Effect of Tannin Extraction on the Performance of Chicks Fed Bird Resistant Sorghum Grain Diets[1]

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ABSTRACT Three experiments were conducted to determine the effects on chick performance of extracting the tannin from bird resistant sorghum grains BR64, IS8260 and IS6992 as well as non-resistant sorghums RS610 and RS671. The procedure, involving alkaline treatment of whole grain followed by hot water washing, removes most of the tannins from bird resistant sorghums and renders them equivalent in tannin content to non-resistant sorghums. Comparisons among sorghum grains were made on an isonitrogenous basis in a sorghum-soybean meal diet at sub-optimal protein levels. Extraction of the tannins from bird resistant sorghums BR64 and IS8260 resulted in significantly better chick growth and feed conversion as compared with that of chicks fed the intact bird resistant sorghum diets. Performance with these treated bird resistant sorghums was approximately equal to that obtained with intact non resistant sorghums. The extraction process did not significantly influence growth rate but did significantly improve feed conversion of the non-resistant sorghum grains. Supplementation of the sorghum IS8260 diet with methionine resulted in a significant improvement in chick performance and an increased incidence and severity of a leg abnormality as compared with the unsupplemented IS8260 diet. Methionine supplementation of a diet containing sorghum IS8260 after extraction of the tannin resulted in similar leg scores and only a slight improvement in performance as compared with the same diet without added methionine.

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INTRODUCTION

STUDIES by several workers (Chang and Fuller, 1964; Connor *et al.*, 1969; Rostagno *et al.*, 1973a; Armstrong *et al.*, 1973) have shown that weight gain and feed conversion were reduced when high-tannin sorghum grains were fed to chicks as compared with chicks fed low-tannin sorghum grains. Sorghum varieties with brown seed color and open heads have been characterized as being high in tannin content. Fuller *et al.* (1966) observed that a dietary level of tannin between 0.64 and 0.83 percent was required to cause a depression in chick performance. These same workers found that the tannin was concentrated in the pericarp or seedcoat of the sorghum grain.

Blessin *et al.* (1963) observed that the anthocyanogens were located in the pericarp and seedtip and were absent from the endosperm of sorghum grains. Studies by Nip and Burns (1969, 1971) showed that the yellow pigments present in sorghum grains with reddish-brown seed coats were not present in the white varieties and hybrids. These workers suggested there was a varietal difference in the kinds and numbers of pigments present in sorghums and that treatment with sodium hydroxide extracted several of these pigments.

Blessin *et al.* (1971) studied the effects of an alkali dehulling procedure on the composition and wet-milling characteristics of sorghum grains. They suggested that since the anthocyanogens were converted into colored substances in acid solutions, bleaching would often be necessary before wet-milling to produce a satisfactory white starch. A whiter appearing starch was observed from dehulled grain. The dehulling process had little effect on the composition of the grains dehulled

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except for a 40% reduction in the fiber content of the sorghum grains due to the loss of much of the pericarp.

The present study was conducted to determine the effects on chick performance of tannin extraction from bird resistant sorghum grain by an alkaline-hot water extraction process. The effects of methionine supplementation of diets containing extracted or non-extracted bird resistant sorghum were also studied.

EXPERIMENTAL PROCEDURE

Male, White Mountain chicks were randomly alotted to treatments at one day of age. Each chick was weighed, wing-banded and placed in an electrically heated battery brooder with raised wire floors. Eight chicks were placed in each pen with four replicates per treatment. No two replicates of the same treatment appeared in the same battery or the same deck level of another battery. Feed and water were provided *ad libitum*. All experiments were conducted for 21 days.

The protein content of the sorghum grains and soybean meal was determined by the Kjeldahl procedure (A.O.A.C., 1960). Tannic acid equivalent of the grains was determined by the Folin-Denis method as described by Burns (1963). The protein and tannic acid equivalents are presented in the table for each experiment. In Experiments 1 and 2, the different grains were compared on an isonitrogenous basis at a 15.3% dietary protein level. Each of the grains which had been extracted was compared on an equal weight basis with the same intact grain. Composition of experimental diets is shown in Tables 1-3.

Extraction of the tannins from the sorghum grains used in this study was based on an alkali dehulling procedure described by Blessin *et al.* (1971). The procedure was expanded to permit extraction of tannins from larger quantities of grain. Forty-pound samples of grain were presoaked in 60° C. water for five minutes with constant stirring. After draining,

TABLE 1.—*Composition of diets and the effect of tannic acid extraction from sorghum grains on weight gain and feed conversion of chicks. (Experiment 1)*

	Diets			
Ingredients (%)	1	2	3	4
Sorghum RS671 (9.4)[1] [0.37][2]	58.51	—	—	—
Sorghum RS671-extracted (9.8) [0.27]	—	56.12	—	—
Sorghum IS8260 (7.6) [2.66]	—	—	72.37	—
Sorghum IS8260-extracted (7.6) [0.25]	—	—		72.37
Corn oil	5.00	5.00	5.00	5.00
Glucose monodydrate	13.86	16.25	—	—
Soybean meal (52.2)	17.75	17.75	17.75	17.75
Premix[3]	4.88	4.88	4.88	4.88
Weight gain (g.)[4]	232[a]	218[a]	128[b]	204[a]
Feed/gain ratios (g.)[4]	2.03[b]	1.85[c]	2.69[a]	1.85[c]

[1]Figures in parenthesis represent the protein contents expressed as a percentage of air-dried feedstuff.

[2]Figures in brackets represent the percent tannic acid equivalents of the sorghum grains.

[3]The premix provided the following ingredients (in %): dicalcium phosphate, 2.1; limestone, 1.5; sodium chloride (iodized), 0.45; manganese sulfate·H_2O, 0.01692; zinc oxide, 0.006; butylated hydroxytoluene, 0.0125; vitamin supplement, 0.7935. The vitamin supplement contained the following (in units/kg.): choline chloride, 2000 mg.; vitamin A, 5000 I. U.; vitamin D_3, 2250 I.U.; riboflavin, 8.8 mg.; calcium pantothenate, 17.6 mg.; niacin, 39.6 mg.; d-alpha tocopherol acetate, 8.8 I.U.; menadione sodium bisuflite, 1.4 mg.; vitamin B_{12}, 11.0 μ.

[4]Mean values for 3-week old chicks. Means bearing the same superscripts are not significantly different. ($P > .05$).

TABLE 2.—*Composition of diets and the effect of tannic acid extraction and* DL*-methionine supplementation of sorghum grains on weight gain, feed conversion and leg score of chicks. (Experiment 2)*

Ingredients (%)	Diets				
	1	2	3	4	5
Sorghum RS610 (9.8)[1] [0.33][2]	57.78	—	—	—	—
Sorghum IS8260 (7.6) [2.66]	—	71.82	71.82	—	—
Sorghum IS8260-extracted (7.6) [0.25]	—	—	—	71.82	71.82
Corn oil	5.00	5.00	5.00	5.00	5.00
Glucose monohydrate	16.69	0.55	0.40	0.55	0.40
Soybean meal (52.2)	17.75	17.75	17.75	17.75	17.75
Premix[3,4]	4.88	4.88	4.88	4.88	4.88
DL-Methionine[5]	—	—	0.15	—	0.15
Weight gain (g.)[6]	216[b]	138[c]	258[a]	216[b]	231[ab]
Feed/gain ratios (g.)[6]	1.94[b]	2.76[a]	2.09[b]	1.90[bc]	1.74[c]
Leg scores[6,7]	1.28[b]	1.28[b]	2.00[a]	1.28[b]	1.39[b]

[1,2,3]See footnotes 1, 2 and 3, Table 1.
[4]B-vitamins were also added as follows (mg./kg. diet): thiamine, 1.8; pyridoxine, 3.0; biotin, 0.09; folic acid, 1.0.
[5]98% pure.
[6]See footnote 4, Table 1.
[7]Values assigned for leg condition: 1—Normal; 2—Slightly abnormal; 3—Severely abnormal; 4—Unable to stand.

the tempered grain was soaked with constant stirring in a 20% solution of sodium hydroxide at 70° C. for eight minutes. A ratio of 1 liter of sodium hydroxide solution for every 0.7 to 0.9 kg. of grain was used. The grain was then poured onto a screen, allowed to drain for approximately 1 minute and transferred into another fiberglass tub. Hot tap water (60° C.) was introduced into the tub and allowed to overflow for a period of 30 minutes. Constant stirring facilitated tannin extraction. After thorough rinsing, any remaining sodium hydroxide was neutralized with a 5% solution of glacial acetic acid. The treated grain was dried in a forced air oven at 70° C. for 18-20 hours, then allowed to equilibrate to room moisture for 24 hours before being weighed for mixing.

It should be noted that the alkali treatment loosens a large amount of pericarp which floats to the surface and is removed in the overflow. However, total removal of pericarp is not essential for extraction of most of the tannin in the grain.

In Experiment 1, the effects of extraction of the tannins from a non-resistant sorghum grain, RS671, and a bird resistant sorghum grain, IS8260, were studied. Diets containing sorghums RS671 and IS8260 before and after extraction of the tannins were compared on an isonitrogenous basis at suboptimal levels of protein (Table 1).

Experiment 2 was conducted to determine the effect of tannin extraction and DL-methionine supplementation of sorghum IS8260 when fed to chicks. Sorghum IS8260, before and after extraction of the tannins, and sorghum RS610, a non-resistant sorghum grain, were compared on an isonitrogenous basis at sub-optimal levels of protein (Table 2). The effect of 0.15% DL-methionine supplementation of intact and extracted sorghum IS8260 diets was also observed. A subjective leg score was used at the end of the 21-day study in an attempt to quantitatively evaluate the extent of an abnormal leg condition described previously (Armstrong *et al.*, 1973). The determination was based on the following: 1—normal; 2—slightly abnormal; 3—severely abnormal; and 4—unable to stand.

TABLE 3.—*Composition of diets and the effect of tannic acid extraction from sorghum grains on weight gain and feed conversion of chicks. (Experiment 3)*

Ingredients (%)	Diets							
	1	2	3	4	5	6	7	8
Sorghum RS671 (10.8)[1] [0.56][2]	57.24	—	—	—	—	—	—	—
Sorghum RS671-extracted (10.8) [0.34]	—	57.24	—	—	—	—	—	—
Sorghum BR64 (8.7) [2.26]	—	—	70.76	—	—	—	—	—
Sorghum BR64-extracted (9.0) [0.49]	—	—	—	70.76	—	—	—	—
Sorghum IS8260 (8.7) [2.27]	—	—	—	—	72.37	—	—	—
Sorghum IS8260-extracted (6.7) [0.34]	—	—	—	—	—	72.37	—	—
Sorghum IS6992 (9.8) [1.60]	—	—	—	—	—	—	62.70	—
Sorghum IS6992-extracted (8.3) [0.42]	—	—	—	—	—	—	—	62.70
Corn Oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Glucose Monohydrate	15.13	15.13	1.61	1.61	—	—	9.67	9.67
Soybean Meal (51.4)	17.75	17.75	17.75	17.75	17.75	17.75	17.75	17.75
Premix[3]	4.88	4.88	4.88	4.88	4.88	4.88	4.88	4.88
Total Protein (%)	15.3	15.3	15.3	15.5	15.3	14.1	15.3	14.3
Weight Gain (g.)[4]	214[b]	205[bc]	135[e]	268[a]	167[cde]	191[bcd]	181[bcd]	154[de]
Feed/gain ratios (g.)[4]	1.94[d]	1.93[d]	2.77[a]	1.82[e]	2.45[b]	2.08[c]	2.18[c]	2.11[c]

[1,2,3,4]See footnotes 1, 2, 3 and 4, Table 1.

Experiment 3 was conducted to determine the effect of tannin extraction from other bird resistant sorghum grains not used in previous experiments. Two birds resistant grains, BR64 and IS6992, were studied in addition to the bird resistant IS8260 and non-resistant RS671 sorghum grains used in previous experiments. Comparisons of the four sorghum grains were made on an isonitrogenous basis at sub-optimal levels of protein (Table 3). Diets containing each of the four extracted grains were compared with the respective intact grain on an equal weight basis (Table 3).

Analysis of variance (Steel and Torrie, 1960) was used to statistically analyze final chick weight gain, feed conversion and leg scores. In testing the main effects, the treatment × replication interaction was used. Individual treatment differences were tested by the Newman-Keuls multiple range test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Significantly better ($P < .05$) weight gain and feed conversion were observed in Experiment 1 by chicks fed a diet containing the non-resistant sorghum RS671 than was observed for chicks fed a diet containing the bird resistant sorghum IS8260 (Table 1). These results agree with earlier work (Chang and Fuller, 1964; Conner *et al.*, 1969; Rostango *et al.*, 1973a; Armstrong *et al.*, 1973) who observed that weight gain and feed conversion were reduced when high tannin sorghum grains were fed to chicks.

Tannin extraction of sorghum RS671 did not significantly influence weight gain ($P > .05$) as compared with intact RS671; however, a significant improvement in feed conversion ($P < .05$) was attained over that noted when the intact sorghum diet was fed. Extraction of the tannins from sorghum IS8260 significantly improved chick weight gain and feed conversion ($P < .05$) as compared with those observed with the intact IS8260 sorghum diet. The weight gain observed when the extracted IS8260 diet was fed was similar to that obtained with the RS671 sorghum diets. These results indicate that extraction of the tannins from bird resistant sorghum IS8260 resulted in almost complete alleviation of the reduced weight gain and feed conversion observed with the intact grain.

The improvement in feed conversion noted when the extracted sorghum RS671 was fed to chicks may have resulted from the lower fiber content as a result of the loss of the grain pericarp during the extraction process. Blessin *et al.* (1971) observed a 30–60% reduction in fiber in their studies depending on the type of grain and variety used. These workers observed little effect of the dehulling procedure on ash, ether extract or protein content.

In Experiment 2, significantly better weight gain and feed conversion ($P < .05$) were observed with a sorghum RS610 diet than with an intact sorghum IS8260 diet (Table 2). Extraction of the tannins from sorghum IS8260 (Diet 4) overcame this reduction in weight gain and feed conversion and gave results similar to those observed with the sorghum RS610 diet. These results agree with those observed in Experiment 1.

Supplementation of the intact IS8260 grain diet with 0.15% DL-methionine (Diet 3) significantly improved weight gain and feed conversion ($P < .05$) when compared with the IS8260 unsupplemented diet (Diet 2). These results agree with previous studies (Chang and Fuller, 1964; Conner *et al.*, 1969; Armstrong *et al.*, 1973) who observed that methionine was beneficial in alleviating some of the growth depressing effect of tannins. Supplementation of the extracted sorghum IS8260 diet with 0.15% DL-methionine resulted in some improvement in weight gain and feed conversion when compared to the extracted IS8260 unsupplemented diet; however, these differences were not statistically significant ($P > .05$). These results agree with previous

work (Armstrong *et al.*, 1973) which showed a greater improvement in performance of chicks from DL-methionine supplementation of high tannin sorghum diets than was noted with low tannin sorghum diets. Supplementation of the intact bird resistant sorghum (IS8260) diet with methionine resulted in a significantly greater incidence ($P < .05$) of an abnormal leg condition as compared with intact, unsupplemented bird resistant or non-resistant sorghum diet. In contrast, methionine supplementation of the extracted bird resistant sorghum diet resulted in only a slight increase in the incidence of leg anomalies.

Thiamine, pyridoxine, biotin and folic acid were added to all diets in this experiment to insure that none of these water soluble vitamins was deficient as a result of the tannin extraction procedure. The similar results obtained in this experiment as compared with earlier studies would suggest that this was not a problem.

In Experiment 3, sorghum RS671, a non-resistant sorghum grain, produced significantly better weight gain and feed conversion ($P < .05$) than did the bird resistant sorghum grain BR64 or IS8260 which were high in tannin content (Table 3). Sorghum IS6992, intermediate in tannin content, supported slightly poorer weight gain and significantly poorer ($P < .05$) feed conversion than did sorghum RS671. Sorghum BR64 produced the poorest weight gain and feed conversion of all the bird resistant sorghum grains. Sorghum IS8260 supported somewhat better weight gain and significantly better ($P < .05$) feed conversion than did sorghum BR64 whereas sorghum IS6992, which had the lowest tannic acid equivalents of the bird resistant sorghum grains, supported the best weight gain and feed efficiency of any of the bird resistant grains.

During the extraction process of sorghums RS671 and BR64 there was little change in the percent protein present in these grains. Therefore, when these extracted grains were compared on an equal weight basis with the intact grain, there was little difference in the dietary protein content of the diets (Table 3). However, during the extraction process of IS8260 and IS6992 there was a 1-2% loss in the protein content of the sorghum grains. It is believed that this loss was in part due to the immaturity of these two sorghum grains. As a result, a reduction in the percent dietary protein was obtained when the extracted grains were compared on an equal weight basis with the intact grains (Table 3).

Extraction of the tannins from sorghum RS671 had little effect on weight gain as was noted in Experiment 1. Chicks fed the extracted BR64 diet showed significantly greater weight gain and feed conversion ($P < .05$) as compared with chicks fed the intact BR64 diet. Improvements in chick performance also resulted when the tannin was extracted from IS8260 even though the diet contained less protein. At equal dietary protein levels, the improvement perhaps would have been statistically significant and closer to that observed with the extracted BR64 diet. Extraction of the tannin from sorghum IS6992 resulted in poorer weight gain and similar feed conversion as compared with the intact IS6992 diet. The reason for this decrease in chick weight gain was not readily apparent; however, one should keep in mind that the extracted IS6992 diet contained less protein than the intact IS6992 diet. Also, chick performance with the sorghum IS6992 diet was the highest of any of chicks fed bird resistant grains.

The alkaline-hot water extraction process was effective in removing most of the tannin from the bird resistant sorghum grains BR64, IS8260 and IS6992, which were relatively high in tannin content. The process seemed to have little effect on the protein content when mature grains were used. When the extracted sorghum grains BR64 and IS8260 were fed to chicks, the growth depressing effect observed with the intact grains was almost

completely overcome, indicating that the poorer performance of chicks fed bird resistant sorghum diets was due to the detrimental effects of the tannins. These results suggest that after extracting most of the tannin, these bird resistant grains are essentially equal in biological value to the non-resistant sorghum grains.

The markedly improved performance of chicks fed the BR64 diet supplemented with methionine in contrast to the slight improvement in performance of chicks fed the extracted BR64 diet with added methionine may be explained by: (1) methionine detoxifies tannins possibly via methyl gallic acid; (2) tannins complex with methionine thereby reducing availability; (3) tannins inhibit proteolytic enzymes and thus decreased protein digestibility. Although the latter phenomenon does appear to occur (Rostagno *et al.*, 1973b) it seems unlikely that this could completely explain the results. Methionine is the first limiting amino acid in this type of diet, but one would not expect complete amelioration of the growth depression since deficiencies of other amino acids would be enhanced by reduced availability.

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