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Overcoming the Nutritionally Harmful Effects of Tannin in Sorghum Grain by Treatment with Inexpensive Chemicals

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Treatment of high-tannin sorghum grain with moist, alkaline conditions was shown to substantially reduce the amount of tannin as measured by three chemical assays. Chicks fed a high-tannin grain (Savannah III), treated as the whole grain with dilute ammonium hydroxide for 30 days, showed 3-week weight gains and feed efficiencies which were statistically equivalent to those of chicks fed an untreated low-tannin control (RS-610). A shorter treatment of a ground high-tannin grain (BR-54) with a 0.5 M aqueous solution of K_2CO_3 resulted in a comparable improvement in weight gains and a substantial improvement in feed efficiencies. Treatment of the same grain with moisture and CaO gave an improvement of a lesser magnitude. Increases in available protein after treatments did not appear sufficient to account for the nutritional improvements.

Tannin in sorghum grain provides a natural protective system against bird depredation (McMillan et al., 1972), weathering (Harris and Burns, 1973), and preharvest germination (Harris and Burns, 1970). In areas where any of these stresses are severe, high-tannin genotypes generally produce higher yields and better quality grain. However, when fed to monogastric animals, high-tannin sorghum grain can result in considerably lower growth rate and feed efficiency than low tannin types (Featherston and Rogler, 1975). [The nutritional significance of tannin in ruminant diets is unclear, some reports indicating that it is harmful, e.g., McGinty (1969), Maxson et al. (1973), but others recommending addition of tannin to protect protein from microbial deamination, e.g., Dreidger and Hatfield (1972), Zelter et al. (1970). The balance of evidence seems to be no effect or a negative effect except at low N intake (Broster et al., 1978).] An economical treatment to neutralize the effect of the tannin would add greatly to the value and marketability of high-tannin grain. The impact could be especially important for certain developing nations which lie in geographical regions where high tannin sorghum genotypes must be grown to obtain satisfactory production.

Ammoniation of high-tannin grain (BR-54) by moistening whole grain with concentrated NH_4OH (28–30% NH_3) has been shown to decrease assayable tannin to approximately 30% of the original level and to greatly improve the growth rate and feed efficiency when fed to rats and chicks (Price et al., 1978a). In this investigation, we have examined the effectiveness of a milder treatment with dilute aqueous ammonia as a chemical detoxicant for high-tannin sorghum grain. We have also determined the effects of other aqueous alkalies on both the chemical assays and nutritional properties of high-tannin grain. In addition to BR-54, this study was broadened to also include another commercial high-tannin sorghum, Savannah III.

Table I. Comparison of Detoxification Treatments[a]

	% reduction in assayable tannin					
	days of treatment					
treatment	2	3	8	11	18	22
water		3		12	19	
conc. NH_4OH	59	70	84			94
2.5 M NH_4OH		53		70	87	
6 M NaOH	93	94	98			99
1 M NaOH	44	59	59			79
0.5 M NaOH		24		52	53	
0.2 M NaOH		38		34	25	
1 M K_2CO_3		68		79	90	

[a] One milliliter of solution mixed with 10 g of BR-54 (whole grain) and stored in a closed container at room temperature. Two-gram samples, removed at indicated times and air-dried overnight, were assayed for tannin by the vanillin assay and compared to the tannin content of the grain before the treatments were begun.

EXPERIMENTAL SECTION

Sorghum grain was supplied by Dr. John Axtell from grain grown at the Purdue University Agronomy Farm in 1975 (RS-610), 1976 (BR-54), and 1977 (Savannah III and

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BR-54). Grain was grown from commercial hybrid seed.

Treatments. The effectiveness of various alkalies (NH_4OH, NaOH, and K_2CO_3, room temperature, Table I) at different concentrations for decreasing the assayable tannin in the grain were studied on a lab bench scale by mixing 10 g of whole grain (BR-54) and 1 mL of aqueous alkali in 35-mL capped plastic bottles. Approximately 2 g was removed at timed intervals and allowed to dry overnight before assaying for tannin by the vanillin assay (Price et al., 1978a). For the lab bench studies to establish conditions for CaO and K_2CO_3 treatments, 40 g of ground BR-54 and 8 mL of H_2O and solid CaO or 8 mL of 0.5 M K_2CO_3 were mixed and stored in capped plastic bottles. Two-gram samples were removed, allowed to dry 2 days, and then reground before assaying as previously described. A similar procedure was used with alkali obtained by soaking 130 g of hardwood ashes overnight in 350 mL of water and filtering, except that 5 g of ground BR-54 and 1 mL of filtrate were mixed and stored at room temperature for 3 days before drying and assaying for tannin.

Larger quantities of grain for the nutritional studies were treated with dilute aqueous ammonia prior to grinding by mixing 20 kg of whole grain and 2 L of dilute ammonia [1 volume of concentrated ammonium hydroxide (28–30% NH_3) and 6 volumes of water] in a sealed plastic bag inside a sealed drum. The liquid was rapidly absorbed by the grain; after a few hours no free liquid remained and the grain handled like untreated grain. The drums were rotated a few times to facilitate mixing. The treatment was continued until 85% of the tannin originally present was no longer detectable by the vanillin assay (30 days). Grain was removed, dried 1 day at 50 °C in a forced-air oven to remove ammonia and excess moisture and spread in a 2-cm layer for 1 day to equilibrate with room humidity before grinding and mixing in the diets. No signs of microbial growth were noted. The smell of ammonia was absent after drying.

Treatment with CaO involved mixing 18 kg of grain, ground with a hammer mill, with 0.36 kg of powdered CaO and 3.6 L of water. Treated grain was sealed in plastic bags inside a sealed drum for 7 days at room temperature and then dried in a 52 °C forced-air oven for 1 day and equilibrated with atmospheric moisture before mixing in the diets.

Treatment with K_2CO_3 involved mixing 18 kg of grain, ground as just described, with 3.6 L of 0.5 M aqueous K_2CO_3. Treated grain was kept in sealed plastic bags for 42 h at 52 °C, followed by 5 days at room temperature. The treatment was probably complete without the extra 5 days but the delay was unavoidable. Grain was dried and equilibrated with atmospheric moisture as described above and then mixed in the diets.

Assay of Tannin Content. Grain was ground to pass a 0.4-mm screen on a Udy cyclone mill (Tecator, Inc., Boulder, CO) equipped with a vacuum attachment. Methanolic extracts were prepared as described by Price et al. (1978) after a preliminary extraction with ether which improved results with the protein precipitation assay. The vanillin assay (Burns, 1971) was used as modified by Price et al. (1978b) using a standard curve prepared with tannin purified from sorghum grain (BR-54) as outlined in the cited paper. The protein precipitation assay followed the procedure of Hagerman and Butler (1978). The Prussian blue assays (Price and Butler, 1977) were run on the methanolic extract and also by a procedure, more specific for tannin, involving subtracting assays run on a 1 M salt water extraction from those run on a water extraction. Calculations of reductions in tannin content were based on a comparison of the tannin in treated grain and tannin in grain before treatments were begun.

Pepsin Digestion. Two grams of grain, ground as described for tannin assays, were digested with 1 g of pepsin (Sigma, 1875 units/mg of protein, lot no. 106C-0159) in 50 mL of 0.075 N HCl with shaking at 37.5 °C. After 6 h, an additional 0.5 g of pepsin was added and incubation was continued for 23 h. The residue was filtered through Celite, washed three times with water at 50 °C, and analyzed for nitrogen content by the Kjeldahl procedure.

Residual Alkalinity in Treated Grain. Five grams of grain, ground as described for tannin assays, were allowed to stand overnight in 400 mL of water. The mixture was then titrated with 0.109 N HCl to pH 5.6 (Lancaster et al., 1974).

Feeding Trials. White Mountain male chicks were randomly alloted to treatments at 1 day of age. Each chick was wing-banded, weighed, and placed in electrically heated battery brooders with raised wire floors. Seven chicks were placed in each pen with four replicates per treatment in the ammoniation experiment (Table II) and eight chicks per pen in the CaO–K_2CO_3 experiment (Table III). Feed and water were provided ad libitum. The experiments were conducted for a 21-day period. Isonitrogenous sorghum–soybean meal diets were prepared as previously described (Featherston and Rogler, 1975), with the exception that, in the CaO–K_2CO_3 experiment, the 2.25% dicalcium phosphate and 1% ground limestone in the basal diet were replaced with 0.5% dicalcium phosphate, 0.5% calcium chloride (dihydrate), and 1.43% monobasic sodium phosphate (monohydrate). Total phosphorus in all diets of the CaO–K_2CO_3 experiment was 0.76%, and total calcium was equalized in all diets at 1.33% by addition of $CaCO_3$. No attempt was made to equalize the potassium content of the experimental diets.

Statistical Analysis. Weight gain and feed efficiency were statistically analyzed by analysis of variance. Comparisons of the means were made by the Neuman–Keuls multiple range test (Steel and Torrie, 1960).

RESULTS

All three aqueous alkalies examined on a lab bench scale (NH_4OH, NaOH, and K_2CO_3) eventually caused a 90% or greater reduction in assayable tannin as seen in Table I. Thus considerably less concentrated solutions can be used than the concentrated NH_4OH that was previously reported (Price et al., 1978a), although the more dilute reagents require longer treatment times. The rate of decrease in assayable tannin in treated grain was somewhat slower at 4 °C than at 52 °C in sealed containers (data not shown). Though it was not studied exhaustively, temperature appears to have a relatively small effect on the rate of the detoxification reaction.

A chick feeding trial was conducted with high- and low-tannin grain treated with dilute NH_4OH to determine whether the decrease in assayable tannin would be accompanied by a corresponding improvement in nutritional quality. The results are shown in Table II.

Treatment of Savannah III (a high-tannin grain) resulted in a 46% increase in 3-week weight gain, which was equivalent to both the treated and untreated low-tannin grain, RS-610. This milder treatment did not harm the RS-610, in contrast to the results of a previous study with concentrated NH_4OH (Price et al., 1978a). Ammoniation improved feed efficiency substantially in chicks fed Savannah III but had little influence on chicks fed RS-610. In contrast to previous results with BR-54 (a high-tannin grain) grown in 1976 and earlier, no depression of weight

Table II. Three-Week Chick Feeding Trial of High-Tannin (BR-54, Savannah III) and Low-Tannin (RS-610) Sorghum Grain Treated for 30 Days with Dilute NH_4OH

	3-week wt gain[a]	3-week feed/gain[a]	% with bowed legs	g of tannin/100 g of dry grain[b]	% reduction in assayable tannin: methanol extract: vanillin[b]	methanol extract: protein precipitation[b]	methanol extract: Prussian blue[b]	aqueous extract: Prussian blue[b]	g of protein/100 g of dry grain: total[b]	g of protein/100 g of dry grain: soluble in pepsin[b]	mequiv of HCl to neutralize 1 g of ground grain
BR-54 control	253_a	2.15_b	28.5	2.6					9.69	5.92	0.000
NH_4OH	263_b	1.93_d	3.6		87	77	73	48		7.37	0.112
Savannah III control	183_b	2.32_a	25.0	2.5					9.20	5.09	0.000
NH_4OH	268_a	1.96_d	0.0		85	72	68	38		6.99	0.107
RS-610 control	250_a	1.87_{cd}	3.6	0.0					10.15	9.22	0.000
NH_4OH	268_a	1.82_c	3.6							9.07	0.109

[a] Values not having the same subscript were significantly different ($P < 0.05$). [b] Values reported are the average of determinations on two samples of grain.

Table III. Three-Week Chick Feeding Trial of CaO and K_2CO_3 Treated High-Tannin (BR-54) and Low-Tannin (RS-610) Sorghum Grain

	3-week wt gain[a]	3-week feed/gain[a]	% with bowed legs	g of tannin/100 g of dry grain[b]	% reduction in assayable tannin: methanol extract: vanillin[b]	methanol extract: protein precipitation[b]	methanol extract: Prussian blue[b]	aqueous extract: Prussian blue[b]	g of protein/100 g of dry grain: total[b]	g of protein/100 g of dry grain: soluble in pepsin[b]	mequiv of HCl to neutralize 1 g of ground grain[b]
BR-54 control	174_c	2.54_a	12.9	2.0					10.8	6.22	0.019
CaO	200_b	2.35_b	9.4		90	88	84	78		7.84	0.345
K_2CO_3	263_a	2.10_c	3.1		99	98	93	84		7.88	0.161
RS-610 control	266_a	1.84_e	0.0	0.0					9.9	9.14	0.022
CaO	223_b	1.94_d	3.1							9.08	0.394
K_2CO_3	218_b	1.97_d	3.2							8.95	0.174

[a] Values not having the same subscripts were significantly different ($P < 0.05$). [b] Values reported are the average of determinations on two samples of grain.

gain occurred with the 1977 crop of BR-54, and consequently no significant improvement by the treatment was observed. The reason for the unusually high nutritional quality of the 1977 BR-54 is unclear. The tannin content was nearly identical with that of Savannah III, approximately 2.5% by weight. The frequency of the leg abnormalities reported earlier (Rostagno et al., 1973; Armstrong et al., 1973) was high for both high-tannin grains and was greatly reduced in both cases by the treatment.

Results from four different methods of analysis used to measure the percent decrease in assayable tannin are arranged in Table II in decreasing order of sensitivity to detection of changes in apparent tannin content upon ammoniation. The vanillin, protein precipitation, and Prussian blue assays on the same methanol extract gave roughly parallel results. Because the Prussian blue assay is based on a redox reaction, it detects not only tannin but all other phenols present in the extract. The smaller decrease in tannin content noted with the Prussian assay, suggests that the nontannin phenolics which are present are less sensitive to ammoniation than are the condensed tannins which precipitate protein and respond to the vanillin assay. When the aqueous Prussian blue technique of Price and Butler (1977) was used, the indicated percent decrease in tannin was considerably less than with the other three methods. This technique involves water extraction of all phenols present and a separate aqueous 1 M NaCl extraction which presumably removes only nontannin phenolics. The latter is subtracted from the aqueous value to obtain a measure of tannin content. The apparent higher tannin content of treated grain observed with this assay suggests that at least some of the treated tannins may be soluble in water and are still oxidizable.

Available protein in the high-tannin sorghums, as estimated by pepsin digestibility, was approximately two-thirds that of the low-tannin control (Table II). Ammoniation caused a considerable increase in protein digestibility of both high-tannin types, but not to the level of the low-tannin control.

A small amount of titratable base remained in each grain after drying (Table II). Because values were nearly the same for all three hybrids, this is presumably not related to tannin content.

In preliminary experiments, it was shown that addition of dry CaO and moisture to ground high-tannin sorghum grain caused a large decrease in assayable tannin. After mixing ground BR-54 grain with 20% water and 0, 0.5, 2.0 and 5.0% CaO, the amount of tannin that could be detected by the vanillin assay was reduced by 20, 49, 88, and 99%, respectively. The time course of this treatment differed strikingly from the previously described alkali treatments. Instead of a gradual decrease in assayable tannin with time, the decrease was nearly instantaneous, but the apparently detoxified tannin "reappeared" to a small extent upon drying. Values after 20 h in a 50 °C forced air oven were 7, 21, 60, and 83%, respectively. By readdition of lost water just before samples were extracted with methanol, values could be instantly lowered to their earlier magnitude.

Conditions for a K_2CO_3 treatment were established on a lab bench scale by treating BR-54 grain with 0.5 M K_2CO_3 at 50 °C or at 22 °C in sealed containers for 18 h. The treatment was more rapid with ground grain (reductions in tannin content as measured by the vanillin assay of 77 and 88%, respectively, at 22 and 50 °C) than with whole grain (reductions of 49 and 50%, respectively). Again, there was only a moderate increase in the rate of loss of assayable tannin at the higher temperature. In contrast to the results with CaO, readdition of lost moisture after drying had little effect on tannin assays.

The results of a feeding trial evaluating the nutritional quality of grain which was ground and then treated with CaO and K_2CO_3 are shown in Table III. Because of the anomalous behavior of the BR-54 from the 1977 crop, as reported in Table II, the previous year's crop was used in this experiment. Both treatments improved the nutritional quality of high-tannin grain, but the K_2CO_3 treatment was most effective. The K_2CO_3 treatment of BR-54 resulted in weight gains which were statistically equivalent to those observed with the untreated low-tannin control (a 51% increase in weight gain). Feed efficiency was considerably improved, but was inferior to that of the low-tannin RS-610. CaO treatment gave a 15% increase in 3-week weight gain and a modest improvement in the feed to gain ratio. Both treatments harmed the nutritional value of RS-610; weight gains averaged 16.2 and 18.0% lower for CaO and K_2CO_3, respectively, and the feed required per gram of grain increased by 5.4 and 7.1%.

The incidence of bowed legs was not as high as had been found in the previous feeding trial, but was diminished by the treatments, especially with K_2CO_3.

Available (pepsin digestible) protein of BR-54 was increased considerably by both treatments, but remained appreciably lower than that of the low-tannin grain.

Using the combined data in Tables II and III, there was a high correlation ($r = -0.90$) between percent change in the feed to gain ratio after treatment and percent change in available protein after treatment, but a poorer correlation ($r = -0.43$) between weight gain and available protein.

Many Third-World farmers would be unable to afford even the relatively inexpensive alkalies that we have examined. We have, therefore, examined wood ash as a source of alkali for detoxification. Within 3 days of treatment with a water extract of hardwood ashes, 85% of the tannin appeared to have been inactivated. Though no feeding trials were done, it would appear that 150 lb (possibly less) of ashes would provide sufficient alkali to detoxify a ton of grain. The method used in Uganda and Rwanda of germinating high-tannin grain in wood ash, malting, and using the product in porridge for human consumption (Doggett, 1977) quite possibly detoxifies all or part of the tannin.

DISCUSSION

It now seems clear that moist, alkaline conditions in general, rather than some unique property of ammonia, are responsible for improved nutritional quality and reduced level of chemically assayable tannin in high-tannin sorghum grain treated as described in this paper and in a previous report (Price et al., 1978). Conditions of the treatment, such as duration, use of whole or ground grain and type and concentration of alkali, can be varied according to the requirements of the processor.

Ammoniation offers an advantage over the other treatments described in that excess NH_3 can be removed by volatilization. In the tropics, where insect damage is often severe, it is possible that treatment with moderately concentrated NH_4OH might serve the dual purposes of treating the high-tannin grain and protecting it from insects, although mold would presumably become a problem in prolonged treatments if the NH_4OH concentration were too dilute. The nonvolatile alkalies could be neutralized by addition of acid, though it is not clear whether the concentrations of alkali used here are harmful. Treatment with alkali obtained by soaking wood ashes in

water may be of special interest to small farmers in Third-World countries where high-tannin genotypes often offer considerable advantages. In such cases, greatly improved yields might be obtained with high-tannin sorghum, which could then be treated to overcome the deleterious nutritional effects of the tannin.

Calcium oxide has the advantage of being readily available and relatively inexpensive in most parts of the world. Calcium is also an essential nutrient for animals. The effectiveness of CaO at improving the nutritional quality of high-tannin grain, however, was less than that predicted by the chemical assays.

This plus the unique time course for the CaO treatment (instantaneous reduction in assayable tannin rather than a gradual reduction over a few days) suggests a different basis for its action, perhaps formation of an insoluble tannin–Ca^{2+} complex. Such a complex has recently been suggested by Wah et al. (1977), who found a 100% reduction in assayable tannin in *sal*-seed meal after treatment with moisture and 4% CaO. They also found "reappearance" of some tannin after oven drying this treatment. Unfortunately no feeding trials were done. It is not unlikely that the gradual effect of moisture and alkalinity may occur in the CaO treatment also, its occurrence being masked in the assays by the complex formation. Thus the moderate improvement in nutritional quality caused by CaO may have the same chemical basis as the other alkaline treatments.

The reason for the reduction in nutritional quality of low-tannin RS-610 caused by some treatments remains unknown. The observation that using less NH_4OH and removing excess NH_3 in a forced-air oven eliminated this negative effect would suggest that alkalinity might be uniquely harmful to RS-610 diets. On the other hand, the CaO-treated RS-610, which had twice as much titratable base as the K_2CO_3-treated grain, did not cause as great a growth depression. The question is one of primarily academic interest, because there is no reason for treating low-tannin grain.

The mechanism by which moist, alkaline conditions detoxify high-tannin sorghum, as well as the mode(s) of action of tannin in lowering the nutritional quality of the diet, have not yet been elucidated. The tannin may become altered in some manner so as to become unreactive both nutritionally and in the assays employed here, perhaps by forming insoluble phlobaphenes (Swain, 1965). Alternatively, they may become permanently bound to some nearby component in the grain, perhaps protein, during treatment, rendering both substances insoluble and nutritionally inert. The observation that available protein in the treated high-tannin grain increased, but not to levels found in low-tannin grain, even though the effect of tannin on weight gain was completely overcome, lends some support to the idea of the formation of an indigestible tannin–protein complex during the treatment. Such a complex was found between aflatoxin and protein when contaminated grain was detoxified by ammoniation (Beckwith et al., 1975).

We have shown that a variety of inexpensive aqueous, alkaline solutions, when used to moisten high-tannin sorghum grain, reduce the content of tannin that is detectable by a number of assays and that this decrease is accompanied by an improvement in the nutritional quality of the grain.

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