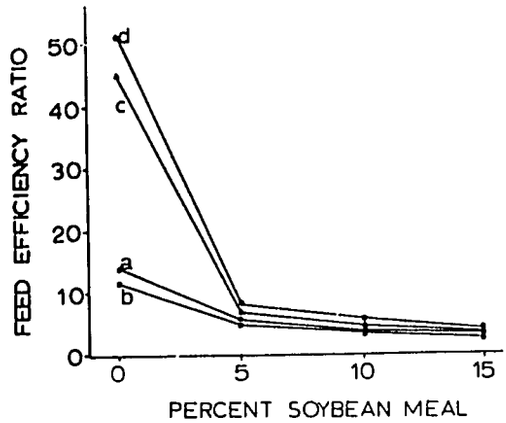

Figure 2. Feed efficiency ratios for rats fed 13 days. a = IS 2549 (low tannin, low protein), b = IS 0062 (low tannin, high protein), c = IS 6992 (high tannin, low protein), and d = IS 1210 (high tannin, high protein).

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, IS 1210, shown in figure 3. 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 content was low and amylopectin was high. This factor may have caused increased feed consumption, and influenced the feed efficiency, 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 fed to weanling rats produced poorer weight gains and poorer feed efficiency than low tannin sorghum grains. 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, and feed consumption indicate that protein from soybean meal at levels of up to 10% 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 greater feed efficiency than the low protein sorghum but the differences were not significant at the 5% level. The increase in weight gain by additions of small amounts of soybean meal to grain sorghum would indicate that the influence of tannins is not a toxic effect, but rather affects the availability of the ration protein. 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 where specific amino acids were to be studied.

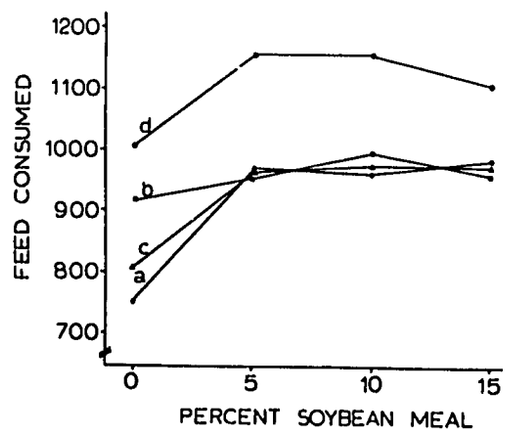

Figure 3. Grams of feed consumed by six rats in 13 days. a = IS 2549 (low tannin, low protein), b = IS 0062 (low tannin, high protein), c = IS 6992 (high tannin, low protein), and d = IS 1210 (high tannin, high protein).

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