SORGHUM RECONSTITUTION: METHODOLOGY STUDY OF SORGHUM TANNIN DETOXIFICATION AND IMPACT OF DETOXIFICATION UPON SORGHUM AMINO ACID BIOAVAILABILITY AND BROILER CHICK PRODUCTIVITY

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in partial fulfillment of the requirements
for the Degree of
Master of Science
July, 1984

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ACKNOWLEDGMENTS

I would like to give my sincere thanks to all those who helped me during the course of my Masters work. Especially I wish to give special thanks to my adviser, Dr. Robert Teeter for his invaluable knowledge, keen insight and constant encouragement. Also I thank the other members of my advisory committee, Dr. Don Wagner, Dr. Charles Maxwell, and Dr. Fred Owens for their advice and many suggestions.

Recognition goes to Dr. Weeks and Dr. Hibberd for their information on statistics and sorghum and to fellow graduate student Mike Smith for his assistance and many man hours. Also to the Poultry Research farm crew, E. Murray, L. Bloom and C. Murray and undergraduate student K. Kovac for their involvement.

Much appreciation goes to my brothers and their wives and to my mother-in-law, Clara Keyes, for their humor and encouragement. My deepest respect, admiration and love is given to my parents, Mahmood and Parvine Sarani who long ago began the dream of education for their sons. Despite the many obstacles and untold sacrifice, giving up many basic necessities of life to see our dreams fulfilled, they persisted.

And finally to my wife Colleen who stood by me during the many endless hours, for her love and patience I dedicate this thesis and all the work that it represents.

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REVIEW OF LITERATURE

Introduction

Sorghum grain is an important crop, ranking fifth worldwide (Mabbayad, 1974). It is the major food crop in the semi-arid tropics, a zone including areas in the Southern United States, part of China, India, and Africa. In the United States the grain is the third largest cereal crop (Maxon et al., 1973; Chibber et al., 1978). Sorghum crops cover 44 million hectares of the earth, 37 million of them are in developing countries (FAO, 1978). Most of the sorghum grown in the United States ends up as livestock feed. In the developing countries, where sorghum may even be the sole crop of some farmers due to climatic, marketing, and farming conditions, sorghum is vital to the human population as a staple and basic source of nourishment (Bullard et al., 1981). It is the principle food crop in Northern Nigeria and is the staple in West Africa (Haikerwal and Mathieson, 1971).

Sorghum production concentrates where high temperatures and lack of moisture deter other cereal crops (Bate-Smith and Rasper, 1969; Niehaus and Schmidt, 1970).

Sorghum's popularity is due to its ability to withstand severe ecological and harsh growing conditions (Doggett, 1970), high per acre yield (Jambunathan and Mertz, 1973), better quality grain (Price et al., 1979b), resistance to preharvest germination (Chibber et al., 1978) and

seed molding (Harris and Burns, 1973). These sorghum qualities are due to the presence of polyphenolic compounds called tannins.

The existence of sorghum grain seed tannins is well documented (Salunkhe et al., 1977; Reichert et al., 1980). Vegetable tannins are water soluble compounds with a range of molecular weights from 500 to 3000 (Bate-Smith and Swain, 1962). There have been varied opinions about tannins' function. Swain (1979) reported that of the secondary metabolites in vegetables, tannins are the most important. Fraenkel (1959) also attributed important biological functions to these compounds. However, Muller (1969) believed them to be metabolic accidents or waste products.

It seems tannins serve a protective function. Tannins are astringents, precipitating salivary proteins which cause the plant tissue to be unpalatable (Gupta and Haslam, 1978). An important aspect of grains with high tannin content is their bird resistance (Goldstein and Swain, 1963; Niehaus and Schmidt, 1970; Chavan et al., 1979; Bullard et al., 1981). Lower tannin sorghum varieties have shown losses of 18-75% (McMillian et al., 1972) up to 100% (Tipton et al., 1970) from bird predators.

Sorghum Kernel Structure and Tannin Content

The sorghum kernel consists of the pericarp, endosperm, and germ (Figure 1, Rooney et al., 1979). Genetic material and lipids make up the germ. The endosperm can be further divided into the peripheral or corneous endosperm and the floury endosperm. The periferal endosperm contains a protein matrix with tightly bound granules resulting in

lowered starch availability. The floury endosperm also contains proteins and starch granules but lacks a matrix (Hibberd, 1982).

The pericarp is made up of several layers, with some dispute as to the exact names and divisions (Rooney et al., 1979). Here they will be referred to as the pericarp, aleurone and testa.

Some sorghum tannins are located in the endosperm (Freeman and Watson, 1969), but the majority are in the testa layer (Freeman and Watson, 1969; Reichert et al., 1980) of the pericarp (Sanders, 1955; Yasumatsu et al., 1965; Nip and Burns, 1969). It is the presence of tannin in the testa (Blessing et al., 1963; Reichert et al., 1980) of high-tannin sorghum varieties that gives the grain its sturdy nature and bird resistant qualities (Wall and Ross, 1970; McGrath et al., 1982). The testa dictates color, however, seed coat color by itself cannot be used to judge nutritional value (Thayer et al., 1957).

Davis and Hoseney (1979) used α-amylase inhibition to measure tannin content preharvest changes during maturity in bird resistant sorghum. Twenty to 30 days following anthesis tannin content is at its highest point, declining as much as 80% at full maturity (Davis and Hoseney, 1979; Price et al., 1979a; Glennie, 1981). McLeod (1974) theorized that the tannin activity decreased with time because the subunits polymerize through maturation.

Protein and Amino Acid Content

Sorghum grain's chemical composition is dependent on moisture, temperature, other growing conditions, conditions during harvest and variety of sorghum (Heller and Seiglinger, 1944).

The protein content ranges between eight and 16% (Klimenko and

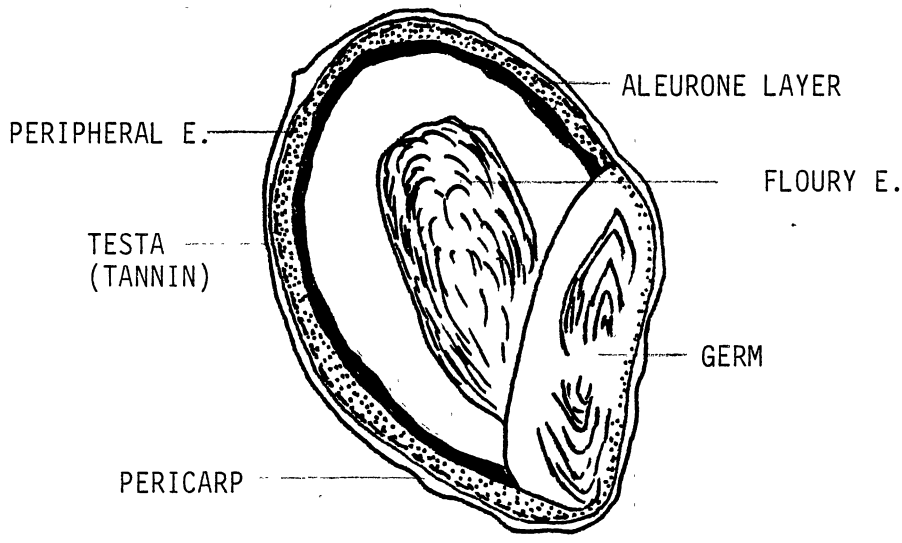

Figure 1. Cross Section of a Sorghum Kernel

Goldenberg, 1960; Rooney and Clark, 1968). As in other cereals, sorghum proteins can be classified as glutelins, globulins, albumins, and prolamines (Jambunathan and Mertz, 1973). Prolamines are alcohol-soluble and very low in lysine (Mertz et al., 1964). The endosperm protein matrix is high in prolamine and similar to the protein in corn, both being first limiting in lysine (Hansen et al., 1946; Jones and Beckwith, 1970). Microscopic granules, thought to be prolamine deposits (Wall and Blessin, 1969) and other protein bodies make up most of the endosperm protein (Seckinger and Wolf, 1973).

The amino acid content has been much studied (Adrian and Sayerse, 1957; Vavich et al., 1959; Horn and Schwartz, 1961; Bressani and Rios, 1962; Deyoe and Shellenberger, 1965). Amino acid composition in the germ has higher proportions of amino acid to protein of histidine, glycine, threonine, valine, aspartic acid, arginine and lysine than that in the whole kernel (Haikerwal and Mathieson, 1971). Bressani and Rios (1962) compared amino acid genetic variation in 25 varieties of sorghum. Like other cereals, sorghums are lysine deficient (Adrian and Sayerse, 1957; Close and Navas, 1958; Pond et al., 1958).

Tannin Chemistry

The vegetable tannins have been divided into two classes according to structural type, hydrolyzable and non-hydrolyzable tannins. Non-hydrolyzable tannins are also called condensed tannins (Gupta and Haslam, 1978). Hydrolytic enzymes, acids and bases cleave the hydrolyzable tannins into a phenolic carboxylic acid (such as gallic acid) plus a sugar (such as glucose) and other polyol (Freundenberg, 1920). Non-hydrolyzable tannins (condensed) do not readily undergo

TABLE 1. Distribution of Tannin and Protein With Sorghum Grain[a]

Component	% by wt	% tannin	% Protein (N x 6.25)
Testa	7.6	22.4 (81.6)[b]	14.7 (9.8)
Pericarp	7.5	4.2 (15.1)	4.1 (2.7)
Endosperm + Germ	84.8	0.08 (3.3)	11.8 (87.5)
Whole Grain	100.0	3.63	11.5

[a]Adapted from Richert et al., 1980.

[b]Proportion of constituent in parentheses.

enzymatic degradations. Condensed tannins do however, when exposed to acid treatment, depolymerize to yield anthocyanidin pigments (Gupta and Haslam, 1978). Sorghum grain tannins are condensed tannins (Strumeyer and Malin, 1975) and are called procyanidins (Figure 2) because cyanidin is released in acid treatment (Watterson and Butler, 1983). Tannins which yield anthocyanidins are likewise called proanthocyanidins (Gupta and Haslam, 1978).

Tannin-Protein Complexity

High tannin sorghum grain has decreased digestibility and nutritional value (Price et al., 1979b) due to tannin-protein binding (Hagemen and Butler, 1981). Antinutritional effects attributable to tannin-protein interactions were observed in non-ruminants fed feeds containing tannin (Tamir and Alumot, 1970; Jambunathan and Mertz, 1973; Schaffert et al., 1974; Martin-Tanguy et al., 1977). Growth depression seen with high tannin sorghums (compared to low tannin sorghums) is well documented (Fuller et al., 1966; Conner et al., 1969; Armstrong et al., 1973; Rostangno et al., 1973a; Armstrong, 1974; Elkin et al., 1978). Sorghum tannins were observed by McGinty (1969) to reduce protein and dry matter digestibility.

Proteins and tannins interact by forming hydrogen bonds between carbonyl groups of the protein and hydroxyl groups on the phenolic component of tannins (Gustavson, 1954; McLeod, 1974). Since protein complexing increases as the number of hydroxyl groups increases (Calderon et al., 1968), a single tannin molecule may crosslink protein chains by forming hydrogen bonds with several peptide groups (Calderon et al., 1968).

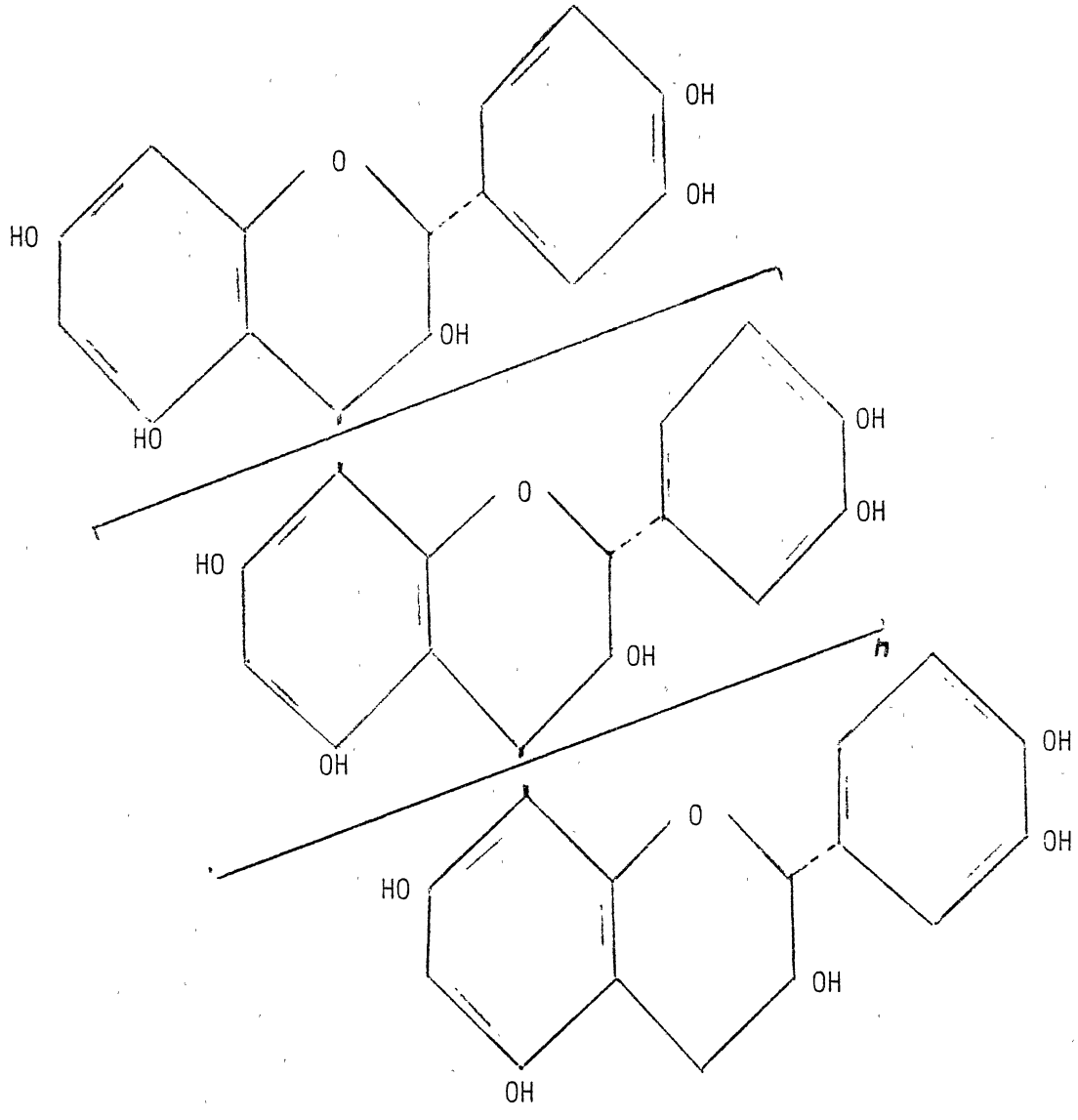

Figure 2. Structure of Procyanidin Polymer

Nutritionally, the proteins in sorghum may be bound by tannins before consumption, during actual digestion (Glick and Joslyn, 1969; Schaffert et al., 1974), or may excessively bind the digestive enzymes (Tamir and Alumot, 1970; Schaffert et al., 1974; Chibber et al., 1978). Sorghum tannins are known to inhibit amylase enzymes (Strumeyer and Malin, 1975) necessary for digestion. Amylase inhibition is also a concern in sorghum beer production as these enzymes are very necessary to the process (Daiber, 1975). Once absorbed, there is a possibility that tannins may exert toxic effects on blood and other body protein components (Potter and Fuller, 1968).

Condensed tannins precipitate proteins. Increased precipitation is seen as protein moelcular weight increases (Van Buren and Robinson, 1969). Also, as the molecular weight or number of phenolic groups in the tannin increases precipitation with gelatin increases (Van Buren and Robinson, 1969) and may be true for other proteins.

Tannin Detoxification

Since high tannin sorghum grains are desirable for their hardy nature and resistance, but undesirable for their nutritional drawbacks, the natural question is how to retain good growing traits and at the same time eliminate the bad feed characteristics. Many methods have been used to treat harvested grains for tannin reduction.

Tannins cannot be removed by screening during commercial milling because they are in the flour (Chavan et al., 1979). Mechanical abrasion dehulling to remove the tannin containing pericarp layer was one solution which resulted in 98% tannin removal. Unfortunately, 37% of the grain itself was lost along with 45% of the protein content (Chibber et

al., 1978).

Chemical treatment, fermentation, and reconstitution (imbibing in water) have shown a resulting decrease in sorghum tannin content. Dilute formaldehyde treatment of the grain reduced tannin content, possibly by some type of constitutent crosslink (Daiber and Taylor, 1982). Price et al. (1978a) removed approximately 70% of tannin when using concentrated NH_4OH to treat sorghum, resulting in improved feed efficiency and growth rate in chicks and rats. Price et al. (1979b) reported that imbibing whole seed in dilute ammonia removed tannin and increased chick feed efficiency and weight gain.

Improved in vitro protein digestibility was observed with 75-85% tannin removal when sorghum was treated with 0.05M NaOH and 0.05M KOH for 24 hours at 30°C (Chavan et al., 1979). Improved feed efficiency and weight gain were seen in rats with aqueous alkali extractions of sorghum to remove tannin (Armstrong et al., 1974). Treatment of ground grain with dilute potassium carbonate decreased tannin content and increased feed efficiency and weight gain in chicks (Price et al., 1978a).

Sullins et al. (1971) observed that storage of reconstituted grain disrupted the protein-starch matrix. Reichert et al. (1980) reduced tannin content of grain sorghum to 0.3% by storing reconstituted sorghum nine days. In the same work, they compared treatment of grain reconstituted in 25% by weight water, 0.8N NaOH and 0.8N HCl stored at 25°C for 2 days. Tannin levels were reduced from 3.63% to 2.2, 0.1, and 0.6%, respectively. Sarani et al. (1983) reported a drastic (85%) decrease in detectable tannin content of high tannin sorghum grain by reconstitution and anaerobic storage for eight days at 32°C.

Sullins et al. (1971) suggested that reconstitution disrupts the

protein matrix of the sorghum endosperm. However, changes in protein and starch due to the reconstitution process are insignificant (Hibberd, 1982). Reconstitution followed by anaerobic storage is a nutritionally effective and energy efficient treatment method (Totusek et al., 1967; Newsom et al., 1968; White et al., 1969).

The improved feed efficiency in ruminants seen with tannin reduction by reconstitution is due to increased starch and protein digestibility (Buchanan-Smith et al., 1968; McNeil et al., 1971; Potter et al., 1971) and the added fermentation from anaerobic storage further reduces tannin content (Cummins, 1971).

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CHAPTER II

THE IMPACT OF RECONSTITUTED-, HCL-, AND NAOH-TREATED HIGH-TANNIN SORGHUM GRAIN UPON TANNIN CONTENT AND LYSINE BIO-AVAILABILITY IN BROILER CHICKS

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Abstract

Two in vitro and in vivo experiments were conducted to evaluate the effect of reconstitution and other chemical treatment of commercial brown sorghum grain on broiler productivity in lysine deficient rations. The commercial type high-tannin brown sorghum grain was reconstituted by imbibing the grain with water to 70% dry matter. The reconstituted sorghum grain (RSG) was then treated with 0.97N HCl or 0.97N NaOH. All three treated grains were incubated for three days at 32°C. Assayable tannin content of commercial brown sorghum (2.41%) decreased by only approximately 7% with reconstitution (2.25%). However, the 0.97N HCl (0.75%) and 0.97N NaOH (0.23%) showed a significant reduction ($P < .01$) in tannin content of approximately 69% and 90% respectively. Reconstituted or chemically treated sorghum grain failed to elicit a growth response when included in a lysine deficient ration through lysine supplementation

increased growth rate and feed efficiency. The low lysine content may have masked the effects of reconstitution in the lysine deficient ration.

Introduction

The detrimental effects of high-tannin sorghum grain upon weight gain and feed utilization for broiler chicks in contrast to low-tannin sorghum grains have been investigated (Chang and Fuller, 1964; Conner et al., 1969; Armstrong et al., 1974; Rostagno et al., 1973a; Elkin et al., 1978). Fuller et al. (1966) reported that dietary tannin levels as low as 0.64% to 0.83% can cause depression in chick performance. To retain the advantages of tannin in bird resistant sorghums and at the same time improve the nutritive value of the grain several detoxification techniques have been utilized including mechanical abrasion (Chibber et al., 1978), supplementation of methyl group donors like methionine or choline to high-tannin diets or addition of polyvinylpyrrolidone to the diet (Fuller et al., 1967; Armstrong et al., 1973) and chemical treatments (Price et al., 1978a, 1979b; Chavan, et al., 1979; Reichert et al., 1980; Muindi and Thomke, 1981). Reconstitution by anaerobic storage of moist grain has been reported to be one sufficient way to detoxify tannin (Lichtenwalner et al., 1979; Sarani et al., 1983).

This study was conducted to evaluate the impact of reconstituted and chemically treated commercial type brown sorghum grain (high-tannin) on tannin content and lysine bioavailability of the grain in broiler chick rations.

Materials and Methods

Experiment 1. The commercial type brown sorghum grain variety (high-tannin) was reconstituted by imbibing the grain in water to 70% dry matter. The grains were also treated with either 0.97N HCl or 0.97N NaOH. All three treated grains were stored in air-tight polyethylene bags and incubated three days at 32°C. Dry matter content of the chemically treated grains equalled the reconstituted grain. A constant number of moles of acid or base (0.02 mol/100g of sorghum) was used. Tannin catechin content was estimated utilizing a procedure by Price et al. (1978a).

Experiment 2. This experiment was conducted to evaluate the impact of reconstitution and chemical treatment of sorghum grains with subsequent three day incubation upon sorghum lysine availability. Arbor Acre X Lancet eight-day-old broiler chicks were randomly assigned to six treatment groups such that there were eight chicks per replicate and three replications per treatment. Methionine supplemented corn-based and sorghum-based rations (Table 1), formulated to be equally first limiting in lysine, constituted treatments 1 and 3, respectively. Substituting reconstituted sorghum grain for untreated sorghum grain formed treatment 4, and supplementing treatment 1 with L-lysine (0.4%) to validate the lysine deficiency formed treatment 2. Treatments 5 and 6 were as treatment 3 except untreated sorghum was substituted with HCl and NaOH treated sorghum, respectively.

Results and Discussion

Incubating reconstituted high-tannin brown sorghum (2.41% tannin) yielded only an approximate 7% reduction (to 2.25% tannin) in detectable

tannin content after a three day incubation (Table 2). However, treated reconstituted brown sorghum (2.25% tannin) with acid or base decreased detectable tannin content by 69 (to 0.75% tannin) and 90% (to 0.23% tannin), respectively, agreeing with Reichert et al. (1980).

Substituting high-tannin brown sorghum for corn grain in Expeiment 2 (Table 3) reduced broiler growth rate by 7%. That the corn ration was first limiting in lysine is indisputable as lysine supplementation increased growth rate by 10%. However, reducing sorghum grain tannin content 7, 69 and 90% through reconstitution, acid, and alkali treatments failed to elicit a favorable broiler growth response. Feed efficiency of birds fed sorghum grain, however was inversely related (r=0.83) with tannin content (Table 3). Lack of a significant tannin response with the sorghum treatments may have been due to several factors including lack of tannin toxicity, too low a lysine concentration to elicit a significant growth rate response, or lysine either failing to be the first limiting nutrient or the presence of a second limiting nutrient other than lysine of nearly equal deficiency. It is possible to speculate that methionine supplemented sorghum grain diets are first limiting in lysine, however, the second limiting amino acid(s), either tryptophane, threonine, or tyrosine, is/are nearly equal in deficiency to lysine.

TABLE 1. Rations Utilized in Experiment 2

Ingredients (%)	Corn-Based		Sorghum-Based			
	1	2	3	4	5	6
Corn, ground (8.9)[a]	72.30	72.30	-	-	-	-
Brown Sorghum (10.3)	-	-	72.30	-	-	-
Treated B. Sorghum (10.3)	-	-	-	72.30	72.30	72.30
Soybean Meal (44.0)	20.70	20.70	20.70	20.70	20.70	20.70
Dicalcium Phosphate	2.50	2.50	2.52	2.52	2.52	2.52
Calcium Carbonate	0.90	0.90	0.88	0.88	0.88	0.88
Sodium Bicarbonate	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin Premix[b]	0.40	0.40	0.40	0.40	0.40	0.40
Polyethylene	0.40	-	0.40	0.40	0.40	0.40
L-Lysine HCl	-	0.40	-	-	-	-
DL-Methionine	0.30	0.30	0.30	0.30	0.30	0.30
Sodium Chloride	0.20	0.20	0.20	0.20	0.20	0.20
Chromium	0.20	0.20	0.20	0.20	0.20	0.20
Trace Mineralized Sales	0.10	0.10	0.10	0.10	0.10	0.10
Protein (%)	15.50	15.50	16.50	16.50	16.50	16.50
Lysine (%)	0.78	1.2	0.78	0.78	0.78	0.78
ME (kcal/kg)	2942.00	2942.00	2898.00	2898.00	2898.00	2898.00

[a]Figures in parentheses denotes percent protein content.
[b]Roche Chemical Division Hoffman-LaRoche, Inc., Nutley, NJ 07410

TABLE 2. Brown Sorghum Tannin Concentration in Experiment 1

Treatments	Incubation Time Days	Tannin %
Brown Sorghum (HT)	3	2.41[a]
Reconstituted	3	2.25[a]
Acid-treated	3	0.75[b]
Base-treated	3	0.23[c]

[a],[b],[c]Significant difference ($P < 0.05$)

TABLE 3. Assayable Tannin, Lysine, Body Weight Gain, and Feed Efficiency for Experiment 2

Treatments	Tannin	Lysine	Nutritional Parameters: Body Weight Gain	Nutritional Parameters: Feed/ Gain
	%	%	(g)	(g)
Corn	0.00	0.78	126	1.63
Corn + Lysine	0.00	1.20	137	1.57
Brown Sorghum	2.41	0.78	118	1.72
B. Reconstituted[a]	2.25	0.78	114	1.69
HCl-treated[a]	0.75	0.78	114	1.63
NaOH-treated[a]	0.23	0.78	115	1.66

[a]Three day incubation time.

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CHAPTER III

SORGHUM RECONSTITUTION: METHODOLOGY AND APPLICATION TO DETOXIFY HIGH TANNIN SORGHUM GRAINS

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Abstract

Three in vivo and in vitro experiments were conducted to determine the appropriate methodology for and effects of reconstituting Darset, Redlan and one commercial hybrid brown sorghum grain variety upon broiler growth rate in threonine deficient and nutritionally complete poultry rations. The reconstitution procedure, which involves imbibing sorghum grain with water to 70% dry matter and subsequent anaerobic incubation at 32°C, removed up to 100% of the chemically detectable tannin. Rate of tannin elimination was dependent upon the variety of sorghum grain used with the commercial brown sorghum variety requiring approximately three days longer for tannin elimination than the Darset. Reconstituting high tannin sorghum grains improved ($p < .05$) growth rate and feed efficiency dramatically in threonine deficient crystalline amino acid fortified rations while reconstituting low tannin sorghum grain was without benefit. The desirable effects of reconstitution appear to be due to the

reduced grain tannin content. Including reconstituted sorghum grains in a practical type broiler ration failed to elicit a weight gain response though feed efficiency was improved by 13% over nontreated sorghum and 3% over corn grain. Lack of a gain response was likely due to excess protein included in the basal diet. Reconstitution offers an alternative method of reducing sorghum grain tannin content and is likely of greatest value in rations containing marginal indispensable amino acid and/or protein levels.

Introduction

Studies have demonstrated that weight gain and feed utilization are reduced when high-tannin sorghum grains are fed to broiler chicks (Chang and Fuller, 1964; Conner et al., 1969; Armstrong et al., 1973) and layers (Sell et al., 1983) in contrast to low-tannin sorghum grains. A portion of tannins toxic effects have been ascribed to reduce nitrogen digestibility and hence retention (Vohra et al., 1966). Rostagno (1973b) observed mean amino acid digestibilities of 73, 41 and 22% for low, intermediate and high-tannin sorghums, respectively.

Several techniques have been utilized to reduce tannin toxic effects. Mechanical abrasion reduced grain tannin content but also decreases yield (Chibber et al., 1978). Supplementing high-tannin sorghum diets with methyl group donors such as methionine and choline partially overcome tannic acid's deleterious effects (Chang and Fuller, 1964; Fuller et al., 1967; Rayudu et al., 1970a; Armstrong, 1973a) as methyl donors presumably aid in detoxifying absorbed tannin (Chang and Fuller, 1964; Fuller et al., 1966, 1967; Conner et al., 1969). Chemically treating sorghum grains with alkali destroys tannins (Price et

al., 1979b) and enhances its water extraction (Armstrong et al., 1974). Reconstituting sorghum grain by imbibing water to 75% dry matter followed by incubation at 25°C for two days has been observed to reduced chemically detectable tannin and elevate rat weight gain and feed efficiency ratios to that observed for low-tannin sorghum varieties (Reichert et al., 1980). Such a process, because of its low cost, may have application to reduce sorghum grain tannin content and enhance the feeding value of bird resistant sorghum to poultry.

The purpose of the study described herein was to evaluate the length of reconstituted sorghum grain incubation upon chemically detectable tannin content, impact of reconstitution and incubation upon threonine availability in sorghum grain in broiler rations.

Materials and Methods

In each of the following experiments sorghum grain was reconstituted by: (1) determining grain dry matter content; (2) adding water to bring grain dry matter to 78%; (3) mixing in a rotary mixer until water was absorbed; (4) storing reconstituted sorghum grain (RSG) in airtight polyethylene bags at 32°C; (5) drying at 55°C to restore grain to as-is dry matter content and (6) grinding grain through a 20 mesh screen. The experiments utilized Arbor Acre X Lancet broiler chicks and were conducted in a thermostatically controlled environment under continuous tungsten filament lighting. Birds were housed in electrically heated batteries with feed and water provided for ad libitum consumption. The amino acids content of the grains were determined using Beckman Amino Acid Analyzer.

Body weight gain, feed consumption and feed efficiency were tallied

at the end of the feeding period. All data were subjected to analysis of variance using the General Linear Model of the statistical analysis system (Barr et al., 1976).

Experiment 1. The impact of incubation time upon RSG tannin content was evaluated in three separate in vitro assays. In assay 1 reconstituted Darset grain (2.53% catechin eq./g) was incubated for 1, 2, 4, and 8 days post reconstitution; in assay 2 Darset, Redlan and commercial brown sorghum grain variety were reconstituted and incubated for 3 days and in assay 3 the three reconstituted grain types were incubated for 3, 6, and 12 days. Tannin content was estimated as catechin equivalents by the method of Price et al. (1978).

Experiment 2. Two high-tannin (Darset, Commercial brown sorghum) and one low-tannin (Redlan) sorghum grain varieties were used in nine treatments (Table 1) to determine the impact of reconstitution and subsequent 12 days incubation upon threonine bioavailability. The day 8 to 16 post-hatching period was utilized in this study with 3 replicates of 8 chicks per replicate used for each treatment. Rations (Table 1) were supplemented with crystalline amino acids (Table 2) such that each was first limiting in threonine. Amino acids were added (insufficient quantity) to bring all essential amino acids to 60% of the chicks requirement (NRC, 1977) for maximal growth except threonine which was approximately 45%. Supplemental threonine was added to treatments 3, 6, and 9 to evaluate threonine as the first limiting nutrient. Choline chloride was provided in each ration to meet the full choline requirement.

Experiment 3. The effects of Darset grain reconstitution and three days incubation in a practical ration were studied during day one to day

28 post hatching feeding trial utilizing 96 chicks. Birds were randomly assigned to 4 replicates of 3 treatment groups. A corn-soy basal ration (Table 3) formed the first treatment with Darset and reconstituted Darset grain incubated for three days substituted for corn (w/w) in treatements 2 and 3, respectively.

Results and Discussion

Incubating reconstituted Darset sorghum grain reduced ($p < .05$) detectable tannin content markedly by 3%/hr during the first day of incubation after which the decline slowed to .3%/hr (Figure 1). Based upo this assay an incubation period of three days appeared sufficient to reduce sorghum grain tannin content. However, in the second assay, incubating a second high-tannin commercial brown sorghum variety yielded only a 7% reduction in tannin content after three days incubation in sharp contrast to the Darset which once again was dramatically reduced (Table 4). The low tannin Redlan had no change in it's catechin equivalent content. This suggests that sorghum grain variety can have a profound impact upon either the benefits derived from reconstitution or the incubation period required to insure tannin elimination. To further evaluate reconstitution-incubation effects upon sorghum grain a third assay was conducted varying incubation to 12 days. Once again differences in the rate of tannin elimination by grain varieties were detected though by 12 days incubation, however, chemically detectable tannin was eliminated (Table 5).

In the second experiment rations were formulated to make threonine the first limiting nutrient in an eight day feeding study. Previous work in our laboratory utilizing lysine as the first limiting nutrient failed

to detect beneficial reconstitution effects upon lysine availability, presumably because sorghum grain lysine content is quite low thereby creating an insensitive nutrient for growth assays. Lysine binding by tannin during the reconstitution process, however, cannot be refuted. The sorghum grains utilized in this study contained sufficient threonine to meet 45% of this aminco acids requirement and was therefore utilized to ensure adequate sorghum concentration of the limiting nutrient. A preliminary experiement validated that the crystalline amino acid mixture used (Table 3) indeed made threonine the first limiting nutrient.

Reconstituting Darset and Brown sorghum grain followed by a 12 day incubation eliminated 100% of the chemically detectable tannin and increased ($p < .01$) body weight gain and feed efficiency (Table 6) for chicks fed rations utilizing threonine as the first limiting nutrient. The reconstitution process reduced both chemically detectable and biologically active tannin content. Supplementing untreated sorghum grains with threonine bringing its' ration content to 60% of the NRC requirement similarly elevated body weight gain ($p < .01$) and feed efficiency ($p < .05$) confirming that threonine was indeed the first limiting nutrient. The reconstitution process increased sorghum bioavailable, but not chemically detectable, threonine as affirmed by amino acid analysis. Reconstituting low-tannin sorghum numerically improved gain and feed efficiency by 5%.

Including untreated and reconstituted sorghum grain in a commercial type broiler ration (Table 7) failed to significantly enhance growth rate in the third experiement. However, feed efficiency of reconstituted grain was enhanced by 13% ($p < .1$) over untreated sorghum grain and by 3% over corn grain. The protein content of these rations exceeded the

broilers protein requirement and conceivably could have allowed a normal growth rate. Including excess dietary protein to eliminate the deleterious effects of tannin upon broiler growth would not be expected to eliminate the negative impact of sorghum tannin upon feed efficiency as the protein-tannin complex thus formed would be indigestible.

Reconstituting sorghum grain and subsequent anaerobic incubation provide an effective method to detoxify tannin in the sorghum grains evaluated. However, to fully utilize the technique in a commercial setting would likely necessitate feeding higher moisture rations to poultry unless economical methods are available to dry reconstituted grain.

TABLE 1. Formulas and analysis of the diets (Experiment 2)

Ingrediments (%)	Darset (11.66)[a]			B.Sorghum (10.32)[a]			Redlan (13.00)[a]		
	1	2	3	4	5	6	7	8	9
Sorghum Grain	90.00	90.00	90.00	90.00	90.00	90.00	90.00	90.00	90.00
Amino Acid Mix	3.96	3.96	3.96	5.11	5.11	5.11	2.81	2.81	2.81
Mineral Mix	5.71	5.71	5.71	5.71	5.71	5.71	5.71	5.71	5.71
Vitamin Premix[b]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Corn Starch	0.13	0.13	----	0.17	0.17	----	0.10	0.10	----
Threonine	----	----	0.13	----	----	0.17	----	----	0.10
Protein (%)	13.80	13.80	13.80	13.80	13.80	13.80	13.80	13.80	13.80
Threonine (%)	0.34	0.34	0.47	0.32	0.32	0.49	0.33	0.33	0.43
ME (Kcal/Kg)	3033.00	3033.00	3033.00	3033.00	3033.00	3033.00	3033.00	3033.00	3033.00

[a]Figures in parentheses denote percent protein content
[b]Included choline chloride to meet 100% of the chicks requirement for maximal growth

TABLE 2. Crystalline amino acid mixture (Experiment 2)

Amino Acids (%)	Darset	Brown	Redlan
L-glutamine	2.80	3.80	1.80
L-lysine HCl	0.47	0.49	0.43
L-arginine HCl	0.42	0.46	0.36
Dl-methionine	0.16	0.17	0.15
L-cysteine	0.09	0.11	0.06
L-tryptophan	0.01	0.04	0.01
L-glycine	----	0.02	----
L-serine	----	0.01	----

TABLE 3. Formulas and Analysis of the Diets (Experiment 3)

Ingredients (%)	Corn	High-tannin Sorghum	Reconstituted Sorghum
Corn, ground (8.9)[a]	53.15	-----	-----
Darset (11.66)[a]	-----	53.15	53.15
Soybean meal (44)[a]	35.95	35.95	35.95
Meat + bone meal (55)[a]	5.00	5.00	5.00
Alfalfa meal (17)[a]	3.00	3.00	3.00
Dicalcium phosphate	1.00	1.00	1.00
Calcium carbonate	0.90	0.90	0.90
Vitamin premix	0.40	0.40	0.40
Sodium chloride	0.30	0.30	0.30
Dl-methionine	0.10	0.10	0.10
Trace minerals	0.10	0.10	0.10
Chromic oxide	0.10	0.10	0.10
Protein	24.00	25.00	25.00
ME (Kcal/kg)	2764.00	2732.00	2730.00

[a]Figures in parentheses denote percent protein content

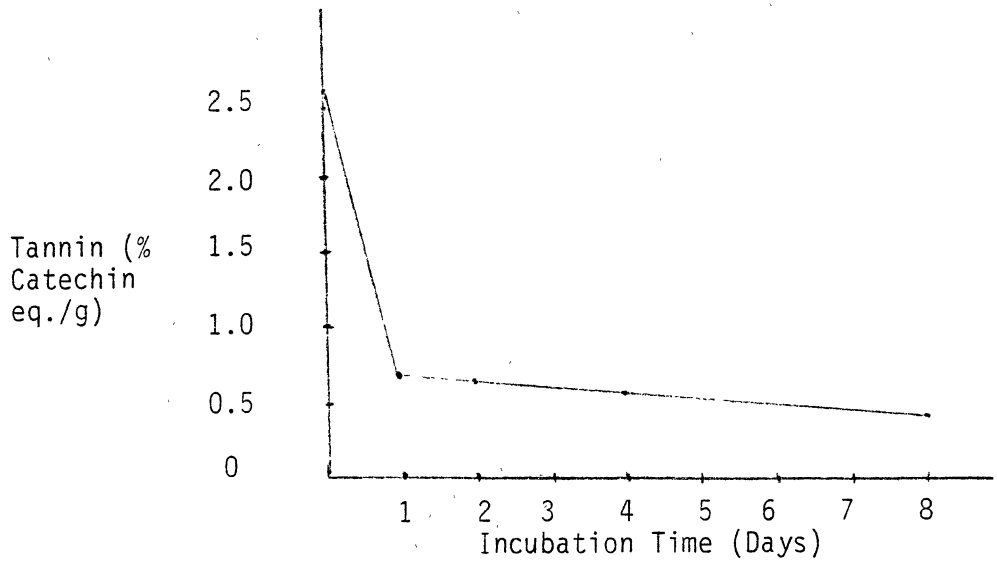

Figure 1. The relationship between Darset sorghum grain tannin concentration and incubation time in assay 1 of experiment 1.

TABLE 4. Darset, Redlan and commercial brown sorghum grain tannin concentration in Assay 2 of Experiment 1

Sample	Incubation time (days)	Tannin (Cat. eq.) g
Darset	0	2.53
Reconstituted Darset	3	0.69
Brown Sorghum	0	2.41
Reconstituted Brown Soybean	3	2.25
Redlan	0	0
Reconstituted Redlan	3	0

TABLE 5. Darset, Redlan and Commercial Brown Sorghum Grain Tannin Concentration in Assay 3 of Experiment 1

Treatments	Tannin (Catechin eq./g) Incubation Time (Days) 0	3	6	12
Darset	2.53[a]	----	----	----
D. Reconstituted	2.53[a]	0.69[c]	0.50[d]	0.00
Brown	2.41[a]	----	----	----
B. Reconstituted	2.41[a]	2.25[a]	1.14[b]	0.00
Redlan	0.00	----	----	----
R. Reconstituted	0.00	0.00	0.00	0.00

[a,b,c,d]Significant difference ($P < 0.05$)

TABLE 6. Assayable tannin and threonine and body weight gain and feed efficiency for Experiment 2.

Treatments	Tannin	Threonine[a]	Weight Gain/Chick	Feed/ Gain
	Cat. eq./g	%	(g)	
Darset	2.53	0.34	218[f]	4.4[a]
D. Reconstituted[b]	0.00	0.34	415[d]	2.7[cd]
Darset + Threonine	2.53	0.47	584[c]	2.5[d]
Brown	2.41	0.32	265[f]	4.1[b]
B. Reconstituted[b]	0.00	0.32	347[e]	3.0[c]
Brown + Threonine	2.41	0.49	641[b]	2.4[d]
Redlan	0.00	0.33	413[d]	3.0[c]
R. Reconstituted[b]	0.00	0.33	436[d]	2.7[cd]
Redland + Threonine	0.00	0.43	851[a]	2.1[e]

[a]Threonine determination made utilizing a Beckman amino acid analyzer.

[b]Incubation for 12 days at 32°C.

[c,d,e,f]Statistically significant at ($P < 0.05$) or ($P < 0.01$)

TABLE 7. Assayable tannin, body weight gain, and feed efficiency ratios for Experiment 3.

Treatments	Tannin (Cat. eq/g)	Weight Gain (g)	Gain/ Feed
Corn	0	892	.61
Darset	2.53	891	.56
Reconstituted Darset[a]	.63	895	.63

[a]Reconstituted and incubated 3 days at 32°C.

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CHAPTER IV

SUMMARY

Four in vitro and in vivo experiments were conducted to determine an appropriate methodology for sorghum tannin detoxification, and to evaluate the impact of detoxificated sorghum strains upon broiler chick productivity in rations first limiting in lysine, threonine, or nutritionally complete rations. The sorghum grains used in these experiments were two high tannin varieties (Darset and commercial hybrid brown) and one low tannin variety (Redlan). To detoxify the harmful effects of tannin a simple and inexpensive reconstitution procedure was used. This involved imbibing sorghum grain with water to 70% dry matter with subsequent anaerobic incubation at 32°C for different lengths of time. Other inexpensive chemicals, such as HCl (0.97N) and NaOH (0.97N) were also added up to the same moisture content for further determination of tannin detoxification and broiler chick productivity.

Experiment I

Three in vitro assays were conducted to determine the impact of incubation time upon reconstituted sorghum grain tannin content. In the fourth in vitro assay the HCl (0.97N) and NaOH (0.97N) were used. Incubating reconstituted Darset sorghum grain reduced ($P < .05$) detectable tannin content markedly to 85% by the eighth day of incubation. Based upon this assay an incubation of three days appeared sufficient to reduce

sorghum grain tannin content significantly. In sharp contrast to Darset, the reconstituted high tannin commercial brown variety showed a 7% reduction in tannin content after three days. Low tannin Redlan had only trace amounts of tannin undetectable by the vanillin-HCl assay, therefore, a zero value was used to express tannin content. These findings suggest that sorghum grain variety can have a profound impact upon either the benefits derived from reconstitution or the incubation period required to insure tannin elimination.

The third assay was conducted to further evaluate reconstitution-incubation effects on sorghum grains. A 12 day incubation showed complete elimination ($P < .01$) of detectable tannin in both Darset and commercial brown high tannin varieties.

In a fourth assay, addition of inexpensive acid (0.97N HCl) and alkali (0.97N NaOH) to commercial brown sorghum potentiated the tannin reduction ($P < .01$) by 69% and 90%, respectively, after a three day incubation. Rate of tannin elimination was dependent upon the variety of sorghum grain used with the commercial brown sorghum variety requiring approximately three days longer for tannin elimination than with Darset.

Experiment II

Three days incubated reconstituted and acid or base treated commercial brown sorghum was used to evaluate the effect of treated sorghum upon sorghum lysine bioavailability in broiler chick ration. That the corn-soy ration was first limiting in lysine is indisputable as lysine supplementation increased growth rate by 10%. Substituting the commercial brown sorghum with corn reduced broiler body weight gain by 7%. However, reconstitution, acid, and alkali treatments failed to

enhance a favorable growth response. Feed efficiency of birds fed commercial brown sorghum grain was inversely related to body weight gain compared with treated sorghum grain. Lack of a significant tannin response with the sorghum treatments may have been due to several factors including lack of tannin toxicity which relates to variety, too low a lysine concentration to elicit a significant growth rate response, or lysine either failing to be the first limiting nutrient or the presence of a second limiting nutrient other than lysine of nearly equal deficiency. Lysine binding by tannin during the reconstitution process, however, cannot be refuted.

Experiment III

This experiment was conducted to evaluate the impact of reconstituted grains upon broiler chick growth rate when the rations were formulated to make threonine rather than lysine the first limiting nutrient. The sorghum grains utilized in this study contained sufficient threonine to meet 45% of this amino acid's requirements. An appropriate crystalline amino acid mixture, with threonine as the first limiting nutrient, was validated in preliminary studies in our laboratory (unpublished data).

The reconstituted high tannin Darset and commercial brown sorghum grains improved ($P < .05$) body weight gain and feed efficiency by approximately 48, 23, 39, and 27%, respectively. However, the reconstituted low tannin Redlan numerically improved weight gain and feed efficiency by only 5%. Supplementing untreated sorghum grains with threonine, bringing its' ration content to 60% of the NRC requirement, similarly elevated body weight gain ($P < .01$) and feed efficiency ($P < .05$).

Experiment IV

A practical ration experiment was designed to evaluate the nutritional value of high tannin Darset sorghum and reconstituted Darset sorghum verses corn in a broiler chick growth study. Including untreated and reconstituted sorghum grain in a commercial type corn-soy broiler ration failed to elicit the growth rate significantly. Feed efficiency of reconstituted grain, however, was enhanced by 13% over untreated sorghum and by 30% over corn.

A calculated protein content in sorghum grain rations exceeded the broiler protein requirement by approximately 10% for the first two weeks and by approximately 20% for the next two weeks.

Including excess dietary protein might eliminate the harmful effects of Darset sorghum tannin content upon broiler productivity.

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Thesis: SORGHUM RECONSTITUTION: METHODOLOGY STUDY OF SORGHUM TANNIN DETOXIFICATION AND IMPACT OF DETOXIFICATION UPON SORGHUM AMINO ACID BIOAVAILABILITY AND BROILER CHICK PRODUCTIVITY

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