VARIATION IN STARCH AND PROTEIN CHARACTERISTICS OF GRAIN SORGHUM AS INFLUENCED BY VARIETY AND THEIR RELATIONSHIP TO DIGESTIBILITY CHARACTERISTICS

by

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PREFACE

The data reported herein represent the fruits of over four years of experimentation in conjunction with this project. The first segment of this endeavor was directed and performed by Mr. Rod Schemm. The following work was performed by Mr. Schemm:

- IVDMD and IVGP of finely, ground and reconstituted grains in Years 1 and 2,
- IVDMD, IVGP and α-amylase digestion of purified starch in Years 1 and 2,
- Wet-milling composition of grains in Years 1 and 2.

Under Mr. Schemm's leadership, many of the experimental procedures utilized in this study were developed and implemented. In addition, valuable insight into the nature and possible solutions to this problem were either set forth or stimulated by Mr. Schemm.

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CHAPTER I

INTRODUCTION

During recent years, environmental and economic conditions have provided an opportunity for increased grain sorghum production in the Southern Great Plains. Although grain sorghum has been considered by many as a suitable concentrate feed, some cattlemen are reluctant to utilize this grain. Major problems associated with grain sorghum are highly variable quality and lower feeding value in relation to corn. The variable quality of grain sorghum may be largely attributed to the wide adaptability of this grain. Characteristics such as bird, lodging, drought and insect resistance have received major emphasis in sorghum breeding programs.

Grain processing has been used in an attempt to improve sorghum grain feeding value. It is generally recognized that grain sorghum must be processed in some manner to maintain an acceptable level of digestibility. Processing methods such as steam flaking or reconstitution have been shown to measurably improve the nutritional quality of sorghum. Moreover, some recent studies have shown certain varieties of grain sorghum to be nutritionally superior to others. The development of highly digestible sorghum varieties should provide an invaluable benefit to the nutritive value of sorghum grain.

The nutrient composition of grain sorghum varies widely across varieties commonly grown but is somewhat similar to corn. Differences

in protein, starch and tannin content between varieties may be large but alone cannot account for the wide ranges noted in digestibility. In addition to variation in chemical content, factors such as the strength or degree of structural and chemical interrelationships between seed constituents may play a role in altering the digestibility of grain sorghum. Furthermore, the effect of grain processing on nutrient composition or structural interrelationships remain to be clearly defined.

Subsequently, the objectives of this study were to 1) determine the relative nutritive value of widely different genetic varieties of grain sorghum and corn, 2) determine the starch and protein characteristics of sorghum as influenced by variety or endosperm type, 3) clarify the role of starch and protein in sorghum varieties which differ widely in digestibility, 4) determine the response to processing of different varieties of grain sorghum and corn.

CHAPTER II

LITERATURE REVIEW

Grain sorghum has been shown to be a valuable substitute for corn in rations for ruminants in many phases of production. The variable quality and apparent lower feeding value of sorghum in relation to corn, however, have caused cattlemen to discriminate against this feedstuff. Special grain processing, however, has been shown to be useful in improving the feeding value of sorghum.

The variability associated with grain sorghum may be attributed in part to the wide adaptability in agronomic traits which have been carefully bred into this species. Logically, the chemical factors within the kernel that contribute valuable agronomic characteristics may also have an effect on the nutritional quality of grain sorghum. In addition, certain varieties of grain sorghum have been shown to be highly digestible and more similar to corn in nutritional acceptability. Consequently, the development of sorghum varieties with high nutritional quality and acceptable agronomic characteristics would appear possible. In order to accomplish this task, a better understanding of the chemical and physical interrelationships affecting various agronomic and nutritional characteristics must be developed. Previous reports have shown strong interrelationships between various seed components (starch, protein, tannin) and digestibility or nutritional quality. These reports provide a basis from which to develop a study of several varie-

ties of grain sorghum and the factors that affect their nutritive value.

Kernel Structure and Composition

Recent developments in the area of grain sorghum endosperm composition and structure warrant a brief discussion of this topic. The endosperm of the sorghum kernel can be divided into three somewhat distinct layers; subaleurone, peripheral endosperm and floury endosperm. The subaleurone layer consists primarily of protein with little or no starch. The thickness of the subaleurone layer may be a variety characteristic directly related to total protein content (Seckinger and Wolf, 1973). Microscopic evaluation suggests that this layer is primarily composed of large protein bodies and small protein bodies (sometimes called spherosomes) which are surrounded by a dense proteinaceous matrix. In this area, the protein matrix is still fairly dense and tight compaction of starch granules and protein bodies within the structural protein is evident. The floury endosperm is the innermost portion of the endosperm and is composed of large starch granules surrounded by a small number of protein bodies and little or no structural protein. The amount of structural protein in the floury endosperm is probably a major factor associated with endosperm hardness in grain sorghum.

The major component of the sorghum kernel is starch which may comprise 70 to 80 percent of the grain (Greenwood, 1970). Sorghum starch is typical of most cereal starches in that it commonly contains approximately 75 percent amylopectin and 25 percent amylose. However, high amylopectin (waxy) sorghums have been discovered whose starch is nearly 100 percent amylopectin. Sorghum starch granules are generally

spherical in shape and may be dimpled if originating from the peripheral endosperm where compaction of the spherical protein bodies during final maturational stages gives the starch a "golf ball" appearance.

The second major component of the sorghum kernel is protein which may account for 8 to 14 percent of the grain. The protein content of the grain may be dependent on variety or level of nitrogen fertilization among other factors. The protein of grain sorghum can be solubilized and categorized in the classical manner; albumins, globulins, prolamines and glutelins. Albumins and globulins are usually enzymatic proteins, prolamines are storage proteins and glutelins are generally very large and perform a structure function in the kernel. The three protein structures of the grain sorghum endosperm can be classified by this scheme. The large protein bodies are alcohol soluble and classified as prolamines (kafirin) whereas the structural protein making up the protein matrix contains the glutelin fraction (Seckinger and Wolf, 1973). The small protein bodies or spherosomes are soluble in saline and may be classified as albumins or globulins. Apparently, the spherosomes are a major storage site for various enzymes and minerals found in the endosperm (Adams and Novellie, 1975).

The Landry and Moureaux Fractionation Sequence D (1970) has been successfully used to separate the proteinaceous components of grain sorghum and corn (Guiragossian et al., 1978; Jambunathan and Mertz, 1973; Misra et al., 1975; Walker and Lichtenwalner, 1977). This method provides a distinct improvement over the antiquated Osborne (1914) technique because more of the total protein (90-95 percent) can be solubilized and quantified using the Landry and Moureaux Technique. In addition, sodium dodecyl sulfate polyacrylamide gel electrophoresis

has been used to validate the selectivity of this technique (Guiragossian et al., 1978; Misra et al., 1976b).

The nutritional quality of the various Landry-Moureaux fractions has been evaluated through amino acid analysis. The lysine content of the albumin and globulin fraction was approximately 4.2 g/100 g protein. Lysine content of the glutelin fractions was 2.2 g/100 g protein whereas the lysine content of the prolamine fraction was only 0.2 g/100 g protein (Guiragossian et al., 1978). It is evident that changing the protein composition of grain sorghum could drastically alter protein quality.

Recent evidence has suggested that digestibility as well as quality of the Landry-Moureaux fractions may be significantly different. Approximate ruminal digestibility of Landry-Moureaux protein fractions was determined by fractionating the sorghum protein both before and after a 24 hour nylon bag incubation. The albumin and globulin fraction was approximately 70 percent digested whereas the prolamine and glutelin fractions showed only about 50 percent digestion after 24 hours (Walker and Lichtenwalner, 1977). Earlier work has shown that the total tract digestibility of corn prolamine (zein) approached only 40 percent (Ely et al., 1967). These studies suggest that any factor, such as variety or processing, which might alter the protein composition of grain sorghum may also affect total protein digestibility. Furthermore, the close structural relationship of protein and starch in the sorghum kernel suggests that alterations in protein composition may also affect starch availability.

The final component of grain sorghum that merits attention is the tannin or polyphenol content. Tannin content of certain bird resistant

sorghums may range as high as 0.2 percent. The structural role of tannins has not been thoroughly elucidated, however, major tannin concentrations have been observed in the subaleurone and peripheral endosperm areas and appears to be primarily associated with the large protein bodies (Chibber et al., 1978; Guiragossian et al., 1978). The tannin-prolamine relationship may decrease the utilization of the prolamine proteins thereby decreasing the nutritive value of the high tannin varieties.

Processing Effects

Numerous factors affect the nutritional quality of grain sorghum. It is commonly accepted that grain sorghum must be processed in some manner prior to feeding in order to achieve efficient utilization of this grain. Consequently, grain processing has been used for many years as a method to enhance the nutritional quality of grain sorghum.

Several processing methods have been developed in recent years in an effort to improve the feeding value of grain sorghum. Initially, grinding or dry rolling were used to increase utilization over whole grain (Totusek and White, 1969). The development of heat and high moisture processing techniques has further enhanced the nutritive value of grain sorghum to levels that are occasionally similar to corn. Steam flaking has been shown to increase grain sorghum utilization over grinding or dry rolling (Garrett et al., 1968; Hale et al., 1966; Hinman et al., 1973; Totusek et al., 1967). Dry heat treatment via the micronization technique has shown increased starch availability (Hinders and Freeman, 1969; Hinman, 1973) and feeding value (Croka and Wagner, 1975) of grain sorghum. High moisture treatments in the form of high

moisture harvesting or reconstitution have also been shown to increase the nutritive value of grain sorghum. Although the response observed for high moisture processed grain sorghum has been somewhat variable, significant increases in feed efficiency have been reported (McGinty et al., 1967; Riggs and McGinty, 1970; Wagner et al., 1971; White and Totusek, 1969).

Increased nutritive value due to processing must be related to chemical or physical changes in the individual seed components. The effect of heat treatment on starch is well documented. When starch granules are heated, they undergo reversible swelling until a critical temperature is obtained. At this temperature, the native structure of the starch granule is ruptured and birefringence is lost. Gelatinization temperatures vary between corn and sorghum and may vary within sorghum varieties as evidenced by differential swelling characteristics for waxy and nonwaxy sorghum starches (Leach, 1965).

The beneficial effect of heat processing on starch digestion and utilization has been observed using in vitro and in vivo procedures. Thirty minute enzymatic digestion with pancreatin showed a 100 percent increase in starch digestion for steam flaked over dry, finely ground grain sorghum (Osman et al., 1970). Highly gelatinized steam flaked starch produced almost four times as much glucose as dry ground or reconstituted starch in an enzymatic starch digestion utilizing amyloglucosidase. In contrast, highly gelatinized micronized starch produced an intermediate amount of glucose (McNeill et al., 1975). These observations led to the suggestion that degree of gelatinization was not the only factor affecting susceptibility of starch to enzyme action.

Evidence for starch granule alteration in high moisture harvested

or reconstituted sorghum grain is limited. Theoretically, starch in high moisture harvested sorghum grain may not have completed its maturational process during the final stages of kernel dehydration thereby leaving the starch granule more susceptible to enzyme attack. Starch granules in reconstituted grain may also be in a very susceptible state due, however, to a different mechanism. The release of gibberellins and subsequent activation of amylolytic enzymes during the early stages of the germinization process (Luchsinger, 1966) may render the starch granules more susceptible to ruminal microbial attack. In addition, fermentative action may also play a role in determining the availability of starch from reconstituted grain.

In digestion trials utilizing processed grain sorghum, total starch digestibility has been improved ($P < .05$) by steam flaking and reconstitution relative to dry ground or micronized sorghum. In addition, ruminal starch digestion was greatest for the steam flaked and reconstituted grains indicating increased starch availability or accessibility for these treatments (McNeill et al., 1971). In contrast, site of digestion studies have shown no difference in ruminal starch digestion for dry ground, dry rolled, micronized and steam flaked sorghum. The dry rolled sorghum showed decreased ($P < .05$) intestinal starch digestion and therefore lower total tract digestion relative to the other treatments (Hinman and Johnson, 1974).

The effect of heat treatment on the protein composition or structure of cereal grains has not been clearly elucidated. Theoretically, chemical or physical changes such as coagulation and/or denaturation of cereal grain protein through heat treatment would be expected to alter the digestion or utilization of these proteins. Potter et al. (1971)

showed that total tract protein digestibility of processed grains was not different for steam flaked, micronized or dry ground sorghums. Ruminal protein breakdown, however, was 62.16 percent for steam flaked, 51.28 percent for dry ground and only 36.11 percent for micronized sorghum. Consequently, although total protein digestibility may not be altered by processing, site of digestion and subsequent utilization can be altered drastically.

Developmental changes in protein structure have been observed microscopically for high moisture harvested corn. Khoo and Wolf (1970) observed that kernel development required at least 50 days before the protein components appeared to be in their final maturational state. In addition, the Landry-Moureaux protein composition of developing corn kernels continues to change as late as 49 days post-pollination (Misra et al., 1975). Although studies of this type have not been performed for grain sorghum, similar patterns would be expected due to the close relationship of grain sorghum and corn.

Disruption of the proteinaceous matrix surrounding the endosperm of grain sorghum has been suggested as part of the mechanism of the reconstitution process. Microscopic evaluation of reconstituted sorghum kernels has illustrated disruption or solubilization of the protein matrix thereby allowing greater starch accessibility (Sullins et al., 1971). Furthermore, Landry-Moureaux protein fractionation has shown a higher concentration of water and salt soluble protein in reconstituted than in untreated grain sorghum (Walker and Lichtenwalner, 1977).

Varietal Effects

One of the major problems confronting sorghum proponents is the

wide variability associated with this grain. Some of this variability may be due to factors such as location or conditions during the growing season. Substantial evidence suggests that endosperm type or, perhaps more precisely, variety is a major factor affecting sorghum variability. Large differences in nutritional characteristics have been observed for waxy, bird resistant and normal varieties.

Modified ruminal nylon bag studies (Walker and Lichtenwalner, 1977) have shown 24-hour digestibilities of waxy and hetero-yellow sorghums to be similar and superior to a normal variety. Saba et al. (1972) illustrated that the in vitro gas production and dry matter disappearance (DMD) of a normal sorghum was almost twice as high as that of a bird resistant sorghum. Twenty two different varieties of grain sorghum showed nylon bag digestibilities ranging from 56.2 to 80.6 percent (Miller et al., 1972). Ten varieties of grain sorghum produced average daily gains in laboratory rats ranging from 0.47 to 1.27 grams per day for a 28 day feeding period (Breuer and Dohm, 1972). Furthermore, Jambunathan and Mertz (1973) showed that low tannin sorghum varieties supported weight gains in rats averaging about 1 gram per day whereas high tannin varieties produced weight gains of only 0.2 grams per day.

In a sheep metabolism trial, the organic matter digestibility of a waxy and white sorghum was similar but was significantly ($P < .05$) decreased for the regular sorghum. Crude protein and NFE digestibilities were not different for the three sorghums but trends favored the waxy and white types (Nishimuta et al., 1969). Net energy values of 2.40, 1.98, 1.78 Mcal per kilogram for maintenance and 1.49, 1.15 and 0.73 Mcal per kilogram for gain have been obtained for corn, normal and bird resistant sorghum, respectively (Maxson et al., 1973). In addi-

tion, net energy values of 1.43 and 1.50 Mcal per kilogram for maintenance and 0.95 and 1.24 Mcal per kilogram for gain have been developed for regular and waxy sorghum, respectively (Sherrod et al., 1969). In feeding trials, average daily gains ranged from 1.76 to 2.23 pounds per day and feed efficiencies from 6.89 to 9.16 pounds of feed per pound of gain for eight grain sorghum hybrids. The poorest performance (ADG and F/G) was observed for a bird resistant hybrid and the highest ADG was observed with a waxy hybrid (McCollough et al., 1972b).

Varietal effects on the nutritive value of grain sorghum must be manifested in factors relating to the starch, protein or tannin content or composition and the interrelationships of these factors in the kernel. Studies comparing the starch characteristics of different grain sorghum varieties have been limited. In gas production studies on purified starch carrying incremental doses of the waxy gene, Lichtenwalner et al. (1978) observed a 28 percent increase in gas production from the nonwaxy (Wx Wx Wx) starch to the waxy (wx wx wx) starch. In addition, in vitro starch hydrolysis (glucoamylase enzyme) showed the waxy starch produced 39 percent more glucose than the nonwaxy starch. Gas production studies utilizing baker's yeast and amyloglucosidase enzyme showed a 35 percent advantage for a waxy sorghum over the average of three normal types. In addition, gas production studies on the purified starch from each variety suggested a 25 percent advantage for the waxy starch over the normal starches (Sullins and Rooney, 1974).

Changes in protein composition between varieties has been suggested as part of the explanation for differing nutritional quality across sorghum types. The Landry-Moureaux protein composition of three normal and three bird resistant sorghums generally indicated that the bird

resistant sorghums contained lower concentrations of albumins, globulins and prolamines and a higher concentration of glutelins than the normal sorghums (Jambunathan and Mertz, 1973). Landry-Moureaux protein fractionation has also shown that a hetero-yellow sorghum contained slightly higher concentrations of albumins and globulins and slightly lower concentrations of prolamines than a waxy or normal sorghum (Walker and Lichtenwalner, 1977). In another study utilizing Kafir and Redlan varieties, Lichtenwalner et al. (1978) observed that the concentration of albumins and globulins increased as incremental doses of the waxy gene were added. This increase in saline soluble protein concentration was followed by a concomittant increase in total soluble protein as the protein concentration in the other fractions remained relatively constant.

The Landry-Moureaux protein composition of a high and low tannin sorghum showed major differences in the glutelin fraction where the high tannin variety contained 80 percent in contrast to the low tannin variety with only 40 percent glutelin (Chibber et al., 1978). In addition, protein fractionation of four sorghum varieties (normal, high lysine-2, and high tannin) illustrated that the normal and high tannin sorghums contained approximately 8 percent albumins and globulins in contrast to the high lysine varieties which averaged almost 25 percent. The increase in ablumin and globulin concentration for the high lysine varieties was followed by a decrease in prolamine concentration. Glutelin concentration for the high tannin variety reached almost 65 percent as compared to approximately 40 percent for the normal and high lysine types. Polyacrylamide gel electrophoresis patterns revealed similar molecular weight profiles for all varieties within each fraction except

for the high tannin variety which showed a major band in the glutelin fraction that was not present for the other varieties (Guiragossian et al., 1978).

Several seed components other than starch and protein probably affect the nutritive value of grain sorghum. Tannin or polyphenol content has been suggested as a partial explanation for the decreased performance of bird resistant varieties. For example, a significant decrease in gas production from washed rumen microorganisms was observed when pericarp from a bird resistant sorghum was incubated with endosperm from a normal sorghum (Saba et al., 1972). It has been suggested, however, that tannin content alone is not the primary factor depressing performance of bird resistant varieties but that an interaction of tannin with certain protein moieties may be responsible (Chibber et al., 1978; Guiragossian et al., 1978). Theoretically, the protein-tannin interaction may decrease the biological value of the protein and thereby decrease the nutritive value of high tannin varieties.

Varietal Effects on Grain Processing

Due to a wide range in digestibility for different varieties of grain sorghum, differential responses to grain processing might be expected. Walker and Lichtenwalner (1977) observed a differential response to reconstitution for three varieties of grain sorghum. Small positive responses were noted for hetero-yellow and Redlan waxy varieties; whereas, a Redlan nonwaxy variety showed a significant decrease in nylon bag digestibility after both 24 and 48 hours of incubation. Saba et al. (1972) observed that *in vitro* dry matter disappearance values

almost doubled for a normal over a bird resistant sorghum when the grains were finely ground. After steam flaking, however, the normal variety showed no response whereas the bird resistant variety was elevated to a level similar to the normal variety. These limited studies suggest that different varieties or types of grain sorghum respond differently to grain processing. The implication of this theory is that sorghum varieties of high nutritive value might be adequately processed by energy and cost efficient methods such as grinding or dry rolling whereas poorly digestible sorghum types may require more intensive expensive processing in the form of reconstitution or steam flaking.

CHAPTER III

THE INFLUENCE OF VARIETY ON NUTRITIVE CHARACTERISTICS OF GRAIN SORGHUM AND CORN

Summary

Nine varieties of grain sorghum differing widely in nutritive and agronomic characteristics and four varieties of corn, most grown in three consecutive crop years, were evaluated for chemical composition, in vitro dry matter disappearance (IVDMD) and in vitro gas production (IVGP). Crude protein content ranged from 11.14 to 16.46 percent and starch ranged from 61.9 to 83.0 percent for the sorghum varieties. Bird resistant (BR) sorghum (brown seed coat) generally contained elevated tannin levels. The corn varieties showed higher IVDMD than the sorghums in Years 1 and 2, although differences were not large except for the bird resistants. The Waxy sorghums were generally similar in IVDMD to the Normal varieties in all three years; however, both were intermediate between the corn and bird resistant types. The Floury-BR Soft Endo performed somewhat comparably to the Waxy types in Years 1 and 2 but showed a depressed ($P < .05$) IVDMD response in Year 3. In Year 2, the Waxy-BR 1133 was similar to the poorly digestible Darset but proved to be more intermediate between the Waxy and bird resistant types in Year 3. Increased digestibility (IVDMD) was generally explained by increased

starch availability (IVGP). This was true for most types except the Floury-BR Soft Endo where the soft endosperm evidently increased IVGP, however, IVDMD was not elevated to the same relative extent, perhaps due to tannin content. These studies show that some varieties of grain sorghum are much more similar to corn in feeding value than other types, especially bird resistants. Furthermore, the wide range in digestibility (IVDMD) observed for these sorghums suggests that the wide variation in feeding value generally considered for grain sorghum may be primarily due to variety or type.

Introduction

Grain sorghum has traditionally been discriminated against due to highly variable quality and an apparent lower feeding value in relation to corn. A portion of the variability may possibly be attributed to environmental conditions during growth and maturation of the kernel. Variety or endosperm type, however, may be a more important factor affecting the variable digestibility of grain sorghum.

The potential of certain sorghum varieties has been demonstrated in digestibility trials where a sorghum variety with the floury endosperm characteristic had a digestibility higher than that of ground corn (Samford et al., 1970). In addition, McCollough and Brent (1972c) observed that the digestibility of three sorghum hybrids was similar ($P < .05$) to three corn hybrids in digestion trials with steers. Varietal differences within sorghum types have been illustrated with a nylon bag procedure which showed digestibilities ranging from 56.2 to 80.6 percent for 21 different sorghum varieties (Miller et al., 1972). Furthermore, McGinty and Riggs (1968) observed that *in vivo* digestion

coefficients for seven different sorghum varieties ranged from 50.01 to 71.58 percent.

Previous studies in this area have suggested large differences in digestibility for different varieties of grain sorghum. However, description and classification of varieties is generally not sufficiently adequate to allow characterization of the feeding value for many different sorghum types. In addition, *in vitro* dry matter digestibility data describing the relative nutritive value of sorghums differing widely in nutritive and agronomic characteristics has not been published. Therefore, the objective of this study was to evaluate the nutritive characteristics of several grain sorghum varieties differing in seed coat color (bird resistance), endosperm color, endosperm hardness and amylopectin content (waxiness) with respect to nutrient composition (protein, starch and tannin content), *in vitro* digestibility and starch availability (measured as gas produced from incubation with amyloglucosidase and baker's yeast).

Materials and Methods

Nine varieties of grain sorghum differing widely in seed characteristics were grown and harvested under similar dryland conditions at the Perkins Oklahoma Agronomy Research Station. Since corn cannot be produced under dryland conditions at this location, four varieties of hybrid field corn were grown and harvested under similar irrigated conditions at the Panhandle Agronomy Research Station located at Goodwell, Oklahoma. Sorghums were grown and harvested for three consecutive crop years but only the Dwarf Redlan, Redlan, Soft Endo and Darset varieties were grown in all three years (Table I). Two different

TABLE I

DESCRIPTIVE CHARACTERISTICS OF GRAIN SORGHUMS AND CORN

	Year	Seed Coat Color	Endosperm			
			Color	Hardness	Waxy or Normal	Classification[b]
Dwarf Redlan[a]	1,2,3	red	white	intermediate	waxy	Waxy
1122	2,3	red	yellow	intermediate	waxy	Waxy
1126	2,3	white	yellow	intermediate	waxy	Waxy
1133	2,3	brown	yellow	intermediate	waxy	Waxy-BR
Redlan[a]	1,2,3	red	white	intermediate	normal	Normal
OK 612	1,2	red	hetero-yellow	intermediate	normal	Normal
Soft Endo[a]	1,2,3	brown	white	soft	normal	Floury-BR
ROKY 78	3	brown	yellow	intermediate	normal	Normal-BR
Darset [a]	1,2,3	brown	white	intermediate	normal	Normal-BR
Pioneer 3149	1	colorless	yellow	intermediate	normal	corn
Pioneer 3306	1	colorless	yellow	intermediate	normal	corn
Pioneer	2	colorless	yellow	intermediate	normal	corn
NK	2	colorless	yellow	intermediate	normal	corn

[a]Represented in all three years.

[b]BR = bird resistant.

corn varieties were grown in Years 1 and 2 only. Four varieties; 1133, Soft Endo, ROKY 78 and Darset, carried the brown seed coat indicating bird resistance. Endosperm color was either white or yellow except for the hetero-yellow OK 612 variety. All sorghums were of intermediate endosperm hardness except for the Soft Endo which carried the soft endosperm characteristic. Four varieties; Dwarf Redlan, 1122, 1126 and 1133 exhibited the waxy endosperm indicating (>90%) amylopectin content. The classification system is based on major characteristics determining in vitro digestibility, i.e. waxiness, bird resistance and endosperm hardness.

Prior to analysis, all grain samples were finely ground through either a 0.4 mm screen in a Udy mill for compositional analysis or a 20-mesh screen in a laboratory Wiley mill for digestibility studies. Crude protein was determined by the Kjeldahl procedure (N X 6.25) and total starch as α-linked glucose polymers by the procedure of Macrae and Armstrong (1968). Tannin content was measured as catechin equivalents by a modified Vanillin-HCL procedure described by Price et al. (1978). In vitro dry matter disappearance (IVDMD) was determined to obtain relative digestibility estimates for the grain smaples. Fresh rumen fluid was obtained from a concentrate-fed (80% corn) steer, strained twice through six layers of cheesecloth and mixed with pre-warmed McDougall's buffer. Thirty milliliters of inoculant (22 ml buffer : 8 ml rumen fluid) were placed in preweighed 50 milliliter centrifuge tubes containing 0.4 g of the grain sample. After either a 6 or 24 hour incubation at 39 C, the tubes were centrifuged, decanted and dried for 48 hours in a 80 C oven.

An in vitro gas production (IVGP) procedure was utilized as a

measure of starch availability of the grain samples. A 0.4 g sample and 0.25 g of commercial baker's yeast were placed in 50 milliliter Erlenmeyer flasks. Ten milliliters of a 0.1 percent (w/v) amyloglucosidase enzyme solution were added to each flask. The flasks were connected to an inverted buret gas recovery system and placed in a 39 C water bath. Gas production was measured after 6 and 12 hours of incubation and results were expressed as milliliters of gas per gram of dry sample.

The data obtained from the IVDMD and IVGP studies can be described by:

$$Y_{ij} = \mu + V_i + R_j + E_{ij}$$

where Y_{ij} is 6 or 24 hour IVDMD or 6 or 12 hour IVGP and where V is variety and R is run. The components μ, V_i and R_j were treated as fixed effects of all records of variety i and run j. Random error effect, E_{ij}, was specific to each observation. The variety by run interaction was assumed to be zero.

Estimated differences between variety means were obtained by method of least squares and significant differences between varietal means were determined by Tukey's HSD test. The error mean squares and corresponding degrees of freedom are presented in Appendix B, Table XXXV for the IVDMD and IVGP analyses in Years 1-3. These analyses represent a subset of a larger data set containing treatment and purified starch components.

Results and Discussion

Chemical Composition

Except for the Redlan variety (15.20% C.P.), the sorghum varieties

in Year 1 were generally similar in crude protein content (12.80 to 13.32%) although some statistical differences were detected (Table II). The corn varieties averaged 9.78 percent crude protein and were lower ($P < .05$) than the sorghums. The OK 612 sorghum variety contained the greatest amount of starch (83.0%) followed by Pioneer 3149 corn (79.0%). Pioneer 3306 corn contained the lowest amount of starch (67.9%); other sorghum varieties were generally intermediate (70.6 to 74.9%). The Darset bird resistant was the only variety that contained significant tannin levels. Although Soft Endo carried the brown seed coat, it did not contain appreciable tannin concentration.

Crude protein content for Year 2 sorghums was similar (11.14 to 12.02%) except for the 1122 (13.78%) and 1126 (16.46%) varieties which showed elevated crude protein levels (Table III). Corn varieties averaged 9.40 percent crude protein and were again lower ($P < .05$) than the sorghums. As in Year 1, OK 612 contained the highest concentration of starch (72.9%). The Normal-BR, Redlan and corn varieties were similar in starch content (67.6 to 71.7%) as were the varieties exhibiting the waxy endosperm (61.9 to 63.8%). High tannin levels were observed for the Darset, Soft Endo and 1133 varieties in association with their brown seed coat.

Crude protein levels in Year 3 (Table IV) were intermediate for the Redlan (13.78%) and Soft Endo (13.76%) as compared to the 1126 (16.30%) or the other varieties represented (11.40 to 12.61%). Starch content was similar for most varieties (72.0 to 79.5%) except the Waxy 1126 (66.6%). As in Year 2, the Waxy 1126 contained the highest protein and lowest starch concentrations. Bird resistant qualities were reflected by increased tannin levels for Darset, ROKY 78 and 1133. As in Year 1,

TABLE II

CHEMICAL COMPOSITION OF WHOLE GRAINS (YEAR 1)

	Protein (%)	Starch (%)	Tannin (catechin equivalents)
Waxy			
Dwarf Redlan	13.12[a,b]	74.3[a,b]	.012[a]
Normal			
Redlan	15.20[c]	74.9[a]	.005[a]
OK 612	12.90[a,b]	83.0[e]	.010[a]
Floury-BR			
Soft Endo	13.32[a]	70.6[b,c]	.002[a]
Normal-BR			
Darset	12.80[b]	74.8[a]	.372[b]
Corn			
Pioneer 3149	8.84[d]	79.0[d]	.001[a]
Pioneer 3306	10.72[e]	67.9[c]	.000[a]
SEM (obs./mean)	0.08(2)	0.8(2)	.002(2)

[a,b,c,d,e]Means in a column with different superscripts are significantly different ($P < .05$).

TABLE III

CHEMICAL COMPOSITION OF WHOLE GRAINS (YEAR 2)

	Protein (%)	Starch (%)	Tannin (catechin equivalents)
Waxy			
Dwarf Redlan	12.23[a]	63.6[a]	.000[a]
1122	13.78[d]	63.8[a]	.001[a]
1126	16.46[e]	61.9[a]	.006[a]
Waxy-BR			
1133	11.71[b]	62.6[a]	.151[b]
Normal			
Redlan	11.82[a,b]	67.6[b]	.017[a]
OK 612	11.61[b,c]	72.9[d]	.000[a]
Floury-BR			
Soft Endo	11.14[c]	69.5[b,c]	.090[c]
Normal-BR			
Darset	12.02[a,b]	71.7[c,d]	.302[d]
Corn			
Pioneer	9.46[f]	70.0[b,c,d]	.000[a]
NK	9.34[f]	70.3[b,c,d]	.000[a]
SEM (obs./mean)	0.09(2)	0.5(2)	.005(2)

[a,b,c,d,e,f]Means in a column with different superscripts are significantly different ($P < .05$).

TABLE IV

CHEMICAL COMPOSITION OF WHOLE GRAINS (YEAR 3)

	Protein (%)	Starch (%)	Tannin (catechin equivalents)
Waxy			
Dwarf Redlan	11.50[a]	79.5[a]	.011[a]
1122	12.61[b]	74.5[a]	.000[a]
1126	16.30[c]	66.6[b]	.002[a]
Waxy-BR			
1133	12.02[d]	75.7[a]	.122[b]
Normal			
Redlan	13.78[e]	77.1[a]	.031[a]
Floury-BR			
Soft Endo	13.76[e]	72.0[a,b]	.020[a]
Normal-BR			
ROKY 78	11.40[a]	79.0[a]	.122[b]
Darset	11.66[a]	77.8[a]	.348[c]
SEM (obs./mean)	0.06(2)	0.6(2)	.005(2)

[a,b,c,d,e]Means in a column with different superscripts are significantly different ($P < .05$).

Soft Endo did not show increased tannin levels in association with the brown seed coat.

In Vitro Dry Matter Disappearance

Six and twenty four hour IVDMD trends are illustrated in Tables V and VI, respectively. Due to the fact that 6 hour trends closely resemble 24 hour trends, only 24 hour values (Table VI) will be discussed. In both Years 1 and 2, the corn varieties tended to be more digestible than the sorghum varieties, however differences were not large except for the bird resistant types. Corn has generally been considered to be of superior feeding value in relation to sorghum. These studies, however, suggest that some sorghum types, i.e. Waxy and Normal, may be more similar to corn than other types such as the bird resistants. This trend is supported by McCollough and Brent (1972c) where apparent dry matter digestibilities determined in digestion trials showed a waxy and 3 of 6 normal sorghum hybrids to be similar to the corn hybrids represented. Furthermore, a bird resistant sorghum showed a significantly depressed response in comparison to the corn hybrids.

Within sorghum types, the Darset bird resistant variety was consistently inferior to the Waxy and Normal types in all three years (Table VI) being significant ($P < .05$) in Years 1 and 3 only. In Year 3, an additional bird resistant (ROKY 78) was also inferior to the Normal and Waxy types. The brown seed coat or bird resistant characteristic has been considered to lower the digestibility of varieties carrying this factor. For example, Saba et al. (1972) also observed decreased IVDMD performance for a bird resistant in comparison to a sorghum with

TABLE V

SIX HOUR IVDMD (%) OF DRY, FINELY GROUND SORGHUM AND CORN GRAIN

	Year 1	Year 2	Year 3
Waxy			
Dwarf Redlan	15.0^{a,b}	33.6^{a}	21.5^{a}
1122	---	31.3^{a,b}	21.7^{a}
1126	---	30.7^{a,b}	21.5^{a}
Waxy-BR[1]			
1133	---	24.4^{b,c}	17.2^{a,b}
Normal			
Redlan	14.9^{a,b}	31.4^{a,b}	15.9^{b,c}
OK 612	11.8^{a,b}	28.0^{a,b}	---
Floury-BR[1]			
Soft Endo	13.9^{a,b}	26.4^{a,b}	13.5^{b,c}
Normal-BR[1]			
Darset	9.3^{b}	17.2^{c}	8.1^{d}
ROKY 78	---	---	11.4^{c,d}
Corn			
Pioneer 3149	16.2^{a}	---	---
Pioneer 3306	15.7^{a,b}	---	---
Pioneer	---	30.6^{a,b}	---
NK	---	28.0^{a,b}	---
SEM (obs./mean)	1.4(4)	1.9(4)	1.1(4)

[1]BR = bird resistant.

^{a,b,c,d}Means within a column with different superscripts are significantly different ($P < .05$).

TABLE VI

TWENTY-FOUR HOUR IVDMD (%) OF DRY, FINELY GROUND SORGHUM AND CORN GRAIN

	Year 1	Year 2	Year 3
Waxy			
Dwarf Redlan	52.4[a]	54.2[a,b,c]	61.6[a]
1122	---	56.0[a,b,c]	59.5[a]
1126	---	58.9[a,b]	57.8[a,b]
Waxy-BR[1]			
1133	---	49.7[c]	54.7[b]
Normal			
Redlan	50.8[a]	52.9[b,c]	59.2[a,b]
OK 612	49.8[a]	55.0[a,b,c]	---
Floury-BR[1]			
Soft Endo	48.7[a]	52.0[b,c]	46.2[c]
Normal-BR[1]			
Darset	29.2[b]	48.8[c]	47.3[c]
ROKY 78	---	---	49.1[c]
Corn			
Pioneer 3149	56.7[a]	---	---
Pioneer 3306	58.6[a]	---	---
Pioneer	---	62.8[a]	---
NK	---	60.4[a,b]	---
SEM (obs./mean)	2.5(4)	2.0(4)	1.0(4)

[1]BR = bird resistant.

[a,b,c]Means within a column with different superscripts are significantly different ($P < .05$).

a red seed coat.

In all three years, the IVDMD performance of the Normal and Waxy varieties were similar ($P > .05$). Although Nishimuta et al. (1969) and Sherrod et al. (1969) showed depressed animal performance for Regular or Normal sorghums in comparison to Waxy types, this work shows them to be quite similar which may be due in part to the close genetic relationship of the Redlan Normal and Dwarf Redlan Waxy varieties. In addition, the hetero-yellow characteristic of the OK 612 may account for some of its increased performance as documented by Walker and Lichtenwalner (1977).

The Soft Endo variety performed somewhat comparably to the Waxy types in Years 1 and 2 but showed a depressed ($P < .05$) response in Year 3. The effect of endosperm hardness was illustrated by Samford et al. (1971) in that the carbohydrate digestion of a floury sorghum type was 15 percent higher than a normal endosperm sorghum. Perhaps the soft or floury nature of the endosperm of this variety increases the nutritive value in spite of the brown seed coat characteristic.

The Waxy-BR 1133 was similar to the poorly digestible Darset in Year 2 but proved to be more intermediate between the Waxy and bird resistant types in Year 3. Very little information is available concerning the combination of agronomic and nutritional characteristics in grain sorghum. These studies suggest that the combination of bird resistance and waxiness may offer an acceptable compromise in terms of digestibility or nutritive value.

In Vitro Gas Production

As with the 6 hour IVDMD values, the 6 hour IVGP values (Table VII)

TABLE VII

SIX HOUR IVGP OF DRY, FINELY GROUND SORGHUM AND CORN GRAIN (ml gas/g DM)

	Year 1	Year 2	Year 3
Waxy			
Dwarf Redlan	65.9[a]	88.1[a,b]	75.5[a]
1122	---	87.4[a,b]	75.5[a]
1126	---	79.6[b,c,d]	58.6[c]
Waxy-BR[1]			
1133	---	76.3[d]	62.2[b,c]
Normal			
Redlan	51.1[b]	84.8[b,c,d]	58.9[c]
OK 612	48.0[b]	85.8[a,b,c]	---
Floury-BR[1]			
Soft Endo	65.4[a]	94.8[a]	68.1[b]
Normal-BR[1]			
Darset	11.2[c]	48.4[e]	37.6[e]
ROKY 78	---	---	48.9[d]
Corn			
Pioneer 3149	55.1[b]	---	---
Pioneer 3306	53.1[b]	---	---
Pioneer	---	77.8[b,c]	---
NK	---	87.3[a,b]	---
SEM (obs./mean)	1.7(4)	1.6(4)	1.4(5)

[1]BR = bird resistant.

[a,b,c,d,e]Means within a column with different superscripts are significantly different ($P < .05$).

will not be discussed due to their similarity to 12 hour IVGP values. Twelve hour IVGP for Year 1 (Table VIII) showed the corn varieties to be similar ($P > .05$) to the Waxy Dwarf Redlan but superior ($P < .05$) to the Darset variety. In Year 2 (Table VIII), the corn varieties were similar to most sorghums except the Darset variety. Increased starch availability (IVGP) may account for some of the positive IVDMD response observed earlier for the corn varieties. The magnitude of the difference in IVGP, however, between the corns and sorghums, suggests that other factors such as protein solubility or degradability may have an effect.

Within sorghum types, the Floury-BR Soft Endo produced more gas than any other variety in Years 1 and 2 and was high in IVGP for Year 3 (Table VIII). Possibly the weak structural nature of the endosperm of the floury type sorghums results in increased starch accessibility which may account for the unexpected increase in gas production. The Darset variety produced less gas ($P < .05$) in all three years than any corn or sorghum variety. In addition, the ROKY 78 produced less gas ($P < .05$) than other sorghums in Year 3. Hinders and Eng (1971) also observed depressed gas production for bird resistant sorghums in comparison to other sorghum types. The magnitude of the depression in gas production for the Darset and ROKY 78 varieties suggests that the factor(s) that limit utilization by ruminal microbes <u>in vitro</u> may also limit starch availability. The performance of the Floury-BR Soft Endo indicates that other factors such as endosperm hardness may mediate the effect of the brown seed coat characteristic.

The Waxy sorghums generally showed greater starch availability than the Normal sorghums in Years 1 and 3 (Table VIII). Hinders and Eng

TABLE VIII

TWELVE HOUR IVGP OF DRY, FINELY GROUND SORGHUM AND CORN GRAIN (ml gas/g DM)

	Year 1	Year 2	Year 3
Waxy			
Dwarf Redlan	88.9^{a,b}	103.7^{b,c}	105.8^{a}
1122	---	103.9^{b,c}	104.7^{a,b}
1126	---	98.1^{c}	87.8^{c}
Waxy-BR[1]			
1133	---	96.5^{c}	96.9^{b}
Normal			
Redlan	75.8^{c}	102.6^{b,c}	87.3^{c}
OK 612	70.3^{c}	105.0^{b,c}	---
Floury-BR[1]			
Soft Endo	96.7^{a}	118.6^{a}	101.6^{a,b}
Normal-BR[1]			
Darset	15.7^{d}	58.9^{d}	61.7^{e}
ROKY 78	---	---	77.2^{d}
Corn			
Pioneer 3149	88.8^{a,b}	---	---
Pioneer 3306	82.1^{b,c}	---	---
Pioneer	---	104.0^{b,c}	---
NK	---	114.6^{a,b}	---
SEM (obs./mean)	2.2(4)	2.6(4)	1.8(5)

[1]BR = bird resistant.

^{a,b,c,d,e}Means within a column with different superscripts are significantly different ($P < .05$).

(1971) also observed increased gas production for Waxy sorghums over other varieties tested. Evidently, the Waxy (high amylopectin) characteristic increases starch availability and subsequent digestibility by: (1) increased enzyme susceptibility due to high amylopectin starch, or (2) increased starch granule accessibility due to higher protein solubility of Waxy sorghums (Lichtenwalner et al., 1978). In Years 2 and 3 (Table VIII), the Waxy 1126 did not perform as well as the other Waxy types. The depressed gas production of this variety may be attributed to extremely low starch and/or high protein content (Tables III and IV).

Gas production of the Waxy-BR 1133 in Years 2 and 3 (Table VIII) was similar ($P > .05$) to the other Waxy varieties. In this instance, the brown seed coat affected IVGP to a lesser degree than in the IVDMD studies. This evidence suggests that factors other than tannin content may be affected by the bird resistant gene. Perhaps protein solubility and/or structural integrity of the kernel are affected by epistatic gene effects of the bird resistant characteristic.

Comparison of varietal responses across years is of interest. Although statistical comparisons among years cannot be made, it is interesting to note that the IVDMD response of the Darset in Year 2 is not depressed to the same magnitude as in Years 1 and 3. Although not well understood, environmental conditions during the growing season could certainly affect starch and/or protein deposition thereby altering nutrient availability in the kernel.

These studies suggest that the range in protein and starch content for different grain sorghum varieties can be substantial. High starch levels are probably beneficial, however the variety with the highest

starch content (OK 612) did not give the highest IVDMD response but was similar to the Waxy varieties. The high amylopectin content of the Waxy types may have boosted their digestibility in spite of decreased starch content.

The relative importance of protein content is difficult to discern. The protein in grain sorghum may play a role in limiting starch granule accessibility, particularly in the peripheral endosperm of the kernel (Seckinger and Wolf, 1973). High protein levels were not related to IVDMD as the Waxy 1126 variety, which had the highest crude protein content, also showed IVDMD performance similar to other Waxy types which contained significantly lower crude protein concentrations. This observation suggests that other factors such as protein composition may play a major role in determining starch availability and subsequent digestibility.

In conclusion, the IVDMD and IVGP studies show the corn varieties to be slightly superior to most of the sorghum varieties. The performance of the Waxy and Normal varieties, however, indicates that some sorghum types may be similar to and perhaps superior to corn. This study also indicates that the nutritive value of grain sorghum is highly dependent on variety or type. Consequently, the consideration of grain sorghum as an entity must underestimate the feeding value of certain sorghums such as the Waxy and Normal types. In addition, the wide range in digestibility and starch availability observed for the sorghum varieties in this study may account for the wide variability in feeding value commonly associated with grain sorghum. Awareness of the nutritional contribution of different seed characteristics should allow for continued genetic improvement of nutritional as well as agronomic traits.

In this regard, the performance of the Waxy-BR 1133 illustrates the potential combination of bird resistant and waxy characteristics to produce adequately digestible types that maintain important agronomic traits.

CHAPTER IV

THE RESPONSE OF DIFFERENT GRAIN SORGHUM AND CORN VARIETIES TO RECONSTITUTION

Summary

In vitro dry matter disappearance (IVDMD) studies were conducted to examine the response to reconstitution of different genetic varieties of grain sorghum and corn. Sorghum types tested were Waxy (Dwarf Redlan, 1122, 1126), Waxy bird resistant (1133), Normal (Redlan, OK 612), Floury bird resistant (Soft Endo) and Normal bird resistant (Darset, ROKY 78). Small positive responses in IVDMD to reconstitution were observed for all varieties in Year 1 except the Darset (bird resistant) which increased significantly. Small to moderate IVDMD responses were observed for all types in Year 2. In Year 3, both positive and negative responses to reconstitution were noted, with the Darset variety again being improved significantly. In all three years, the range in IVDMD among varieties was decreased by reconstitution compared to pretreatment results. *In vitro* gas production (IVGP) studies were utilized to assess changes in starch availability of the reconstituted grains. Large, positive increases in IVGP were observed for all varieties in Years 1 and 3. The response in IVGP ranged from negative to highly positive in Year 2. The magnitude of the response in IVGP was highly dependent on variety in all three years. In general, as for IVDMD, the more poorly digestible bird resistants gave a larger response than the

more highly digestible types. This study suggests that the response to reconstitution is highly dependent on variety or type. In support of this observation, significant ($P < .01$) treatment by variety interactions were observed for Years 1 and 3 of the IVDMD and Years 1, 2 and 3 of the IVGP studies. In addition, the variety dependent response increased the poorly digestible bird resistant types more than the more highly digestible non-bird resistant types thereby producing an equalizing effect in nutritive value across varieties.

Introduction

Reconstitution has been recognized as a low energy grain processing alternative for many years. This process has been shown to increase dry matter digestibility of grain sorghum as much as 20 percent (Buchanan-Smith et al., 1968; McGinty et al., 1967). Hinders (1976) summarized numerous feeding trials and observed changes in average daily gain ranging from -5.1 to +11.6 percent and changes in feed efficiency ranging from -4.3 to +28.1 percent for reconstituted as compared to dry ground or rolled sorghum. This summary suggests that the response to reconstitution has not been as consistent as some research data would imply. The reason for these ranges in response is not clear; however, differences in experimental procedure or, more likely, source of grain (variety) might be suspect. In most cases, the variety or type of sorghum is either not reported or unknown.

Differential varietal responses to grain processing across sorghum types have been observed but not directly investigated. Using a modified nylon bag procedure, Walker and Lichtenwalner (1977) observed 24-hour responses to reconstitution over dry ground to be +5 percent for

a hetero-yellow, +3 percent for a Redlan Waxy and -11 percent for a Redlan nonwaxy variety. Saba et al. (1972) observed a significant difference in dry matter digestibility between a red seed coat and a bird resistant variety in the dry ground form. After steam flaking, both varieties were increased to similar levels ($P > .05$) thereby demonstrating a differential varietal response. Studies have not been implemented to determine the magnitude or repeatability of the varietal dependent response to processing. The information currently available can only be inferred from studies originally performed for other reasons. Consequently, the objective of this study was to investigate the response to reconstitution of several varieties of grain sorghum and corn grown in three consecutive crop years that differ widely in agronomic and nutritive characteristics.

Materials and Methods

The grain sorghum and corn varieties utilized are the same as described previously (Chapter III). Each grain sample was processed using a laboratory reconstitution procedure. A 200 gram sample of whole grain was placed in a glass bottle and water added to a moisture level of 30 percent. The bottles were then flooded with carbon dioxide and sealed for a 21-day storage period. After reconstitution, the grain samples were ground through a 20-mesh screen in a laboratory Wiley mill. Dry ice was used to facilitate grinding of the moist samples. Untreated grain samples were also ground through a 20-mesh screen in a laboratory Wiley mill for baseline comparison to the reconstituted samples.

In vitro dry matter disappearance (IVDMD) studies were performed

to evaluate the relative digestibilities of the reconstituted and finely ground samples. In vitro gas production (IVGP) studies were utilized to determine the effect of reconstitution on starch availability. The IVDMD and IVGP procedures were described in Chapter III.

The data obtained from the IVDMD and IVGP studies can be described by:

$$Y_{ijk} = \mu + V_i + R_j + T_k + VT_{ik} + VR_{ij} + RT_{jk} + E_{ijk}$$

where Y_{ijk} is either 6 or 24-hour IVDMD or 6 or 12-hour IVGP and where V is variety, R is run (procedure repeated on different days), T is treatment (processing method), VT is variety by treatment interaction, VR is variety by run interaction and RT is run by treatment interaction. The components μ, V_i, R_j, T_k, VT_{ik}, VR_{ij} and RT_{jk} were treated as fixed effects of all records of variety i, run j and treatment k. Random error effect, E_{ijk}, was specific to each observation. The three-way interaction VRT_{ijk} was assumed to be zero. The error mean squares and corresponding degrees of freedom are presented in Appendix B, Table XXXVI for the IVDMD and IVGP analyses in Years 1-3.

Estimated differences between variety by treatment means were obtained by a method of least squares. Significant differences between variety by treatment means were detected using Tukey's HSD test.

Results and Discussion

In Vitro Dry Matter Disappearance

Six and twenty four hour IVDMD results are presented in Tables IX and X, respectively. Because 6-hour trends closely resemble 24-hour trends, discussion will be oriented towards 24-hour results unless

TABLE IX

SIX HOUR IVDMD RESPONSE TO RECONSTITUTION OVER DRY GROUND SORGHUM AND CORN IN YEARS 1-3

	Year 1		Year 2		Year 3	
	IVDMD (%)	Response[1]	IVDMD (%)	Response[1]	IVDMD (%)	Response[1]
Waxy						
Dwarf Redlan	15.1[a]	+0.1	26.2[a]	-7.5	15.6[b]	-5.8*
1122	----	----	25.8[a]	-5.5	16.5[b]	-5.1*
1126	----	----	26.5[a]	-5.2	23.4[a]	+1.9
Waxy-BR[2]						
1133	----	----	22.9[a]	-1.4	16.8[b]	-0.3
Normal						
Redlan	14.9[a]	0.0	25.2[a]	-6.2	15.3[b]	-0.6
OK 612	16.2[a]	+4.4	26.4[a]	-1.6	----	----
Floury-BR[2]						
Soft Endo	17.9[a]	+4.1	23.7[a]	-2.8	15.5[b]	+2.0
Normal-BR[2]						
Darset	14.9[a]	+5.7	17.4[a]	+0.2	17.7[b]	+9.6*
ROKY 78	----	----	----	----	14.0[b]	+2.6
Corn						
Pioneer 3149	16.6[a]	+0.4	----	----	----	----
Pioneer 3306	17.8[a]	+2.1	----	----	----	----
Pioneer	----	----	18.3[a]	-12.3*	----	----
NK	----	----	21.4[a]	-6.7	----	----
SEM (obs./mean)	1.3(4)		1.7(4)		0.9(4)	

[1]Numerically equal to percentage units change in IVDMD.

[2]BR = bird resistant.

[a,b,c]Means within a column with different superscripts are significantly different ($P < .05$).

*Significant response to reconstitution ($P < .05$).

TABLE X

TWENTY-FOUR HOUR IVDMD RESPONSE TO RECONSTITUTION OVER DRY GROUND SORGHUM AND CORN IN YEARS 1-3

	Year 1		Year 2		Year 3	
	IVDMD (%)	Response[1]	IVDMD (%)	Response[1]	IVDMD (%)	Response[1]
Waxy						
Dwarf Redlan	53.3[a]	+1.0	65.5[a,b]	+11.2	54.7[a,b]	-6.9
1122	----	----	58.8[a,b,c]	+2.8	56.7[a,b]	-2.8
1126	----	----	61.3[a,b,c]	+2.5	58.9[a,b]	+1.1
Waxy-BR[2]						
1133	----	----	54.7[a,b]	+5.0	57.4[a,b]	+2.7
Normal						
Redlan	51.4[a]	+0.6	58.1[a,b,c]	+5.2	56.6[a,b]	-2.6
OK 612	53.3[a]	+3.5	60.3[a,b,c]	+5.3	----	----
Floury-BR[2]						
Soft Endo	51.3[a]	+2.6	53.7[c]	+1.7	42.0[c]	-4.3
Normal-BR[2]						
Darset	49.2[a]	+20.0*	55.4[b,c]	+6.6	61.4[a]	+14.1*
ROKY 78	----	----	----	----	50.3[b,c]	+1.2
Corn						
Pioneer 3149	59.2[a]	+2.6	----	----	----	----
Pioneer 3306	60.2[a]	+1.5	----	----	----	----
Pioneer	----	----	65.3[a,b]	+2.6	----	----
NK	----	----	68.3[a]	+7.9	----	----
SEM (obs./mean)	2.4(4)		2.1(4)		1.7(4)	

[1]Numerically equal to percentage units change in IVDMD.

[2]BR = bird resistant.

[a,b,c]Means within a column with different superscripts are significantly different ($P < .05$).

*Significant response to reconstitution ($P < .05$).

otherwise specified. The IVDMD studies for Year 1 (Table X) illustrate only small positive responses to reconstitution for the corn (+1.5 to +2.6 percentage units) and all sorghums (+0.6 to +3.5 percentage units) except the Normal-BR Darset (+20.0 percentage units). A significant ($P < .01$) treatment by variety interaction verified the observed varietal dependent reconstitution response. The reconstituted corn varieties tended to show higher IVDMD values than the sorghums although the difference was not statistically significant. Within the sorghum group, reconstitution increased the value of the Darset to a level very similar ($P > .05$) to the other sorghums represented.

In Year 2, the 24-hour IVDMD results (Table X) indicate a positive response to reconstitution for all varieties of corn (+2.6 to +7.9 percentage units) and sorghum (+1.7 to +11.2 percentage units). The 6-hour IVDMD trends (Table IX) showed a decreased response for most types, however, this difference had disappeared by twenty four hours (Table X). In contrast to Year 1, the 24-hour IVDMD treatment by variety interaction was not significant ($P > .5$). As in Year 1, the corn varieties tended to be higher than most sorghums except the Dwarf Redlan variety. The Darset variety did not show the same degree of response as in Year 1, possibly due to its higher initial IVDMD in Year 2.

A mixed IVDMD response to reconstitution was observed across varieties in Year 3 (Tables IX and X). The 24-hour IVDMD treatment by variety interaction term was very highly significant ($P < .001$) in relation to this mixed response. As in Years 1 and 2, the Darset variety responded favorably ($P < .05$) to reconstitution. In contrast to Years 1 and 2, however, the Dwarf Redlan showed a negative response to reconstitution which was significant ($P < .05$) at 6 hours of digestion. Small

positive responses ($P > .05$) to reconstitution were observed for the ROKY 78, 1133 and 1126 varieties and small negative responses ($P > .05$) were observed for the Soft Endo, Redlan and 1122 varieties. After reconstitution the Darset variety was similar ($P > .05$) to the Waxy and Normal types. The other bird resistant types (ROKY 78 and Soft Endo) did not show a similar response suggesting that the Darset variety may be peculiar in this respect.

IVDMD patterns indicate response to reconstitution is, in part, variety dependent. Walker and Lichtenwalner (1977) showed a mixed response to reconstitution with hetero-yellow, Redlan waxy and Redlan nonwaxy varieties. It does appear, however, that factors other than variety (i.e. environment) may be additionally responsible for this phenomenon. Although the Darset variety responded favorably in all three years, the magnitude of the response was variable. This point is further illustrated by the Dwarf Redlan variety which showed a small, positive response in Year 1, a larger positive response in Year 2 and a negative response in Year 3.

In Vitro Gas Production

As with the IVDMD studies, the 6-hour IVGP results (Table XI) will not be addressed directly unless major discrepancies from 12-hour results merit attention. Reconstitution increased IVGP in Year 1 (Table XII) for the sorghum (+24.8 to +89.5 ml gas/g DM) and corn (+20.8 to +39.6 ml gas/g DM). Similar to the IVDMD studies for Year 1 (Table X), the Darset variety showed the greatest increase ($P < .05$) in IVGP, whereas a lesser response was observed for the other sorghum and corn varieties. The differential varietal response to reconstitu-

TABLE XI

SIX HOUR IVGP RESPONSE TO RECONSTITUTION OVER DRY GROUND SORGHUM AND CORN IN YEARS 1-3

	Year 1		Year 2		Year 3	
	IVGP	Response[1]	IVGP	Response[1]	IVGP	Response[1]
Waxy						
Dwarf Redlan	87.0[a]	+21.1*	84.3[a]	-3.8	84.8[a,b]	+9.3*
1122	----	----	75.5[b,c,d]	-11.9*	87.3[a,b]	+11.8*
1126	----	----	80.3[a,b,c]	+0.7	82.3[b,c]	+23.7*
Waxy-BR[2]						
1133	----	----	83.4[a,b]	+7.0	88.8[a]	+26.5*
Normal						
Redlan	80.0[a,b]	+28.8*	68.9[d,e]	-15.9*	77.4[c,d]	+18.5*
OK 612	75.6[b,c]	+27.6*	69.1[d,e]	-16.7*	----	----
Floury-BR[2]						
Soft Endo	83.7[a,b]	+18.3*	73.5[c,d]	-21.3*	88.0[a,b]	+20.0*
Normal-BR[2]						
Darset	66.1[c]	+54.9*	56.8[f]	+8.4	75.7[d]	+38.0*
ROKY 78	----	----	----	----	72.4[d]	+23.6*
Corn						
Pioneer 3149	67.6[c]	+12.5*	----	----	----	----
Pioneer 3306	78.2[a,b]	+25.0*	----	----	----	----
Pioneer	----	----	59.2[f]	-18.6*	----	----
NK	----	----	60.9[e,f]	-26.4*	----	----
SEM (obs./mean)	2.0(4)		1.6(4)		1.2(5)	

[1]Numerically equal to units change in IVGP (ml gas/g DM).

[2]BR = bird resistant.

a,b,c,d,e,f Means within a column with different superscripts are significantly different ($P < .05$).

*Significant response to reconstitution ($P < .05$).

TABLE XII

TWELVE HOUR IVGP RESPONSE TO RECONSTITUTION OVER DRY GROUND SORGHUM AND CORN IN YEARS 1-3

	Year 1		Year 2		Year 3	
	IVGP	Response[1]	IVGP	Response[1]	IVGP	Response[1]
Waxy						
Dwarf Redlan	121.8[a]	+32.9*	123.5[a]	+19.9*	120.3[a,b]	+14.5*
1122	----	----	110.3[a,b,c]	+6.4	124.2[a]	+19.6*
1126	----	----	112.5[a,b,c]	+14.4	115.4[b,c]	+27.6*
Waxy-BR[2]						
1133	----	----	114.8[a,b]	+18.2*	122.1[a,b]	+25.2*
Normal						
Redlan	119.2[a]	+43.4*	110.5[a,b,c]	+7.8	116.2[b,c]	+28.9*
OK 612	115.9[a,b]	+45.6*	107.4[b,c,d]	+2.4	----	----
Floury-BR[2]						
Soft Endo	121.5[a]	+24.8*	107.7[b,c,d]	-10.9	120.4[a,b]	+18.8*
Normal-BR[2]						
Darset	105.2[b]	+89.5*	92.6[d]	+33.7*	115.4[b,c]	+53.6*
ROKY 78	----	----	----	----	111.5[c]	+34.3*
Corn						
Pioneer 3149	109.5[a,b]	+20.8*	----	----	----	----
Pioneer 3306	121.7[a]	+39.6*	----	----	----	----
Pioneer	----	----	94.7[d]	-9.3	----	----
NK	----	----	99.2[c,d]	-15.4	----	----
SEM (obs./mean)	2.3(4)		2.8(4)		1.5(5)	

[1]Numerically equal to units change in IVGP (ml gas/g DM).

[2]BR = bird resistant.

a,b,c,d,e,f Means within a column with different superscripts are significantly different ($P < .05$).

*Significant response to reconstitution ($P < .05$).

tion was confirmed by a highly significant ($P < .0001$) treatment by variety interaction. Although some statistical differences between varieties were observed after reconstitution, the differential varietal response decreased the total range in IVGP as compared to the pre-treatment range.

Changes in IVGP due to reconstitution in Year 2 (Table XII) ranged from -10.9 to +33.7 ml gas/g DM for the sorghums and -9.3 to -15.4 ml gas/g DM for the corn varieties. Significant positive responses ($P < .05$) were observed for the Dwarf Redlan, 1133 and Darset varieties only. The Soft Endo and corn varieties actually showed depressed responses to reconstitution although these differences were not significant. As in Year 2, a highly significant ($P < .0001$) treatment by variety interaction corresponded to the highly variable varietal responses observed. Changes in IVGP did not correspond as favorably to the IVDMD in Year 2 as in Year 1. This observation suggests that factors other than starch availability may also be affected by reconstitution. In contrast to Year 1, the reconstituted grains showed a wider range in IVGP (92.6 to 123.5 ml gas/g DM) compared to the pretreatment range. Although this range is not tremendously broad, the pretreatment range in Year 2 was also fairly uniform.

Reconstitution increased IVGP for all sorghums (+14.5 to +53.6 ml gas/g DM) in Year 3 (Table XII). As in Years 1 and 2, the Darset variety showed the greatest response to processing. Again, a highly significant treatment by variety interaction ($P < .0001$) supported the variety dependent response. In contrast to Years 1 and 2, the Dwarf Redlan and Darset varieties were similar in IVGP. In concurrence with Year 1, however, the range in IVGP after reconstitution was much less

than the pretreatment range.

In general, varieties that showed a positive IVDMD response to reconstitution also showed increased IVGP values. This observation suggests that increased starch availability (IVGP) may be the major result of the reconstitution process. Sullins et al. (1971) suggested that the favorable response often noted for reconstitution could be due to a weakening of the protein matrix in the peripheral endosperm causing release of protein and starch. Consequently, increased starch availability may also indicate increased protein digestion. Although not directly measured in this study, increased protein digestibility for reconstituted grains has been observed (Potter et al., 1971; Riggs and McGinty, 1970).

The results of the IVDMD and IVGP studies suggest that different varieties of grain sorghum respond differently to reconstitution. The treatment by variety interaction was significant in Years 1 and 3 of the IVDMD studies and in all three years for the IVGP studies. Although varietal dependent responses to processing have not previously been clearly demonstrated, some evidence for this effect has been presented in both reconstituted and steam-flaked sorghum grains (Walker and Lichtenwalner, 1977; Saba et al., 1972).

The increase in IVDMD and IVGP after reconstitution was especially constant for the Darset bird resistant variety. This observation would suggest that reconstitution may be especially useful for increasing digestibility of certain bird resistant types. Furthermore, it would appear that the factor(s) limiting starch availability and subsequent digestibility of the bird resistant types is destroyed, inactivated or removed during the reconstitution process.

IVDMD and IVGP studies suggest that, after reconstitution, the range in nutritive value across all varieties was much less than before treatment. This phenomenon is primarily due to a large increase in nutritive value for poorly digestible varieties such as the Darset whereas small positive and negative changes were observed for the initially highly digestible types, e.g. Waxy and Corn varieties. In effect, the reconstitution process appears to exert an equalizing effect in terms of nutritive value across varieties; however, this response may not occur for all sorghum types.

In conclusion, the response of grain sorghum to processing in the form of reconstitution appears to be variety dependent. Secondly, the large response observed for the bird resistant Darset and smaller responses observed for varieties with higher initial nutritive value would suggest that the reconstitution process produces an equalizing effect in nutritive value across certain sorghum types.

CHAPTER V

DIGESTIBILITY CHARACTERISTICS OF PURIFIED GRAIN SORGHUM AND CORN STARCH

Summary

Purified starch was isolated via a wet-milling procedure from several varieties of grain sorghum and corn grown in three consecutive crop years. *In vitro* dry matter disappearance (IVDMD) was not significantly different for any purified varietal starch in Years 1, 2 and 3. *In vitro* gas production (IVGP) utilizing amyloglucosidase and commercial baker's yeast showed that purified sorghum starch was superior to purified corn starch in Years 1 and 2. Within sorghum types, the purified Waxy (high amylopectin) starch was generally favored over the nonwaxy starches. Some differences in IVGP between nonwaxy starches were apparent, suggesting that other factors such as granule size or chain length may affect starch digestibility. Enzymatic digestion with α-amylase generally favored the Waxy starches although differences between varietal starches were not as broad. Therefore, enzymatic digestibility of starch may be highly dependent on variety or starch type, e.g. Waxy *vs* nonwaxy. Ruminal microbes, however, do not show an apparent preference for any particular starch. Consequently, once starch has been released in the rumen, digestion of that starch should proceed at a rate independent of source. This observation would suggest that factors other than starch, i.e. protein or tannins, may

play a major role in determining the digestibility of grain sorghum and corn in ruminants.

Introduction

Variety or endosperm type of grain sorghum has been suggested as a major source of variability for this feedstuff. Theoretically, differences between individual varieties should be manifested in differences in chemical or structural composition. The largest constituent of most cereal grains, including grain sorghum, is starch (Horan and Heider, 1946). It has been illustrated that differences in total starch content are not a major factor influencing in vitro digestibility (Chapter III). Plausibly, however, differences in starch digestibility could greatly influence digestibility of grain sorghum.

Sullins and Rooney (1974) showed that the 12-hour carbon dioxide gas production of ground whole grain was much higher for a Waxy sorghum over Floury and Normal sorghums. Purified starch from these varieties showed similar differences in gas production suggesting that Waxy starch is digested more rapidly than other starches. Different types of starch may be subject to different types of hydrolytic attack from both pancreatic α-amylase and rumen microorganisms as visualized by electron microscopy (Davis and Harbers, 1974). In addition, pancreatic α-amylase hydrolysis of purified starches showed that a Waxy starch was digested at a greater rate than bird resistant starch.

Differences in starch digestibility in the ground whole grain and purified starch for a few sorghum types have been observed. However, widely different sorghum types have not been utilized and additionally, the ruminal digestibility of different varietal starches has not been

estimated. Therefore, the objective of this study was to compare the relative digestibility of purified sorghum and corn starches using *in vitro* enzymatic and rumen microbial digestion studies.

Materials and Methods

Nine varieties of grain sorghum and four varieties of corn, most grown in three consecutive crop years, were utilized in this study. The varietal characteristics and classification were described previously (Chapter III). Purified, isolated starch was obtained in duplicate from each variety utilizing a modification of the laboratory wet-milling procedure described by Norris and Rooney (1970), see Appendix A, Table XXXIV. Purified starch from each duplicate was blended and homogenized by gentle mixing with a mortar and pestle prior to analysis.

The amylose content of the purified starches was determined by a modified procedure (see Appendix A, Table XXXIII) reported by McCready and Hassid (1943). Purified starch from each sample was subjected to an *in vitro* dry matter disappearance (IVDMD) procedure to determine relative digestibility. The procedure was exactly the same as described previously (Chapter III) except that urea was added at a level of 20 milligrams per tube to simulate nitrogen concentration in the intact kernel. An *in vitro* gas production (IVGP) procedure was utilized to determine the relative enzymatic digestibility of each starch sample (Chapter III). A second enzymatic digestion (α-amylase) was also performed on this purified starch (see Appendix A, Table XXXI). In this procedure, 400 mg of purified starch was placed in 50 ml Erlenmeyer flasks. An acetate buffer solution containing α-amylase (0.001% enzyme, w/v) was added to each flask and the solutions incubated for 1.5 hours

at 39 C in a shaker water bath. After incubation, the amount of glucose liberated was determined by Nelson's test for reducing sugars.

The data obtained from these digestion studies can be described by:

$$Y_{ij} = \mu + V_i + R_j + E_{ij}$$

where Y_{ij} is 6 or 24 hour IVDMD or 6 hour IVGP or whole starch digestion and where V is variety and R is run. The components μ, V_i and R_j were treated as fixed effects of all records of variety i and run j. Random error effect, E_{ij}, was specific to each observation. The variety by run interactions was assumed to be zero. Estimated differences between varietal means were obtained by method of least squares. Significant differences between varietal means were determined using Tukey's HSD test. The error mean square and respective degrees of freedom are presented in Appendix B, Table XXXVII.

Results and Discussion

Purified Starch Characteristics

Amylose content of purified, isolated starches averaged less than 5 percent for the Waxy starches in Years 1 through 3 (Table XIII). The nonwaxy starches ranged from 16.44 to 21.84 percent amylose in all three years and were generally similar except for Year 3 where some significant differences were observed. The crude protein content of purified starches averaged 0.32 percent in Year 1 and 0.25 percent in Year 2 suggesting that the starch utilized in this study was relatively pure.

In Vitro Dry Matter Disappearance

Six hour IVDMD of purified starch for Year 1 (Table XIV) ranged

TABLE XIII

AMYLOSE CONTENT OF PURIFIED, ISOLATED SORGHUM AND CORN STARCHES IN YEARS 1-3 (% AMYLOSE)

	Year 1	Year 2	Year 3
Waxy			
Dwarf Redlan	2.46[a]	3.66[a]	0.00[a]
1122	---	3.64[a]	0.03[a]
1126	---	3.28[a]	0.00[a]
Waxy-BR[1]			
1133	---	4.20[a]	0.00[a]
Normal			
Redlan	16.75[b]	20.54[b]	18.15[b,c]
OK 612	19.44[b]	21.10[b]	---
Floury-BR[1]			
Soft Endo	19.08[b]	18.16[b]	21.82[d]
Normal-BR[1]			
Darset	18.04[b]	20.54[b]	16.44[b]
ROKY 78	---	---	20.60[c,d]
Corn			
Pioneer 3149	18.28[b]	---	---
Pioneer 3306	18.22[b]	---	---
Pioneer	---	20.54[b]	---
NK	---	21.84[b]	---
SEM (obs./mean)	1.02(2)	0.92(2)	0.53(2)

[1]BR = bird resistant.

[a,b,c,d]Means within a column with different superscripts are significantly different ($P < .05$).

TABLE XIV

SIX HOUR IVDMD OF PURIFIED, ISOLATED STARCH
IN YEARS 1-3 (% IVDMD)

	Year 1	Year 2	Year 3
Waxy			
Dwarf Redlan	25.8[a]	24.6[a]	13.4[a,b]
1122	----	31.7[a]	12.0[a,b]
1126	----	30.9[a]	15.2[a]
Waxy-BR[1]			
1133	----	22.4[a]	11.8[a,b]
Normal			
Redlan	22.9[a]	23.0[a]	11.0[a,b]
OK 612	23.9[a]	27.2[a]	----
Floury-BR[1]			
Soft Endo	26.1[a]	27.2[a]	11.2[a,b]
Normal-BR[1]			
Darset	22.3[a]	25.8[a]	9.8[b]
ROKY 78	----	----	10.3[a,b]
Corn			
Pioneer 3149	25.1[a]	----	----
Pioneer 3306	26.3[a]	----	----
Pioneer	----	28.3[a]	----
NK	----	27.5[a]	----
SEM (obs./mean)	1.5(6)	3.2(5)	1.1(4)

[1]BR = bird resistant.

[a,b,c]Means within a column with different superscripts are significantly different ($P < .05$).

from 22.3 to 26.3 percent. No significant differences were observed for any of the purified starches. In Year 2, the 6 hour IVDMD for purified starch ranged from 22.4 to 31.7 percent. Again no significant differences between varietal starches were observed. Six hour IVDMD for Year 3 ranged from 9.8 to 15.2 percent. In contrast to Year 2, the Waxy 1126 starch was significantly more digestible than the Darset starch, other purified starches were intermediate.

Twenty four hour IVDMD of purified starches in Year 1, 2 and 3 (Table XV) showed no significant differences. This data agrees with the 6 hour IVDMD results except that the statistical differences observed in Year 3 had disappeared by 24 hours of digestion. This data suggests that rumen microbes do not show a preference for any particular varietal starch. It is possible that preferences would be found if isolated, pure cultures of individual rumen microbes were tested, however this effect may be masked by the large number of microbial species normally encountered in the rumen.

In Vitro Gas Production

Six hour IVGP for Year 1 (Table XVI) showed the sorghum starches (114.4 to 138.8 ml gas/g DM) to be superior ($P < .05$) to the corn starches (107.2 to 107.8 ml gas/g DM). Within sorghum starches, the Waxy and Floury-BR starches were similar and superior ($P < .05$) to the Normal starches and the Normal-BR starch which was more intermediate. In Year 2, most sorghum starches were again superior to the corn starches. In contrast to Year 1, the Waxy starches were somewhat superior to all other starches. The Normal-BR starch was again intermediate, however the Floury-BR starch was much lower in comparison to Year 1.

TABLE XV

TWENTY-FOUR HOUR IVDMD OF PURIFIED, ISOLATED STARCH IN YEARS 1-3 (% IVDMD)[1]

	Year 1	Year 2	Year 3
Waxy			
Dwarf Redlan	60.8	72.0	53.2
1122	----	74.1	52.0
1126	----	74.9	53.9
Waxy-BR[2]			
1133	----	71.3	54.4
Normal			
Redlan	58.0	66.9	50.5
OK 612	58.6	64.0	----
Floury-BR[2]			
Soft Endo	57.9	69.5	54.0
Normal-BR[2]			
Darset	56.9	65.1	55.6
ROKY 78	----	----	54.2
Corn			
Pioneer 3149	55.0	----	----
Pioneer 3306	60.1	----	----
Pioneer	----	68.6	----
NK	----	69.7	----
SEM (obs./mean)	2.0(6)	2.7(5)	2.1(4)

[1]No significant differences ($P > .05$) within year.

[2]BR = bird resistant.

TABLE XVI

SIX HOUR IVGP OF PURIFIED, ISOLATED STARCH
IN YEARS 1-3 (ml gas/g DM)

	Year 1	Year 2	Year 3
Waxy			
Dwarf Redlan	138.8[a]	103.4[a,b,c,d]	112.5[a,b]
1122	----	115.7[a]	114.7[a]
1126	----	111.4[a,b]	109.1[a,b]
Waxy-BR[1]			
1133	----	106.6[a,b,c]	101.9[b,c]
Normal			
Redlan	114.4[d,e]	92.5[d,e]	86.8[d]
OK 612	118.0[c,d]	98.0[c,d]	----
Floury-BR[1]			
Soft Endo	131.2[a,b]	94.0[c,d,e]	102.4[b,c]
Normal-BR[1]			
Darset	125.8[b,c]	99.1[b,c,d]	83.9[d]
ROKY 78	----	----	92.0[c,d]
Corn			
Pioneer 3149	107.2[e]	----	----
Pioneer 3306	107.8[e]	----	----
Pioneer	----	81.6[e]	----
NK	----	82.2[e]	----
SEM (obs./mean)	2.1(4)	2.8(6)	2.4(5)

[1]BR = bird resistant.

[a,b,c,d,e]Means within a column with different superscripts are significantly different ($P < .05$).

In Year 3, the Waxy starches excluding the 1133 were superior ($P < .05$) to the Normal and Normal-BR starches. The Waxy-BR and Floury-BR starches were intermediate in IVGP.

The IVGP studies suggest that different varietal starches respond differently to enzymatic attack by amyloglucosidase. As expected, the high amylopectin (Waxy) types tended to be superior to the normal starches (Sullins and Rooney, 1974). The depressed performance of the corn starches in comparison to the nonwaxy sorghum starches suggests that other factors such as amylose chain length or starch granule size may affect enzymatic attack of corn starch.

Alpha-Amylase Digestion

Glucose released from α-amylase digestion in Year 1 (Table XVII) was highest for the corn, Waxy, Normal-BR and Normal Redlan starches. The Normal OK 612 and Floury-BR Soft Endo showed significantly depressed ($P < .05$) glucose release. In Year 2, the Waxy 1126 and Normal-BR Darset starches produced more glucose ($P < .05$) than any other sorghum or corn starch. The Normal starches produced the lowest quantity of glucose whereas other starches represented were intermediate. The Waxy 1126 produced the most glucose in Year 3 and was similar ($P < .05$) to other Waxy starches. Other starches released less glucose than the Waxy starches but were not markedly reduced.

The α-amylase digestion studies showed a general trend for the Waxy starches to be superior. The performance of the Normal-BR starch in Years 1 and 2 along with other starches in Years 2 and 3 illustrates that the superiority of the Waxy starches is not always expressed. Possibly, the mode of attack of α-amylase is such that highly branched

TABLE XVII

ALPHA-AMYLASE DIGESTION OF PURIFIED WHOLE STARCH IN YEARS 1-3 (μ MOLES GLUCOSE EQUIVALENTS/ml/MIN)

	Year 1	Year 2	Year 3
Waxy			
Dwarf Redlan	.388[a,b]	.367[b,c]	.440[a,b]
1122	----	.432[b]	.472[a,b]
1126	----	.828[a]	.628[a]
Waxy-BR[1]			
1133	----	.431[b]	.553[a,b]
Normal			
Redlan	.364[b]	.322[c]	.430[b]
OK 612	.166[e]	.282[c]	----
Floury-BR[1]			
Soft Endo	.246[d]	.420[b]	.426[b]
Normal-BR[1]			
Darset	.404[a]	.830[a]	.381[b]
ROKY 78	----	----	.388[b]
Corn			
Pioneer 3149	.319[c]	----	----
Pioneer 3306	.362[b]	----	----
Pioneer	----	.433[b]	----
NK	----	.351[b,c]	----
SEM (obs./mean)	.007(2)	.019(2)	.034(2)

[1]BR = bird resistant.

[a,b,c,d,e]Means within a column with different superscripts are significantly different ($P < .05$).

amylopectin starches are not always preferred to linear amylose starches.

In general, the enzymatic studies (IVGP and α-amylase digestion) suggest that there are major differences between varietal starches. For example, the highly branched amylopectic Waxy starches were generally superior to the straight chain amylose starches. The highly branched nature of amylopectin would be expected to be digested more rapidly, especially with the amyloglucosidase enzyme which contains high β-amylase activity (Radley, 1968). The digestion of amylopectin by α-amylase may be more dependent on the number and size of starch molecules thereby explaining the variable response of the α-amylase digestion. Increased α-amylase digestion of Waxy sorghum starches has been observed, although conditions of digestion were somewhat different from our study (Leach and Schoch, 1961; Sandstedt et al., 1962).

Increased gas production of the Waxy sorghums has been observed. In one study, Waxy starch produced more gas than other sorghum starches in both the ground, whole grain and purified forms (Sullins and Rooney, 1974). As mentioned previously, the high β-amylase activity of amyloglucosidase may account for its preference for high amylopectin starches.

The IVDMD studies suggest that ruminal microbes show no particular preference for any purified sorghum or corn starch. Although Davis and Harbers (1974) illustrated differential attack by microbes on different starch types, this difference is apparently not manifested in differential starch digestion and may be overcome by the wide variety of microorganisms present in the rumen. The impact of this discovery is that ruminal digestion of starch should be similar across all sorghum types

once the starch is accessible. Consequently, starch digestibility of grain sorghum should be enhanced by the development of sorghum varieties (types) or processing techniques that would maximize the rate at which starch is exposed to ruminal microbial attack.

In conclusion, differences in digestibility of grain sorghum and corn varieties cannot be accounted for by differences in starch digestibility especially with respect to ruminal digestion. Post-ruminal starch digestion, however, may be variety dependent as evidenced by the IVGP and α-amylase digestions. Thus, the factors affecting ruminal digestion of sorghum and corn must be related to seed components other than starch, i.e. protein and/or tannins. The extent of degradation or removal of these factors ruminally may be the major factor affecting the overall digestion of grain sorghum and corn. Consequently, differences in the total digestibility of grain sorghum and corn varieties will probably be better understood once the role of factors other than starch, such as protein or tannins, has been elucidated.

CHAPTER VI

THE RELATIVE SOLUBILITY AND MOLECULAR WEIGHT DISTRIBUTION OF PROTEINS ISOLATED FROM SEVERAL VARIETIES OF GRAIN SORGHUM AND CORN

Summary

The protein composition of several varieties of grain sorghum of different genetic type and corn grown in two consecutive crop years was determined using a modified Landry and Moureaux procedure. Generally, the poorly digestible bird resistant types contained less highly soluble protein (Fraction I) and more insoluble protein (Fractions II and IV) than the Waxy types. The highly digestible Waxy varieties contained higher levels of highly soluble Fraction I protein and lower levels of insoluble Fraction II and IV protein than the bird resistant types. A Waxy bird resistant type showed intermediate digestibility and intermediate protein concentrations in comparison to the Waxy and bird resistant types. A Normal variety similar in digestibility to the Waxy types also showed protein composition similar to the Waxy types. A moderately digestible Floury (nonwaxy) bird resistant type showed protein composition intermediate to the Waxy and bird resistant types. Thus, sorghum varieties with higher concentrations of highly soluble protein and lower concentrations of poorly soluble protein will probably be more digestible than sorghums containing less soluble protein. Sodium dodecyl

sulfate-polyacrylamide gel electrophoresis of protein fractions isolated from each variety showed no major differences in molecular weight distribution for Fractions I, II and III. The molecular weight profile of Fraction V extracts showed a series of high molecular weight species (> 45,000 MW) for the bird resistant types that were not observed in the non-bird resistant types. This data suggests that high molecular weight proteins in addition to elevated insoluble protein levels may interact to decrease the digestibility of the bird resistant sorghums.

Introduction

Wide ranges in the digestibility of grain sorghum may be influenced by variety, endosperm type, growth conditions and/or other factors. Such differences in nutritive value should theoretically be manifested in biochemical constituents of the kernel. The peripheral endosperm of grain sorghum contains a highly structured protein matrix which encapsulates starch granules. The thickness of the protein matrix in the peripheral endosperm may be a variety characteristic related to total protein content. In addition, the structural or matrix protein of grain sorghum is apparently composed of highly insoluble glutelins; whereas, the protein bodies contained within the matrix are primarily kafirin (Seckinger and Wolf, 1973).

Seckinger and Wolf's (1973) work suggests that any factor (variety, environment, etc.) that would alter protein composition of grain sorghum may also affect the strength and thickness of the protein matrix and subsequent starch availability. Jambunathan and Mertz (1973) demonstrated that bird resistant sorghums, which produced only small gains in laboratory rats, contained lower levels of albumin and globulin proteins

and much higher levels of glutelins than their non-bird resistant counterparts. Decreases in highly soluble proteins and sizeable increases in glutelin protein have been observed for a bird resistant in contrast to a normal sorghum (Guiragossian et al., 1978). In addition, these workers demonstrated with SDS-PAG electrophoresis that the glutelin fraction of the bird resistant sorghum contains protein species that are not present in the non-bird resistant glutelins. To date, most work dealing with sorghum protein composition or molecular weight profiles has been limited to a few varieties that display only a few characteristics. In addition, adequate relationships between digestibility or nutritive value and protein composition have not been elicited. Consequently, the objective of this study was to evaluate several varieties of grain sorghum differing widely in nutritive and agronomic characteristics in terms of protein composition and the molecular weight distribution of the various protein components.

Materials and Methods

Several varieties of grain sorghum that differed widely in nutritive and agronomic traits were utilized to compare protein characteristics. Seven sorghum varieties from Year 2 and eight varieties from Year 3, described previously (Chapter III), were utilized in addition to two corn varieties from Year 2 which were used as standards. Each sample was freshly ground through a laboratory Udy mill and defatted in 100 ml of petroleum ether for one hour prior to analysis.

Protein composition was determined for each sample in duplicate utilizing a slightly modified (see Appendix A, Table XXVIII) Landry and Moureaux Fractionation Sequence D (1970). Ten grams of each sample

were placed in 250 ml plastic centrifuge bottles. One hundred milliliters of the specified reagent were added and the solution was stirred for the specified time period (Table XVIII). After the extraction period, the samples were centrifuged at 3,000 X g for 15 minutes and then decanted into appropriate volumetric flasks. After diluting each extract to volume with the appropriate solvent, ten milliliters of thoroughly mixed extract were pipetted in duplicate for micro-Kjeldahl analysis.

In order to compare the molecular weight distribution within each protein fraction, the extraction procedure was repeated on Year 3 sorghums only. After extraction, Fractions I, IV and V were dialized against ten volumes of distilled water changed three times during the 48-hour dialysis period. Fractions II and III were flash evaporated after which all fractions were frozen and lyophilized. Micro-Kjeldahls were performed on each extract and the samples were then resolubilized at a level of 10 milligrams protein per milliliter in a solution of one percent sodium dodecyl sulfate (SDS) and one percent β-mercaptoethanol in 8 molar urea. Fraction IV extracts required heating for 3 hours in a 60 C water bath in order to achieve complete resolubilization. A protease inhibitor (phenylmethylsulfonylflouride) was added to the Fraction I extracts at a level of 0.1 mmoles/liter to minimize proteolysis damage. All extracts within the same protein fraction were then electrophoresed on a vertical slab gel system using 12 percent polyacrylamide gels containing 1.6 percent sodium dodecyl sulfate (see Appendix A, Table XXIX). A set of standard molecular weight proteins, solubilized in the same manner as the protein extracts, were electrophoresed with each fraction to determine the relative molecular weight

TABLE XVIII

DESCRIPTION OF REAGENTS AND PROTEINS SOLUBILIZED BY THE PROTEIN FRACTIONATION PROCEDURE[a]

Fraction	Reagent	Content
I	0.5 M NaCl, H_2O	Saline soluble -albumins and globulins
II	70% isopropanol (v/v)	Alcohol soluble -kafirins
III	70% isopropanol (v/v) 0.6% β-mercaptoethanol (v/v)	Alcohol soluble with reducing agent -kafirin-like
IV	Borate buffer with NaCl (pH 10, 0.5μ) 0.6% β-mercaptoethanol (v/v)	Alkaline soluble -glutelins
V	Borate buffer with NaCl (pH 10, 0.5μ) 0.6% β-mercaptoethanol (v/v) 0.5% sodium dodecyl sulfate (w/v)	Alkaline soluble -glutelins

[a]See Appendix for complete procedure (Table XXXIII).

(MW).

The data obtained from the protein fractionation can be described by:

$$Y_{ij} = \mu + V_i + E_{ij}$$

where Y_{ij} is Fraction I, II, III, IV or V and where V is variety. The components μ and V_i were treated as fixed effects of all records of variety i. Random error effect, E_{ij}, was specific to each observation. Estimated differences between variety within fraction means were obtained by method of least squares and significant differences were determined using Tukey's HSD test. The error mean squares and corresponding degrees of freedom for each respective protein fraction in Years 2 and 3 are presented in Appendix B, Table XXXVIII.

Results and Discussion

Protein Composition

Average protein recovery for the protein fractionation in Year 2 (Table XIX) was 93.8 percent suggesting that the extraction was essentially complete. The corn reference contained the greatest concentration ($P < .05$) of Fraction I protein in Year 2. Although differences within the sorghum types were not significant, a trend indicated a higher concentration of albumin and globulin protein for the highly digestible Dwarf Redlan, 1122, Redlan and Soft Endo varieties. The poorly digestible Darset variety contained the lowest concentration of Fraction I proteins, whereas the 1126 and 1133 varieties were intermediate. The highest concentration of Fraction II (kafirin) protein was observed for the corn reference. Within sorghums, the poorly digestible

TABLE XIX

LANDRY-MOUREAUX PROTEIN COMPOSITION
OF SORGHUMS IN YEAR 2

	FI(%)[1]	FII(%)	FIII(%)	FIV(%)	FV(%)	Total Recovery(%)
Waxy						
Dwarf Redlan	14.7^{b}	$13.5^{b,c}$	$27.7^{a,b}$	5.1^{d}	33.2^{a}	94.2
1122	14.1^{b}	$13.6^{b,c}$	23.2^{b}	$6.1^{c,d}$	36.5^{a}	93.5
1126	11.0^{b}	$14.0^{b,c}$	31.2^{a}	5.3^{d}	30.7^{a}	92.2
Waxy-BR[2]						
1133	10.2^{b}	13.3^{c}	21.8^{b}	$8.0^{b,c}$	39.6^{a}	92.9
Normal						
Redlan	14.4^{b}	$17.0^{b,c}$	$28.6^{a,b}$	5.4^{d}	32.1^{a}	97.6
Floury-BR						
Soft Endo	14.0^{b}	$18.4^{b,c}$	$24.6^{a,b}$	5.3^{d}	30.8^{a}	93.1
Normal-BR						
Darset	8.1^{b}	$21.2^{a,b}$	21.2^{b}	8.4^{b}	33.8^{a}	92.7
Corn						
NK	24.8^{a}	28.9^{a}	9.4^{c}	11.2^{a}	20.4^{b}	94.6
SEM (obs./mean)	1.3(2)	1.4(2)	1.3(2)	0.4(2)	1.7(2)	

[1] FI = Fraction I, represented as a percent of total protein.

[2] BR = bird resistant.

^{a,b,c,d} Means within a column with different superscripts are significantly different ($P < .05$).

Darset along with the moderately digestible Soft Endo and Redlan varieties contained the highest level of kafirin. All varieties carrying the waxy characteristic showed lower concentrations of Fraction II protein. Fraction III (kafirin-like) protein was mostly highly concentrated in the Dwarf Redlan, 1126 and Redlan varieties. The 1122, 1133, Soft Endo and Darset varieties contained intermediate levels, whereas the corn reference contained the lowest concentration ($P < .05$) of Fraction III protein. The corn reference contained the highest concentration ($P < .05$) of Fraction IV protein followed by the poorly digestible Darset and 1133 varieties. Other varieties contained lower concentrations of Fraction IV proteins. The lowest concentration ($P < .05$) of Fraction V (glutelin) proteins was observed for the reference corn variety. Within sorghums, trends showed that the 1133 contained the highest concentration of glutelins followed by the 1122 variety. All other varieties contained lower glutelin concentrations.

Protein recovery for Year 3 (Table XX) averaged 90.6 percent. The highest concentration ($P < .05$) of Fraction I protein was observed for the reference corn variety. Within sorghums, trends showed that Waxy varieties contained the highest Fraction I concentration followed by Redlan and Soft Endo varieties. Intermediate concentrations of Fraction I protein were observed for the 1133 and ROKY 78 varieties. As in Year 2, the lowest concentration of Fraction I protein was observed for the poorly digestible Darset variety. The highest concentration of Fraction II (kafirin) protein was observed for the Soft Endo followed by the 1133, Redlan and Darset varieties. The lowest concentration of kafirin was observed for the Dwarf Redlan and 1126 varieties. The 1122, ROKY 78 and corn reference were all intermediate in prolamine content. The 1126

TABLE XX

LANDRY-MOUREAUX PROTEIN COMPOSITION
OF SORGHUMS IN YEAR 3

	FI(%)[1]	FII(%)	FIII(%)	FIV(%)	FV(%)	Total Recovery(%)
Waxy						
Dwarf Redlan	17.4^{b}	9.6^{b}	$26.7^{a,b,c}$	$5.5^{b,c}$	$29.9^{b,c}$	89.1
1122	17.9^{b}	$14.0^{a,b}$	$20.1^{b,c,d}$	$5.3^{b,c}$	$32.2^{a,b}$	89.5
1126	18.1^{b}	8.8^{b}	34.5^{a}	4.8^{c}	$29.6^{b,c}$	95.8
Waxy-BR[2]						
1133	$13.0^{b,c}$	$16.8^{a,b}$	$20.8^{b,c}$	9.4^{a}	$34.5^{a,b}$	94.6
Normal						
Redlan	15.8^{b}	$15.2^{a,b}$	$29.5^{a,b}$	4.2^{c}	$29.7^{b,c}$	94.5
Floury-BR						
Soft Endo	15.1^{b}	19.4^{a}	$20.0^{b,c,d}$	3.8^{c}	$30.1^{b,c}$	88.4
Normal-BR						
Darset	8.1^{c}	$16.5^{a,b}$	$24.2^{a,b,c}$	9.7^{a}	$30.2^{b,c}$	88.7
ROKY 78	$13.6^{b,c}$	$13.9^{a,b}$	$16.6^{c,d}$	$8.1^{a,b}$	41.3^{a}	93.6
Corn						
Pioneer	31.1^{a}	$12.7^{a,b}$	7.7^{d}	8.6^{a}	21.2^{c}	81.3
SEM (obs./mean)	1.0(2)	1.5(2)	2.3(2)	0.5(2)	1.9(2)	

[1] FI = Fraction I, represented as a percent of total protein.

[2] BR = bird resistant.

^{a,b,c,d} Means within a column with different superscripts are significantly different ($P < .05$).

variety contained the highest concentration of Fraction III protein followed by the Redlan variety. Moderately high concentrations of Fraction III protein were observed for the Dwarf Redlan and Darset varieties. The corn reference contained the lowest concentration of Fraction III protein, whereas other varieties were intermediate. The highest concentration of Fraction IV protein was observed for the poorly digestible 1133, Darset and ROKY 78 varieties. The corn reference also contained high concentrations of Fraction IV protein, whereas the Waxy, Redlan and Soft Endo types were much lower. The highest concentration of Fraction V (glutelin) protein was observed for the ROKY 78 variety and the lowest concentration for the reference corn variety. All other sorghum varieties contained intermediate levels of glutelin protein.

Molecular Weight Distribution

Sodium dodecyl sulfate-polyacrylamide gel (SDS-PAG) electrophoresis showed a wide range of protein species for Fraction I in Year 3. Major bands common to most varieties are numerically characterized in Figure 1. This range (11,900 to 43,200 MW) is typical of albumin and globulin proteins in that many different functional proteins are represented in this fraction. Differences in the protein composition of different varieties are not clearly discernable except for some high molecular weight species (> 45,000 MW) in the 1133, Soft Endo and Darset varieties. Electrophoresis of Fraction II (kafirin) proteins showed a major band at approximately 25,000 MW (Figure 2). This observation is consistent with literature reports for zein (Misra et al., 1976) and kafirin protein (Guiragossian et al., 1978). In addition, there were no protein species peculiar to any one variety suggesting that Fraction II proteins are

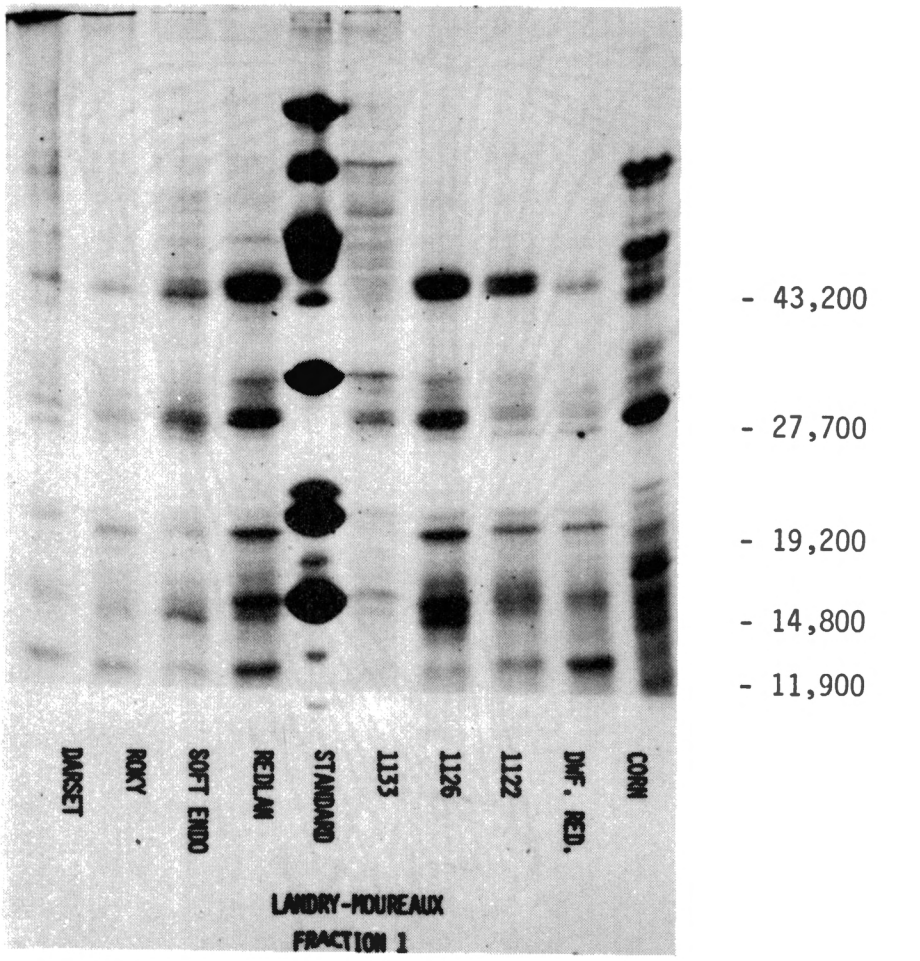
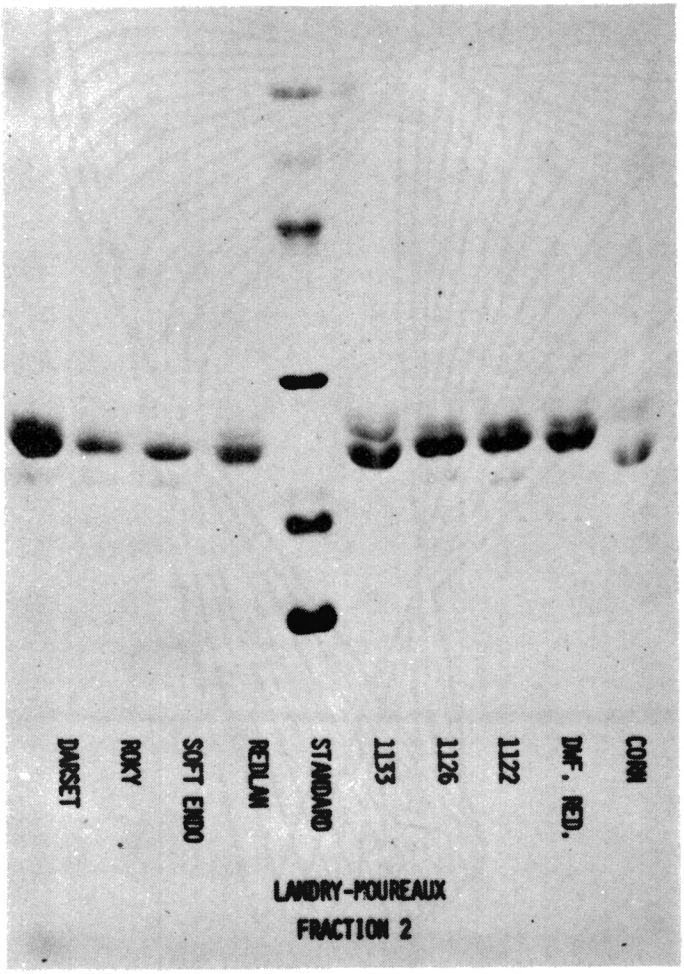


Figure 1. Molecular Weight Profile of Protein From Fraction I.

Relative
Molecular
Weight (MW)



- 25,000

Figure 2. Molecular Weight Profile of Protein From Fraction II.

similar across all varieties.

The molecular weight profile of Fraction III proteins (Figure 3) showed a major band at approximately 22,800 MW. Minor protein species were also observed at 20,000 and 21,900 MW along with some higher molecular weight species around 52,000 MW. As in Year 2, major protein bands were represented across all varieties and no unusual bands unique to any one variety were observed.

Fraction IV proteins were electrophoresed in the same manner as the other fractions; however, no individual protein bands were resolved, therefore, this gel is not presented. Fraction V (glutelin) proteins showed several major protein species in the 21,000 to 25,000 MW range (Figure 4). These bands were present for all varieties but were not as pronounced in the Dwarf Redlan. In addition, a significant concentration of high molecular weight species (45,000 to 86,700 MW) were observed for all varieties with the bird resistant characteristic (Darset, ROKY 78, Soft Endo and 1133).

The results of the protein composition studies suggest that protein composition may be closely related to digestibility or nutritive value. For example, the poorly digestible bird resistant varieties (Darset and ROKY 78) generally showed lower Fraction I and higher Fraction II and IV concentrations that the more highly digestible types. In contrast, the highly digestible Waxy varieties (Dwarf Redlan, 1122 and 1126) generally showed higher Fraction I and lower Fraction II and IV concentrations than the bird resistant types. The concentration of Fraction III and V were not apparently closely related to digestibility.

The performance of the other sorghum types was generally intermediate between the Waxy and bird resistant types. The moderately

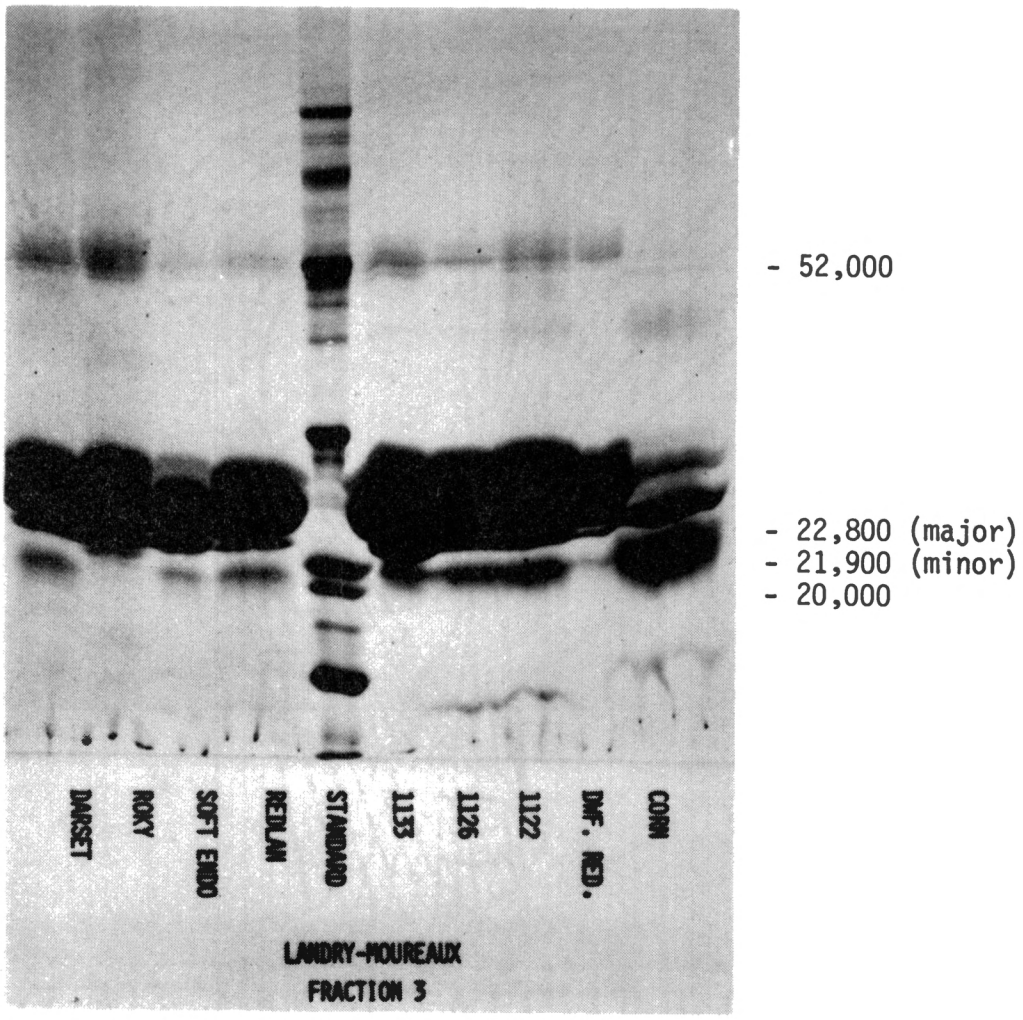


Figure 3. Molecular Weight Profile of Protein From Fraction III.

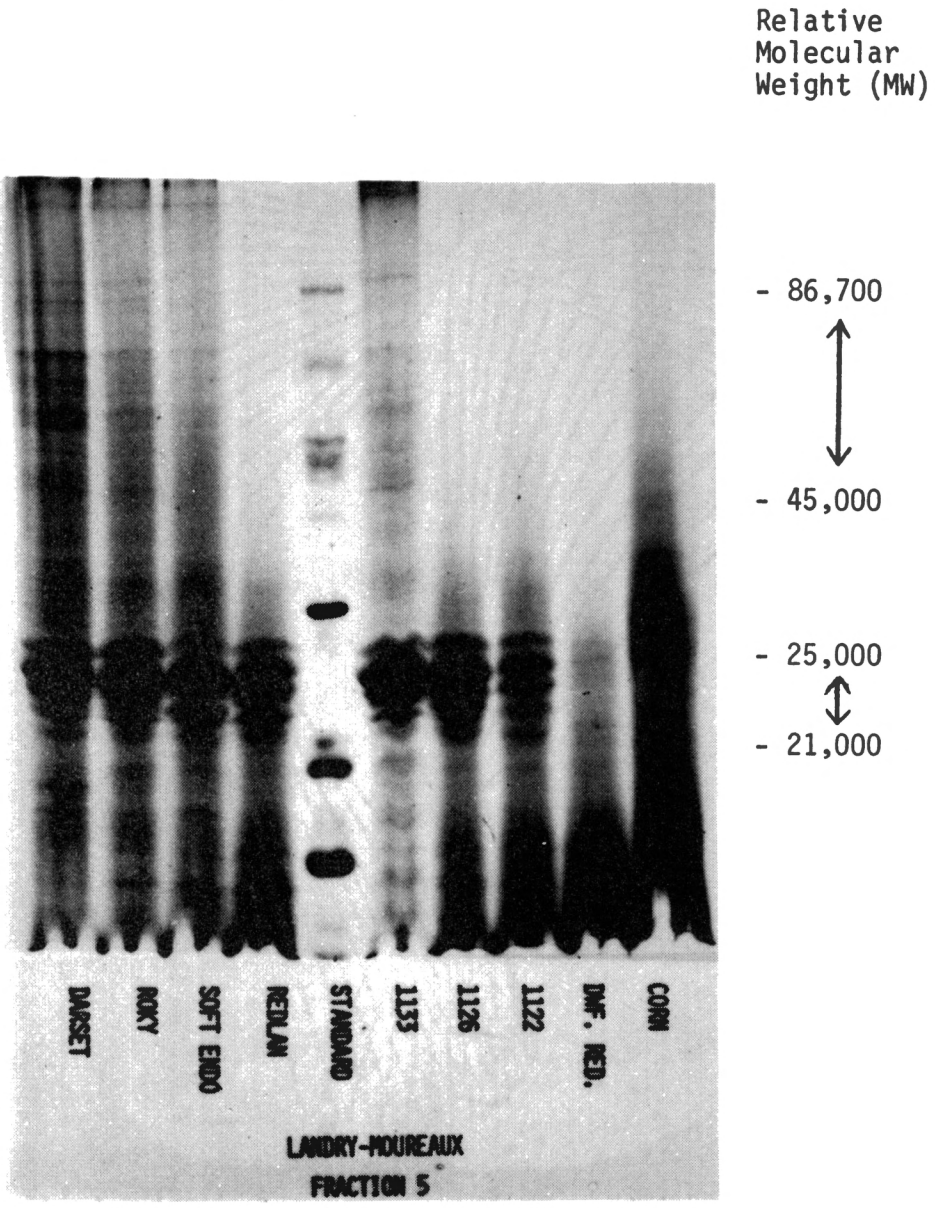


Figure 4. Molecular Weight Profile of Protein From Fraction V

digestible Redlan normal variety was generally intermediate in protein composition between the Waxies and the bird resistants, but tended to be more similar to the Waxy types. The moderately digestible Soft Endo was also intermediate in protein composition, possibly exhibiting the divergent effects of the soft endosperm and brown seed coat. The Waxy bird resistant (1133) showed qualities similar to both the Waxy and bird resistant characteristics. This variety was intermediate in Fraction I, low in Fraction II , high in Fraction IV and also showed higher than average Fraction V concentration.

A relationship between protein composition and digestibility may exist (see Appendix B, Table XXXX). These data suggest that varieties with more soluble protein (Fraction I) will probably be more digestible, while varieties with more insoluble protein (Fraction II and IV) will probably be less digestible. Previous studies have suggested a similar relationship (Jambunathan and Mertz, 1973; Guiragossian et al., 1978). Although differences between Waxy and Normal sorghums may be small, Lightenwalner et al. (1978) utilized varieties differing only in dosage of waxy (wx) gene and illustrated that waxy (wx wx wx) types had a higher nylon bag dry matter digestibility and Fraction I protein concentration than a nonwaxy (Wx Wx Wx) counterpart. Although these relationships are not perfect, our studies suggest that protein composition may be a major factor in determining the digestibility of grain sorghum.

The electrophoresis studies showed that the molecular weight distribution is generally similar across all sorghum types for Fractions I, II and III. However, the series of high molecular weight species observed for the bird resistant types suggest another mechanism by which digestibility may be altered. The glutelin proteins appear to be a

major component of protein matrix of grain sorghum (Seckinger and Wolf, 1973). If the structural matrix of bird resistant sorghums is composed of larger protein molecules than the non-bird resistant types, the accessibility of starch granules in bird resistant sorghums could be greatly hindered by the decreased rate of degradability of large, insoluble proteins. Moreover, if the large proteins are accompanied by an increase in glutelin concentration (Guiragossian et al., 1978), the effect on digestibility may be increased to an even greater extent. It is not known at present if these large protein species are in fact discrete protein molecules or if they are complexes of smaller proteins held together by polyphenols (Guiragossian et al., 1978; Chibber et al., 1978). In either case, the restriction on digestibility may very well be similar.

CHAPTER VII

THE WET-MILLING COMPOSITION OF SEVERAL VARIETIES OF GRAIN SORGHUM AND CORN

Summary

Nine varieties of grain sorghum and four varieties of corn, most grown in three consecutive crop years, were subjected to a wet-milling procedure to determine percent bran and germ, peripheral endosperm cells (PEC), gluten and starch. Wet-milling composition showed that the corn varieties contained lower bran and germ, PEC and gluten and higher starch concentrations than the sorghum types in Years 1 and 2. In addition, more of the total starch in corn was liberated by wet-milling than in the sorghums suggesting decreased structural integrity in the corn varieties. Within sorghum types, differences in wet-milling composition were not large but some differences were apparent. Major changes were observed in the relationship of PEC or gluten content and total starch recovery. In general, sorghum varieties with depressed PEC and/or gluten content showed an increase in total starch recovery suggesting an inverse relationship between PEC or gluten content and total starch recovery. Year effects on digestibility may be partially explained by increased total starch recovery in Year 2 as compared to Years 1 and 3. Protein recovery in each wet-milling constituent was decreased for the corn varieties in Years 1 and 2, implying that more corn protein was solubilized during the steeping process than in the sorghum types.

Total protein recovery was increased for the bird resistant sorghums in Year 2 indicating decreased protein solubilization for these types. This trend, however, was not visible in Years 1 and 3. These studies suggest that wet-milling composition may affect the digestibility of grain sorghum and corn, especially in relation to total starch recovery. In addition, protein recovery, as an indirect measure of protein solubility, may affect the ease with which starch granules are released and digested.

Introduction

The wide variability in nutritive quality of grain sorghum should be related to the biochemical and structural components of the sorghum kernel. Previously, the effects of concentration and digestibility or degradability of major seed constituents such as starch and protein have been discussed (Chapter V and VI). The relationships of starch and protein in regard to total sorghum digestibility are somewhat ambiguous and suggest that other factors may interact. Perhaps one of the most important factors yet to be considered is the structural interrelationships which most certainly occur between starch granules and protein bodies and/or matrix protein. Such a relationship is easy to conceptualize but difficult to measure.

Perhaps the easiest method of measuring the degree of structural interrelationships is by means of the wet-milling procedure. This procedure allows separation of the kernel into four components; bran and germ, peripheral endosperm cells (PEC), gluten and purified starch. Watson et al. (1955), showed that starch yield from a laboratory wet-milling procedure was dependent on endosperm hardness. These workers

also suggested an inverse relationship between starch yield and PEC content. In addition, Norris and Rooney (1970) demonstrated that starch yield and PEC content were inversely related for most sorghum types used in their study. Although conditions for digestion of grain in ruminants do not closely parallel those of the wet-milling procedure, the amount of starch recovered and the concentration of other wet-milling components may be related to starch accessibility and subsequent digestibility. To date, the results of wet-milling studies have been interpreted only in terms of industrial starch recovery. The potential relationship between wet-milling composition and grain sorghum digestibility has not been investigated. Therefore, the objective of this study was to determine the wet-milling characteristics of several varieties of grain sorghum and corn that differ widely in nutritive and agronomic characteristics.

Materials and Methods

Nine varieties of grain sorghum and four varieties of corn, most grown in three consecutive crop years, were utilized to study wet-milling composition. The descriptive characteristics and classification of these varieties were presented previously (Chapter III). Duplicate grain samples from Year 1 (400 grams), Year 2 (300 grams) and Year 3 (200 grams) were cleaned prior to analysis. The laboratory wet-milling procedure was implemented essentially as described by Norris and Rooney (1970), see Appendix A, Table XXXIV. Each grain sample was subjected to a two-phase steeping period for 48 hours and then refrigerated overnight. The steeped grain was ground in a Waring blender with the blades reversed to minimize starch granule damage. This slurry was

washed over a series of U.S. #80, 230 and 325 sieves. The bran and germ remained on the first (#80) sieve, the peripheral endosperm cells on the second (#230) sieve and the coarse gluten on the third (#325) sieve. The solution recovered under the #325 sieve contained a slurry of starch and gluten. This slurry was poured down an aluminum starch table 2.67 meters long with a pitch of 2.54 cm for its entire length. The gluten flowed off and was collected at the end of the table as the denser starch settled on the surface of the table. The starch cake was allowed to air-dry on the table, afterwhich it was removed and dried in a 40 C oven for approximately 48 hours. Other wet-milling components were dried in a 100 C oven for 24 hours. The yield of starch, bran and germ, peripheral endosperm cells and gluten was calculated as a percent of initial sample weight on a 100 percent dry matter basis. Crude protein content was determined on homogenized duplicates of each sample using the Kjeldahl procedure. Protein recovery is presented as a percent of total protein.

The data obtained from the wet-milling procedure can be described by:

$$Y_{ij} = \mu + V_i + E_{ij}$$

where Y_{ij} is bran and germ, PEC, gluten or starch and where V is variety. The components μ and V_i were treated as fixed effects of all records of variety i. Random error effect, E_{ij}, was specific to each observation.

Estimated differences between varietal means were obtained by method of least squares. Significant differences between varietal means were determined by Tukey's HSD test. The error mean squares and respective degrees of freedom for the wet-milling composition are presented in Appendix B, Table XXXIX.

Results and Discussion

Wet-Milling Composition

Wet-milling composition in Year 1 (Table XXI) showed the corn varieties to be lower in bran and germ, peripheral endosperm cells - PEC ($P < .05$) and gluten ($P < .05$) and higher in starch ($P < .05$) than any sorghum variety. Within sorghums, the Soft Endo contained the highest quantity of bran and germ, whereas the Dwarf Redlan was the lowest. Peripheral endosperm cell content was highest for the Redlan and lowest for the Dwarf Redlan. The Redlan variety contained the highest gluten concentration and OK 612 the lowest. Starch yield from wet-milling was highest for the Dwarf Redlan and OK 612 varieties and lowest for the Redlan variety. Starch recovered as a percent of total starch was highest for the corn varieties which were followed by the Dwarf Redlan and Soft Endo sorghums. Wet-milling recovery was essentially similar for all sorghum and corn varieties.

As in Year 1, the corn varieties in Year 2 (Table XXII) contained lower concentrations of bran and germ ($P < .05$), PEC and gluten ($P < .05$) and higher concentrations of starch ($P < .05$) than the sorghum varieties. The Waxy 1122 and 1126 varieties contained the most bran and germ ($P < .05$). Peripheral endosperm cell content was highest for the Waxy 1122 and 1126 in contrast to OK 612 and 1133 which contained the lowest PEC concentration ($P < .05$). Gluten content was highest for the 1133 and Darset varieties, whereas other sorghums were generally similar and lower. Starch yield from wet-milling was similar for all sorghums except the 1122, 1126 and Darset varieties which showed decreased starch yield. The highest total starch recovery was observed for the corn

TABLE XXI

WET-MILLING COMPOSITION OF SORGHUMS AND CORN IN YEAR 1

	Bran & Germ (%)	PEC[1] (%)	Gluten (%)	Starch (%)	Starch[2] Recovery (%)	Wet-Milling[3] Recovery (%)
Waxy						
Dwarf Redlan	16.60^{a}	3.20^{c}	11.00^{b,c}	59.00^{b}	79.4	89.80
Normal						
Redlan	18.60^{a}	6.25^{a}	12.90^{a}	53.65^{c}	71.6	91.40
OK 612	16.65^{a}	5.20^{a,b}	10.65^{c}	58.95^{b}	71.0	91.45
Floury-BR[4]						
Soft Endo	19.00^{a}	4.75^{a,b,c}	11.15^{b,c}	54.40^{b,c}	77.0	89.30
Normal-BR[4]						
Darset	17.70^{a}	4.35^{b,c}	12.20^{a,b}	54.90^{b,c}	73.4	89.15
Corn						
Pioneer 3149	15.05^{a}	1.15^{d}	7.60^{d}	65.15^{a}	82.5	88.95
Pioneer 3306	14.90^{a}	1.10^{d}	7.85^{d}	64.65^{a}	95.2	88.50
SEM (obs./mean)	0.98(2)	0.33(2)	0.24(2)	0.84(2)		

[1]PEC = Peripheral Endosperm Cells.

[2]Starch Recovery = (Starch from Wet-Milling/Total Starch Content) 100.

[3]Wet-Milling Recovery = Bran and Germ + PEC + Gluten + Starch.

[4]BR = Bird Resistant.

^{a,b,c,d}Means within a column with different superscripts are significantly different ($P < .05$).

TABLE XXII

WET-MILLING COMPOSITION OF SORGHUMS AND CORN IN YEAR 2

	Bran & Germ (%)	PEC[1] (%)	Gluten (%)	Starch (%)	Starch[2] Recovery (%)	Wet-Milling[3] Recovery (%)
Waxy						
Dwarf Redlan	16.80^{d}	3.45^{c}	13.60^{b,c,d}	61.00^{b}	95.9	94.85
1122	23.15^{b}	4.60^{a}	12.10^{d}	54.00^{c}	84.6	93.85
1126	26.20^{a}	4.40^{a,b}	14.70^{a,b,c}	47.00^{d}	75.9	92.30
Waxy-BR[4]						
1133	16.90^{d}	1.20^{e}	16.70^{a}	59.20^{b}	94.6	94.00
Normal						
Redlan	16.50^{d}	3.50^{c}	13.10^{c,d}	60.30^{b}	89.2	93.40
OK 612	15.80^{d}	2.20^{d}	13.20^{c,d}	61.65^{b}	84.6	92.85
Floury-BR[4]						
Soft Endo	20.10^{c}	3.50^{c}	12.75^{c,d}	59.70^{b}	85.9	96.05
Normal-BR[4]						
Darset	20.10^{c}	3.95^{b,c}	15.85^{a,b}	53.25^{c}	74.3	93.15
Corn						
Pioneer	14.50^{e}	0.90^{e}	6.45^{e}	70.40^{a}	100.6	92.25
NK	14.30^{e}	1.15^{e}	6.45^{e}	70.15^{a}	99.8	92.05
SEM (obs./mean)	0.19(2)	0.10(2)	0.44(2)	0.52(2)		

[1]PEC = Peripheral Endosperm Cells.

[2]Starch Recovery = (Starch from Wet-Milling/Total Starch Content) 100.

[3]Wet-Milling Recovery = Bran and Germ + PEC + Gluten + Starch.

[4]BR = Bird Resistant.

^{a,b,c,d,e}Means within a column with different superscripts are significantly different ($P < .05$).

varieties followed by the Dwarf Redlan and 1133 sorghums. Depressed total starch recovery was noted for the 1126 and Darset varieties.

As in Year 2, the highest ($P < .05$) bran and germ concentrations in Year 3 (Table XXIII) was observed for the Waxy 1126 variety. The lowest concentration of bran and germ was observed for the Dwarf Redlan. The Redlan variety contained the most PEC ($P < .05$) followed by the Normal-BR Darset and ROKY 78. As in Year 2, the lowest PEC concentration was observed for the Waxy-BR 1133. Gluten content was highest in the 1133 and ROKY 78 varieties. The Soft Endo contained the lowest gluten content, somewhat similar to Year 2. Starch yield from wet-milling was similar to most sorghums except the ROKY 78 and 1126 ($P < .05$) which showed depressed recovery. Total starch recovery was similar for most sorghums except the Soft Endo which showed an increase and the ROKY 78 which showed a depression in total starch recovery in relation to the other sorghums.

The wet-milling studies for Years 1 and 2 suggest that corn varieties may contain lower concentrations of bran and germ, PEC and gluten than sorghum varieties. In addition, more starch was recovered during wet-milling for the corn varieties than the sorghums. Major differences appear to be concentrated in the PEC and gluten portions which would suggest that less starch is tied up in these fractions in corn than in sorghum. The increase in total starch recovery for the corn varieties may help explain why corn is usually somewhat more digestible than sorghum.

The wet-milling studies also illustrate that the concentration of bran and germ, PEC and gluten was generally similar for each sorghum variety across years. The starch yield from wet-milling, however, was more variable within varieties across years and may explain some of the

TABLE XXIII

WET-MILLING COMPOSITION OF SORGHUM IN YEAR 3

	Bran & Germ (%)	PEC[1] (%)	Gluten (%)	Starch (%)	Starch[2] Recovery (%)	Wet-Milling[3] Recovery (%)
Waxy						
Dwarf Redlan	16.65^{d}	2.50^{b,c,d}	12.10^{b,c}	61.35^{a}	77.2	92.60
1122	19.35^{c}	2.30^{c,d}	11.05^{c}	58.45^{a,b}	78.4	91.15
1126	24.85^{a}	2.10^{c,d}	13.15^{a,b}	50.45^{d}	75.8	90.55
Waxy-BR[4]						
1133	17.15^{d}	1.75^{d}	14.70^{a}	58.35^{a,b}	77.1	91.95
Normal						
Redlan	18.10^{c,d}	4.80^{a}	13.20^{a,b}	56.95^{b,c}	73.9	93.05
Floury-BR[4]						
Soft Endo	18.70^{c,d}	2.05^{d}	10.50^{c}	58.55^{a,b}	81.3	89.80
Normal-BR[4]						
Darset	17.90^{c,d}	3.40^{b}	13.30^{a,b}	60.55^{a}	76.6	95.15
ROKY 78	22.15^{b}	3.15^{b,c}	14.25^{a}	54.60^{c}	70.2	94.15
SEM(obs./mean)	0.38(2)	0.19(2)	0.32(2)	0.54(2)		

[1]PEC = Peripheral Endosperm Cells.

[2]Starch Recovery = (Starch from Wet-Milling/Total Starch Content) 100.

[3]Wet-Milling Recovery = Bran and Germ + PEC + Gluten + Starch.

[4]BR = Bird Resistant.

^{a,b,c,d}Means within a column with different superscripts are significantly different ($P < .05$).

differences in relative sorghum digestibility that can be observed across years (Chapter III).

Perhaps more pertinent relationships to digestibility can be drawn from the total starch recovery data. In general, the poorly digestible bird resistants and intermediately digestible Normal sorghums showed less total starch recovery than the Waxy types. In addition, total starch recovery appeared to be fairly closely related to PEC and, to a certain extent, gluten content. Although these relationships do not appear to be strong, an apparent inverse relationship exists between PEC or gluten content and total starch recovery (Norris and Rooney, 1970). It is also noteworthy that total starch recovery was much higher for most sorghums in Year 2 than in Years 1 and 3. This difference in total starch recovery may also help explain some of the year effects observed for sorghum digestibility (Chapter III).

The wet-milling recovery is simply the total of the four wet-milling components; bran and germ, PEC, gluten and starch. The unaccounted for portion would probably be soluble sugars and proteins that were extracted during the steeping process. One might expect large differences in this unaccounted for fraction due to differences in protein solubility observed across different sorghum types (Chapter VI). Differences in wet-milling recovery across sorghum and corn types were not apparent thereby suggesting that this fraction may not plan an important role in determining digestibility of grain sorghum and corn.

Protein Recovery

Protein recovery as a percent of total protein for Year 1 (Table XXIV) showed that the bran and germ and PEC fractions of corn accounted

TABLE XXIV

PROTEIN RECOVERY IN WET-MILLING COMPONENTS
FOR YEAR 1

	Bran & Germ[1] (%)	PEC[1,2] (%)	Gluten[1] (%)	Total Protein Recovery[3] (%)
Waxy				
Dwarf Redlan	20.92	6.31	42.76	69.99
Normal				
Redlan	20.45	9.14	40.08	69.67
OK 612	20.99	8.07	38.06	67.12
Floury-BR[4]				
Soft Endo	24.28	8.53	39.52	72.33
Normal-BR[4]				
Darset	22.69	8.09	35.87	66.65
Corn				
Pioneer 3149	16.33	2.56	39.79	58.68
Pioneer 3306	14.22	1.83	35.95	52.00

[1]Protein Recovery = $\left[\frac{\% \text{ of Component X Crude Protein Content of Component}}{\text{Total Crude Protein Content}}\right]100$.

[2]PEC = Peripheral Endosperm Cells.

[3]Total Protein Recovery = Bran and Germ + PEC + Gluten.

[4]BR = Bird Resistant.

for less of the total protein than in the sorghums. Protein recovery in the gluten fraction was similar for the corn and sorghum types. This difference is reflected in decreased total protein recovery for the corn in relation to the sorghum varieties. Within sorghums, the Soft Endo recovered more protein in the bran and germ and the Dwarf Redlan recovered less protein in the PEC than other varieties represented.

Protein recovery in Year 2 (Table XXV) again showed that the corn varieties contained lower protein recoveries in the bran and germ and PEC fractions than the sorghums. In contrast to Year 1, protein recovery in the gluten fraction was also much lower than most sorghums resulting in an extremely depressed total protein recovery for the corn in relation to the sorghums. The most striking difference within the sorghum types was the low PEC and high gluten protein recovery for the Waxy-BR 1133. Total protein recovery was highest for the 1133, Soft Endo and Darset varieties which all carried the bird resistant characteristics.

In Year 3 (Table XXVI), protein recovery was generally similar for most varieties. The Waxy-BR 1133, however, did show decreased PEC and increased gluten recovery as in Year 2. Total protein recovery was similar for most sorghum types except the Soft Endo which showed depressed protein recovery in Year 3.

Protein recovery in Years 1 and 2 illustrates that the corn varieties used in this study had more protein solubilized during steeping than the sorghum. Increased protein solubilization can probably account for the high starch recoveries observed for the corn varieties during wet-milling. Within sorghum types, the depressed PEC and increased gluten protein recovery for the Waxy-BR 1133 suggests that different

TABLE XXV

PROTEIN RECOVERY IN WET-MILLING COMPONENTS
FOR YEAR 2

	Bran & Germ[1] (%)	PEC[1,2] (%)	Gluten[1] (%)	Total Protein Recovery[3] (%)
Waxy				
Dwarf Redlan	25.72	5.77	46.46	77.95
1122	34.61	7.02	35.22	76.85
1126	34.41	9.36	34.16	77.93
Waxy-BR[4]				
1133	21.94	2.75	57.76	82.45
Normal				
Redlan	24.11	7.36	44.58	76.05
OK 612	22.45	5.02	45.49	72.96
Floury-BR[4]				
Soft Endo	31.90	8.45	50.45	90.80
Normal-BR[4]				
Darset	31.37	9.95	42.52	83.84
Corn				
Pioneer	15.36	2.16	30.50	48.02
NK	14.64	2.49	29.47	46.60

[1]Protein Recovery = $\left[\frac{\text{\% of Component X Crude Protein Content of Component}}{\text{Total Crude Protein Content}}\right]100$.

[2]PEC = Peripheral Endosperm Cells.

[3]Total Protein Recovery = Bran and Germ + PEC + Gluten.

[4]BR = Bird Resistant

TABLE XXVI

PROTEIN RECOVERY IN WET-MILLING COMPONENTS
FOR YEAR 3

	Bran & Germ[1] (%)	PEC[1,2] (%)	Gluten[1] (%)	Total Protein Recovery[3] (%)
Waxy				
Dwarf Redlan	24.89	6.13	44.30	75.32
1122	29.02	6.09	40.48	75.59
1126	30.84	6.06	41.22	78.12
Waxy-BR[4]				
1133	23.84	3.64	49.99	77.47
Normal				
Redlan	22.44	9.06	42.99	74.49
Floury-BR[4]				
Soft Endo	20.94	5.26	38.45	64.65
Normal-BR[4]				
Darset	27.21	8.20	46.01	81.42
ROKY 78	29.75	6.61	42.35	78.71

[1]Protein Recovery = $\left[\frac{\%\text{ of Component X Crude Protein Content of Component}}{\text{Total Crude Protein Content}}\right]100.$

[2]PEC = Peripheral Endosperm Cells.

[3]Total Protein Recovery = Bran and Germ + PEC + Gluten.

[4]BR = Bird Resistant.

structural relationships may occur in this variety in comparison to the other sorghums. Total protein recovery was similar for most sorghums in Years 1 and 3 but the bird resistant types showed higher total protein recovery in Year 2. Although this response was not observed in Years 1 and 3, depressed soluble protein content could theoretically account for decreased digestibility of bird resistant types.

CHAPTER VIII

SUMMARY

Several varieties of grain sorghum and corn were utilized to: (1) evaluate the relative nutritive value of sorghums that differ widely in nutritive and agronomic characteristics and (2) evaluate major factors such as starch and protein as to their effect on relative nutritive value. Nine varieties of dryland sorghum and four varieties of irrigated corn were obtained for three consecutive crop years, however not all varieties were represented in each year. Five different sorghum types were represented; Waxy, Waxy-BR (BR=bird resistant), Normal, Floury-BR and Normal-BR.

In vitro dry matter disappearance (IVDMD) studies revealed that the corn varieties were generally superior in relative digestibility to the sorghum types, especially the Normal-BR sorghums. The Waxy and Normal sorghums were similar in IVDMD and somewhat intermediate to the corn and Normal-BR sorghums. The Floury-BR performed similar to the Waxies in Years 1 and 2 but showed a depressed response in Year 3. The Waxy-BR sorghum was generally intermediate in value in Years 2 and 3. In addition, the range in digestibility was different for all three years being wide in Years 1 and 3 and relatively narrow in Year 2.

In vitro gas production (IVGP) studies were implemented to investigate the relative starch availability of the grains. In general, trends observed in relative digestibility (IVDMD) were supported by

similar trends in IVGP with the exception of the Floury-BR which showed extremely high starch availability. Evidently the beneficial effect of the soft endosperm of this variety was mediated by the presence of the brown seed coat. Generally, the IVDMD and IVGP studies suggested that some grain sorghum varieties are much more similar to corn than others. In addition, the wide range in relative digestibility observed for the sorghum varieties may explain some of the wide variability often associated with this grain.

In order to determine if all sorghum types responded the same to processing, all grain samples were reconstituted and evaluated for IVDMD and IVGP. The IVDMD studies revealed that the bird resistant sorghums responded much more favorably to reconstitution than the non-bird resistant types. IVGP studies showed large increases in starch availability for all grains but again the bird resistants were improved to the greatest extent. This variety dependent response was verified by significant ($P < .01$) treatment by variety interactions for all studies except Year 2 IVDMD. These studies suggest that the reconstitution response is variety dependent and that this process has an equalizing effect on nutritive value across varieties due to the fact that bird resistants were improved to a greater extent than other sorghum types.

The major constituent of most cereal grains is starch. Major differences in the digestibility or nutritive value of starch obtained from different varieties might be expected to alter whole grain digestibility. Results of IVDMD studies on purified, isolated starch suggested that rumen microbes show no preference for any particular varietal starch. Gas production studies utilizing amyloglucosidase enzyme, however, showed that sorghum starch was generally superior to corn starch. In addition,

Waxy sorghum starches were generally favored over non-waxy sorghum starches. Alpha-amylase digestion of purified starches generally favored the Waxy starches although differences were not as great. These results suggest that enzymatic digestion of purified starches may be highly dependent on starch source or type. Ruminal digestion, as exhibited by IVDMD, was not different for any particular starch suggesting that once starch granules are exposed or released in the rumen, the rate of starch digestion should proceed independent of variety or type. In addition, these studies suggest that other factors, such as protein or tannins, may have a major effect on determining the degree of starch granule accessibility and subsequent digestibility.

The second largest component of most cereal grains is protein. Total crude protein content, however, does not appear to have a distinguishable effect on digestibility. In our studies, Landry-Moureaux fractionation of the sorghum and corn varieties for Years 2 and 3 showed that the bird resistant sorghums generally contained lower concentrations of highly soluble albumins and globulins and higher concentrations of insoluble kafirin and glutelin protein than the more highly digestible Waxy sorghums. Other sorghum types showing intermediate digestibility also showed protein composition intermediate to the bird resistant and Waxy sorghums. These observations suggest that sorghum varieties with more soluble protein will probably be more digestible than sorghums with more insoluble protein.

The molecular weight distribution of proteins within each Landry-Moureaux fraction was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis for Year 3 sorghums. The electrophoretic profiles showed no unusual protein species in the albumin and globulin or

kafirin fractions for any sorghum variety. In the glutelin fraction, however, any variety carrying the bird resistant characteristic showed a series of high molecular weight (> 45,000 MW) species that were not observed in the non-bird resistant sorghums. Hypothetically, the high molecular weight proteins in conjunction with higher glutelin concentrations may interact to depress the digestibility of bird resistant sorghums.

Another potential factor that may influence the digestibility of grain sorghum is the degree of structural integrity in the kernel. A laboratory wet-milling procedure was utilized to determine the concentration of bran and germ, peripheral endosperm cells (PEC), gluten and recoverable starch in the sorghum and corn varieties. The greatest difference was observed between the corn and sorghum varieties where the corn showed decreased bran and germ, PEC and gluten and increased starch in comparison to the sorghums. In addition, more total starch was recovered during wet-milling for the corn varieties. Within sorghums, there appeared to be an inverse relationship between PEC or gluten content and starch recovery. Protein recovery in each wet-milling fraction showed that the corn varieties also recovered less of their total protein in the bran and germ, PEC and gluten fractions suggesting that more corn protein is solubilized during wet-milling than in sorghums. These studies suggest that wet-milling composition, as a measure of structural integrity, may have an effect on sorghum and corn digestibility. Moreover, protein recovery, as an indirect measure of protein solubility, may be a major factor indicating the ease with which starch granules are released from the kernel.

The results of these studies suggest that some questions regarding

the nutritive value of grain sorghum have been answered to an extent, but that many more questions remain. For example, the role of purified starch and protein composition appears to be major, but the quantification of these effects and their inference in relation to other sorghums of similar type remains to be determined. In addition, the role of other major seed constituents such as tannins has yet to be elucidated. Differences in total digestibility across years appear to be substantial, however the effects of environmental factors such as precipitation or fertilization remain undefined. Also important, is the further classification and characterization of many sorghum varieties of different types which is needed to develop a practical system for analyzing and classifying grain sorghum in terms of nutritive value. Finally, the challenge remains to the animal scientist to develop new and more efficient methods of processing grain sorghum and to the plant breeder to develop new genetic strains of grain sorghum that combine the best characteristics of agronomic and nutritive value.

LITERATURE CITED

Adams, C. A., and L. Novellie. 1975. Composition and Structure of Protein Bodies and Spherosomes Isolated From Ungerminated Seeds of Sorghum Bicolor (Linn.) Moench. Plant Physiol. 55:1.

Breuer, L. H., Jr., and C. K. Dohm. 1972. Comparative Nutritive Value of Several Sorghum Grain Varieties and Hybrids. J. Agr. Food. Chem. 20:83.

Buchanan-Smith, J. G., R. Totusek and A. D. Tillman. 1968. Effect of Methods of Processing on Digestibility and Utilization of Grain Sorghum by Cattle and Sheep. J. Anim. Sci. 27:525.

Chibber, Bakshy A. K., E. T. Mertz and J. D. Axtell. 1978. Effects of Dehulling on Tannin Content, Protein Distribution and Quality of High and Low Tannin Sorghum. J. Agr. Food Chem. 26:679.

Croka, D. C. and D. G. Wagner. 1975. Micronized Sorghum Grain. II. Influence on in vitro Digestibility, in vitro Gas Production and Gelatinization. J. Anim. Sci. 40:931.

Davis, A. B., and L. H. Harbers. 1974. Hydrolysis of Sorghum Grain Starch by Rumen Microorganisms and Purified Porcine α-Amylase as Observed by Scanning Electron Microscopy. J. Anim. Sci. 38:900.

Ely, D. G., C. O. Little, P. G. Woolfolk and G. E. Mitchell, Jr. 1967. Estimation of the Extent of Conversion of Dietary Zein to Microbial Protein in the Rumen of Lambs. J. Nutr. 91:314.

Garrett, W. N., G. P. Lofgreen and R. L. Hull. 1968. Steam Pressure Processed Sorghum Grain for Steers. J. Anim. Sci. 27:1164 (Abstr.).

Greenwood, C. T. 1970. Starch and Glycogen. In W. Pigman and D. Horton (Ed.). The Carbohydrates. Vol. IIB. Academic Press, New York.

Guiragossian, V., B. A. K. Chibber, S. Van Scoyoc, R. Jambunathan, E. T. Mertz and J. D. Axtell. 1978. Characteristics of Proteins from Normal, High Lysine and High Tannin Sorghums. J. Agr. Food Chem. 26:219.

Hale, W. H., L. Cuitin, W. J. Saba, B. Taylor and B. Theurer. 1966. Effect of Steam Processing and Flaking Milo and Barley on Performance and Digestion by Steers. J. Anim. Sci. 25:392.

Hinders, R. G. and K. Eng. 1971. Starch Availability of Various Grain Sorghums. J. Anim. Sci. 33:285 (Abstr.).

Hinders, R. G. and J. Freeman. 1970. Effects of Processing Techniques on Gelatinization and Starch Availability of Grain Sorghum. Proc. West. Sect. ASAS. 20:253.

Hinman, D. D. 1973. Influence of Processing Method on Site and Extent of Milo Starch Digestion in Ruminants. Okla. Agr. Exp. Sta., Ph.D. Thesis.

Hinman, D. D. and R. R. Johnson. 1973. Site and Extent of Milo Starch Digestion as Influenced by Degree of Processing. J. Anim. Sci. 37:345 (Abstr.).

Hinman, D. D. and R. R. Johnson. 1974. Influence of Processing Methods on Digestion of Sorghum Starch in High Concentrate Beef Cattle Rations. J. Anim. Sci. 39:417.

Horan, F. E. and M. F. Heider. 1946. A Study of Sorghum and Sorghum Starches. Cereal Chem. 23:492.

Jambunathan, Ramamurthi and E. T. Mertz. 1973. Relationship Between Tannin Levels, Rat Growth and Distribution of Proteins in Sorghums. J. Agr. Food Chem. 21:692.

Khoo, U. and M. J. Wolf. 1970. Origin and Development of Protein Granules in Maize Endosperm. Amer. J. Bot. 57:1042.

Landry, J. and Therese Moureaux. 1970. Heterogeneite Des Glutelines Du Grain De Mais: Extraction Selective Et Composition en Acides Amines Des Trois Fractions Isolees. Bull. Soc. Chim. Biol. 52:1021.

Leach, H. W. 1965. In R. L. Whistler (Ed.). Starch: Chemistry and Technology. Vol. I. Academic Press, New York.

Leach, H. W. and T. J. Schoch. 1961. Structure of the Starch Granule. II. Action of Various Amylases on Granular Starches. Cereal Chem. 38:34.

Lichtenwalner, R. E., E. B. Ellis and L. W. Rooney. 1978. Effect of Incremental Dosages of the Waxy Gene of Sorghum on Digestibility. J. Anim. Sci. 46:1113.

Luchsinger, W. W. 1966. Carbohydrases of Barley and Malt. Cereal Sci. Today. 11:69.

Macrae, J. C. and D. G. Armstrong. 1968. Enzyme Method for Determination of α-Linked Glucose Polymers in Biological Materials. J. Sci. Fd. Agric. 19:578.

Maxson, W. E., R. L. Shirley, J. E. Bertrand and A. Z. Palmer. 1973. Energy Values of Corn, Bird-Resistant and Non-Bird-Resistant Sorghum Grain in Rations Fed to Steers. J. Anim. Sci. 37:1451.

McCollough, R. L. 1972a. Hybrid Sorghum and Corn Characteristics and Methods Used to Nutritionally Evaluate Them. Kan. Agr. Exp. Sta. Bull. 557:15.

McCollough, R. L., C. L. Drake and G. M. Roth. 1972b. Feedlot Performance of Eight Hybrid Sorghum Grains and Three Hybrid Corns. Kan. Agr. Exp. Sta. Bull. 557:21.

McCollough, R. L. and B. E. Brent. 1972c. Digestibility of Eight Hybrid Sorghum Grains and Three Hybrid Corns. Kan. Agr. Exp. Sta. Bull. 557:27.

McCready, R. M. and W. Z. Hassid. 1950. The Separation and Quantitative Estimation of Amylose and Amylopectin in Potato Starch. Anal. Chem. 22:1154.

McGinty, D. D., L. H. Breuer and J. K. Riggs. 1967. Digestibility of Dry and Reconstituted Sorghum Grain by Cattle. J. Anim. Sci. 26:223.

McGinty, D. D. and J. K. Riggs. 1968. Variation in Digestibility of Sorghum Grain Varieties. J. Anim. Sci. 27:1170 (Abstr.).

McNeill, J. W., G. D. Potter and J. K. Riggs. 1971. Ruminal and Postruminal Carbohydrate Utilization in Steers Fed Processed Sorghum Grain. J. Anim. Sci. 33:1371.

McNeill, J. W., G. D. Potter, J. K. Riggs and L. W. Rooney. 1975. Chemical and Physical Properties of Processed Sorghum Grain Carbohydrates. J. Anim. Sci. 40:335.

Miller, F. R., R. S. Lowrey, W. G. Monson, G. W. Burton and H. J. Cruzado. 1972. Estimates of Dry Matter Digestibility Differences in Grain of Some Sorghum Bicolor (L.) Moench Varieties. Crop Sci. 12:563.

Misra, P. S. and E. T. Mertz. 1975. Studies on Corn Proteins VII. Developmental Changes in Endosperm Proteins of High Lysine Mutants. Cereal Chem. 52:734.

Misra, P. S., E. T. Mertz and D. V. Glover. 1976a. Studies on Corn Proteins. IX. Comparison of the Amino Acid Composition of Landry-Moureaux and Paulis-Wall Endosperm Fractions. Cereal Chem. 53:699.

Misra, P. S., E. T. Mertz and D. V. Glover. 1976b. Studies on Corn Proteins. X. Polypeptide Molecular Weight Distribution in Landry-Moureaux Fractions of Normal and Mutant Endosperms. Cereal Chem. 53:705.

Nishimuta, J. F., L. B. Sherrod and R. D. Furr. 1969. Digestibility of Regular, Waxy and White Sorghum Grain Rations by Sheep. Proc. West. Sect. ASAS. 20:259.

Norris, J. R. and L. W. Rooney. 1970. Wet-Milling Properties of Four Sorghum Parents and Their Hybrids. Cereal Chem. 47:64.

Osborne, T. B. and L. B. Mendel. 1914. Nutritional Properties of Proteins of Maize Kernel. J. Biol. Chem. 18:1.

Osman, H. F., Brent Theurer, W. H. Hale and S. M. Mehen. 1970. Influence of Grain Processing on in vitro Enzymatic Starch Digestion of Barley and Sorghum Grain. J. Nutr. 100:1133.

Potter, G. D., J. W. McNeill and J. K. Riggs. 1971. Utilization of Processed Sorghum Grain Proteins by Steers. J. Anim. Sci. 32:540.

Price, M. L., S. Van Scoyoc and L. G. Butler. 1978. A Critical Evaluation of the Vanillin Reaction as an Assay for Tannin in Sorghum Grain. J. Agr. Food Chem. 26:1214.

Radley, J. A. 1968. Starch and its Derivatives (4th Ed.). Chapman and Hall, Ltd., London.

Riggs, J. K. and D. D. McGinty. 1970. Early Harvested and Reconstituted Sorghum Grain for Cattle. J. Anim. Sci. 31:991.

Saba, W. J., W. H. Hale and B. Theurer. 1972. In Vitro Rumen Fermentation Studies With a Bird Resistant Sorghum Grain. J. Anim. Sci. 35:1076.

Samford, R. A., J. K. Riggs, G. D. Potter, L. W. Rooney and Janice Coon. 1970. In Vitro and In Vivo Digestibility of Sorghum Grain Varieties. Tex. Agr. Exp. Sta. Prog. Rpt. PR - 2784.

Sandstedt, R. M., D. Strohen, S. Verda and R. E. Abbot. 1962. The Digestibility of High-Amylose Corn Starches Compared to that of Other Starches. The Apparent Effect of the AE Gene on Susceptibility to Amylase Action. Cereal Chem. 39:123.

Seckinger, H. L. and M. J. Wolf. 1973. Sorghum Protein Ultrastructure as it Relates to Composition. Cereal Chem. 50:455.

Sherrod, L. B., R. C. Albin and R. D. Furr. 1969. Net Energy of Regular and Waxy Sorghum Grains for Finishing Steers. J. Anim. Sci. 29:997.

Sullins, R. D. and L. W. Rooney. 1974. Microscopic Evaluation of the Digestibility of Sorghum Lines That Differ in Endosperm Characteristics. Cereal Chem. 51:134.

Sullins, R. D., L. W. Rooney and J. K. Riggs. 1971. Physical Changes in the Kernel During Reconstitution of Sorghum Grain. Cereal Chem. 48:567.

Totusek, R., L. Franks, W. Basler and R. Renbarger. 1967. Methods of Processing Milo for Fattening Cattle. Okla. Agr. Exp. Sta. Misc. Publ. 79:79.

Totusek, R. and D. White. 1969. Methods of Grain Processing and the Effects on Feed Efficiency and Rate of Gain. Okla. Cattle Feeders' Seminar Proceeding, p. 1-F.

Wagner, D. G., R. Christensen and W. Holloway. 1971. Influence of Storage Time and Moisture Level on Feeding Value of Whole Reconstituted Milo for Fattening Cattle. Okla. Agr. Exp. Sta. MP - 85:64.

Walker, R. D. and R. E. Lichtenwalner. 1977. Effect of Reconstitution on Protein Solubility and Digestibility of Waxy Sorghum. J. Anim. Sci. 44:843.

Watson, S. A., E. J. Sanders, R. D. Wakely and C. B. Williams. Peripheral Cells of the Endosperms of Grain Sorghum and Corn and Their Influence on Starch Purification. Cereal Chem. 32:165.

White, D. and R. Totusek. 1969. The Effect of Moisture Level on the Feeding Value of Reconstituted Milo. Okla. Agr. Exp. Sta. Misc. Publ. MP - 82:43.

APPENDIX A

PROCEDURES

TABLE XXVII

VANILLIN-HCL PROCEDURE FOR TANNINS

Reagents:

1. 8% HCL in methanol.
2. 4% vanillin in methanol (fresh daily).
3. Vanillin HCL reagent:
   ---mix equal volumes of 1 & 2 immediately before use.
4. Extraction reagent:
   ---1% HCL in methanol
5. Standards:
   ---100 mg catechin in 50 ml methanol

Std.	MeOH
8 ml	2 ml
6 ml	4 ml
4 ml	6 ml
2 ml	8 ml
0 ml	10 ml

Procedure:

1. Place 1 g sample in 125 ml Ehrlenmeyer.
2. Add 50 ml methanol: 1% HCL, stopper.
3. Shake for 24 (20-28) hours.
4. Centrifuge at 2000 x g for 15 minutes.
5. Pipet 1 ml of each sample or standard into test tubes in duplicate, place in 30^{o}C water bath.
6. At timed intervals, add 5 ml vanillin-HCL to each.
7. After 20 minutes, read at 500 nm (525 nm).
   ---Use vanillin-HCL blank

Source: Price et al., 1978.

TABLE XXVIII

MODIFIED LANDRY-MOUREAUX PROCEDURE

			Ext. Time
	1.	Weigh approximately 20 g sample.	
	2.	Add 200 ml petroleum ether for 1 hour, stir occasionally, filter.	
	3.	Weigh 10 g defatted sample, (4 places), place in 250 ml centrifuge bottle.	
FI	4.	Add 100 ml cold 0.5 M NaCL, stir 1 hour at 4°C., centrifuge, decant, save supernatant and repeat for two more 30 minute extraction periods.	60 30 30
	5.	Add 100 ml cold H_2O, stir for 15 minutes at 4°C, centrifuge, decant and repeat for 15 minutes.	15 15
FII	6.	Add 100 ml 70% Isopropanol for three 30 minute extraction periods following same procedure as #4.	30 30 30
FIII	7.	Add 100 ml Isopropanol-mercaptoethanol for two 30 minute periods same as #4.	30 30
FIV	8.	Add 100 ml Borate-mercaptoethanol for one 60 minute and one 30 minute extraction period, same as #4.	60 30
FV	9.	Add 100 ml Borate-mercaptoethanol-SDS for one 60 minute, one 30 minute and one 15 minute extraction period, same as #4.	60 30 15
	10.	Perform micro-Kjeldahl analysis on duplicate samples of each extract.	

Source: Landry and Moureaux, 1970.

TABLE XXIX

SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

SDS Polyacrylamide Slab Gels

Wash plates with soap and water, final rinse is with deionized water. Before clipping plates together wash with MeOH.

Apply a thin layer of vaseline along the edges of the large square plate. Place the spacers on this line. Apply a second layer of vaseline along the edges of the spacers. Place small notched plate on spacers and secure with large binder clips.

Quickly add complete resolving gel solution with pipet. Leave 3/4 inch space between the bottom of the notched area and the top of the gel solution. This space will be filled with stacking gel. (The stacking gel provides sample wells and allows initial stacking of proteins).

Gently tap the bottom of the plates to remove air bubbles. Layer resolving gel with butanol. Allow 7.5% gels one hour to polymerize and gradient gels should polymerize over night.

Pour off butanol and remove remaining butanol with blotter paper. Insert sample well comb and pour in stacking gel solution, this will polymerize in $\simeq$ 15 minutes.

Remove comb and insert small strips of blotter paper into each well to remove excess liquid.

Remove bottom spacer and load sample wells, fill the remaining space in well with Tris-Glycine buffer. Apply a heavy line of vaseline around notched area of plate for adhesion to electrophoresis unit.

Place notched plate facing unit and secure to unit with rubber hoses. Gently pour Tris-Glycine buffer in top chamber. (Watch for leakage, buffer level must be above notched area of small plate).

Fill bottom chamber, buffer level must be above bottom of gel. Remove trapped bubbles between plates.

Run samples through stacking gel at 8 mAMP. When dye front reaches resolving gel portion increase current to 12-15 mAMP.

If running gel overnight, set current at 3 mAMP for stacking and resolving gel portions.

TABLE XXIX (continued)

When gels are finished place in the following series of washings:

1.	25% i-ProH + 10% Hac	60 minutes
2.	25% i-ProH + 10% Hac + .025% R-250 Coomassie Blue	30 minutes
3.	10% Hac + Foam Sponge	90 minutes
4.	10% MeOH (2 washings)	30 minutes each
5.	10% MeOH + 1% glycerol (2 washings)	30 minutes each

STOCK SOLUTIONS

Acrylamide

30% acrylamide
8% bis acrylamide

Tris-Glycine buffer pH 8.3

.025 M Tris base (THAM)
.192 M Glycine (not HCl-glycine)
.10% SDS

Resolving gel buffer pH 8.8

18.5g Tris HCl
77.0g Tris base
2.0g SDS/500 ml H_2O

Stacking Gel Solution pH 6.8

7.5g Tris HCl
.368 Tris base
.4g SDS/100 ml H_2O

COMPLETE SOLUTIONS

Stock	Stacking Solution	7.5%	12%
Stacking gel	6.25	--	--
Resolving gel	--	10	20
Acrylamide solution	3.3	10	20
H_2O	15.25	15	10

To calculate different percent gels:

Resolving gel buffer		25%
30% Acrylamide		3.33 x % gel
H_2O		QS
APS (.1g/ml)		.25%
temed	(for up to 10%)	.03%
	(for 10-20%)	.02%

TABLE XXX

SOLUBLE STARCH ASSAY (α-AMYLASE)

1. Weigh 100 ± 0.5 mg starch for each sample plus 100 mg of soluble starch (Lintner) into 150 ml Berzelius beaker. Waxy starches may require 500 to 800 mg to get adequate soluble starch concentrations.

2. Add 100 ml of Acetate Buffer and stir bar, cover with foil and heat just until boiling. Remove beaker to stir plate to cool. After cooling, centrifuge samples at about 3500 rpm for 15 minutes. A white pellet of amylopectin should form at the bottom of each tube. Return supernatant to cleaned beaker, material may be covered with parafilm and stored overnight in refrigerator if necessary.

3. Starch-Iodine Assay: Pipet 0.0, 0.1, ... 1.0 ml of soluble starch sample in triplicate for standard curve. Dilute all standards to 1 ml total volume. Pipet 1.0 ml of each starch sample in triplicate also. Add 1.0 ml of dilute Iodine reagent to each tube and vortex. Read absorbance at λ = 620 nm, determine mls of each sample needed to adjust starch concentration to that of lowest sample.

4. Pipet calculated milliliters of each sample into 6 tubes and label 3 with enzyme and 3 without enzyme. Dilute with acetate buffer to a volume of 1 ml. Place all tubes in 30 C water bath, separating with from without enzyme tubes. Add 100 microliters of α-amylase (100 mg/ 20 ml) to each enzyme tube for a 5 minute incubation. After incubation, add 1.0 ml of Nelson's Reagent (A+B) at same timed intervals to stop reaction. Add 1.0 ml of Nelson's Reagent (A+B) to without enzyme tubes also and proceed with Nelson's Assay step 2 (Table XXXII). XXXVII). In calculations, use without enzyme tubes to correct for reducing sugars present before α-amylase digestion.

5. Reagents:

   Enzyme:

   100 mg α-amylase/20 ml acetate buffer.
   Store in refrigerator or freezer, place in ice during assay.

   Iodine reagent (stock):

   0.1776 g I
   + 0.8300 g KI in 200 ml of 0.05 N HCl
   Store in brown bottle, dilute 7 X with 0.05 N HCl before use.

   Acetate buffer (0.05 M):

   2.88 ml glacial acetic acid, dilute to 1 l with distilled water.

TABLE XXXI

WHOLE STARCH ASSAY (α-AMYLASE)

---

1. Weigh 50, 100, 200, 400, 800 and 1200 mg of starch sample in duplicate into 50 ml Erlenmeyer flasks. Weights should be within ± 0.2 mg for each sample. Label one with and one without enzyme.

2. Add 25 ml of Acetate Buffer to each and place in shaker-incubator at 37C. Add 100 microliters of α-amylase (100 mg/20 ml) to each flask at 10 minute intervals for a 90 minute digestion period.

3. After 90 minute digestion, remove both with and without enzyme flasks for each sample and filter at least 10 ml through Whatman #50 filter paper. (Can use water aspirator to aid filtration). Pipet 1.0 ml of each sample in triplicate from with and without enzyme filtrates (0.5 ml sample + 0.5 ml acetate buffer for > 200 mg starch levels). Immediately add 1 ml of Nelson's Reagent (A+B) and proceed with Nelson's Assay step 2 (Table XXXII). In calculations, use without enzyme samples to correct for reducing sugars present before α-amylase digestion.

4. Reagents:

   See Soluble Starch Assay (Table XXX).

TABLE XXXII

NELSON'S ASSAY FOR REDUCING SUGARS

1. Standard Curve - Pipet 0.0, 0.1, 0.2, ... 1.0 ml of stock glucose solution in triplicate. Dilute all samples to 2 ml total volume.

2. Mix Nelson's Reagent (50 ml of A and 2 ml of B). Add 1 ml of Nelson's Reagent to each sample or standard tube and vortex. Place tubes, capped with marbles, in steam bath for 20 minutes. Remove tubes simultaneously and cool in cold water. After cooling, add 1 ml of arsenomolybdate reagent to each tube and vortex. After approximately 5 minutes, dilute each tube to a total of 10 ml with distilled water. Read absorbance at λ = 510 nm as soon as possible.

3. Reagents:

   Nelson's A reagent:

   12.5 g Na_2CO_3 (anhydrous)
   + 12.5 g $KNaC_4H_4O_6 \cdot 4H_2O$
   + 10.0 g $NaHCO_3$
   + 100.0 g Na_2SO_4(anhydrous) added sequentially to 350 ml H_2O diluted to a final volume of 500 ml.

   Nelson's B reagent:

   7.5 g $CuSO_4 \cdot 5H_2O$ in 50 ml H_2O, plus 1 drop conc. H_2SO_4.

   Arsenomolybdate reagent:

   25 g $(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$ in 450 ml H_2O, add 21 ml of conc. H_2SO_4.
   3.0 g $Na_2HAsO_4 \cdot 7H_2O$ in 25 ml H_2O and add to above solution, incubate at 37 C for 24 hours.
   Store in brown bottle, reagent should be yellow with no green tint.

   Glucose standard (0.5 mM):

   45 mg glucose in 500 ml H_2O.
   Store in refrigerator or freezer.

TABLE XXXIII

DETERMINATION OF AMYLOSE-AMYLOPECTIN RATIO

1. Weigh 0.1 g of each starch sample in duplicate and place in 100 ml volumetric flasks. Weighed graded amounts of amylose and amylopectin for standards:

amylose	amylopectin
0.00 g	0.10 g
0.01 g	0.09 g
....	....
0.10 g	0.00 g

   Include a water blank.

2. Wet each sample and standard with 1 ml 95% ethanol and 10 ml distilled water. Add 2 ml of 10% NaOH. Heat flasks on steam bath for 90 minutes shaking every 20 minutes. Cool flasks for 10 minutes and dilute to volume, mix well.

3. Place 5 ml of solution from 100 ml volumetric into a 500 ml volumetric flask. Add 100 ml of distilled water plus 3 drops of 6N HCl. Mix well. Add 5 ml of Iodine reagent, mix well. Dilute to volume and measure absorbance at λ = 643 nm.

4. Reagents:

   See Soluble Starch Assay (Table XXX).

TABLE XXXIV

WET-MILLING PROCEDURE FOR CEREAL GRAINS

---

1. Clean approximately 1 kg of grain and weigh 200-400 g in duplicate.

2. Soak each sample in 1.5 l of steep I for 40 hours at 50 C. Strain through cheesecloth and soak in steep II for 8 hours. Strain through cheescloth and refrigerate steeped grain for 12 hours or overnight.

3. Grind the first sample in a Waring blender with the blades reversed. Use voltage regulator set at 85 volts or 72% of maximum. Add enough water to cover the grain and grind corn for 1 minute and sorghum for 1.5 minutes.

4. Pour ground slurry onto screens (#80, 230 and 325) with bottom pan to catch liquid. Wash each screen with distilled water from a wash bottle or spray mister until white starch is gone. When washing, pull screens apart to create suction and set screen at an angle for final wash. As bottom pan fills, pour off into large beaker. Recover material from each screen and regrind and rewash material from top screen by repeating the washing procedure. Place recovered material in labelled pans and dry in 100 C oven for at least 24 hours.

5. Wash starch table with spray mister and leave damp. Set a 500 ml separatory funnel in a ring stand about 6 inches from top end of table. Stir liquid recovered from bottom pan and pour into separatory funnel. Open funnel to let liquid slowly run down table. Use spray mister to spread the liquid across the width of the table by spraying against the flow of the liquid. Place large catch beaker under far end of table and regulate liquid flow so that starch settles on the table and gluten flows off the end. Occasionally, wash the far end of the table with the spray mister to remove gluten and use glass rod held vertically to aid gluten removal. Also use fine mist down the length of the table to keep liquid moving. Rinse first beaker and separatory funnel with distilled water and run down table.

6. Centrifuge liquid in catch beaker at 2500 rpm for 10 minutes to remove gluten. Place gluten in same pan as material off #325 screen and dry in 100 C oven for 24 hours. Let starch dry on table for 60-90 minutes and then scrape all but last 6 inches of table using a large spatula. Remove as much starch as possible with spatula. Wash residual starch and last 6 inches of table into beaker and then filter using Buchner apparatus. Dry purified starch and filtered starch at 40 C for 48 hours. Weigh purified starch and filtered starch separately and then discard filtered starch.

TABLE XXXIV (continued)

7. Flash evaporate steeps to a volume of 50 ml or less and lyophilize.

Reagents:

Ingredient	Amount/3000 ml
Steep I	
$Na_2S_2O_5$	2.226 ml
Lactic Acid (20%)	225.0 ml
Distilled Water	2775.0 ml
Steep II	
$Na_2S_2O_5$	4.460 g
Lactic Acid (20%)	75.0 ml
Distilled Water	2925.0 ml

Source: Norris and Rooney, 1979

APPENDIX B

ACCESSORY DATA TABLES

TABLE XXXV

ERROR MEAN SQUARE (EMS) AND CORRESPONDING DEGREES OF FREEDOM (d.f.) FOR IVDMD AND IVGP ANALYSES, YEARS 1-3

	IVDMD		IVGP	
	d.f.	EMS	d.f.	EMS
6 hour				
Year 1	18	8.0122	18	11.9421
Year 2	27	14.3335	27	10.3754
Year 3	21	4.6145	28	10.0671
12 or 24 hour				
Year 1	18	25.7522	18	19.5960
Year 2	27	16.8097	27	26.2953
Year 3	21	3.6011	28	15.9786

TABLE XXXVI

ERROR MEAN SQUARE (EMS) AND CORRESPONDING DEGREES OF FREEDOM (d.f.) FOR IVDMD AND IVGP ANALYSES, YEARS 1-3

	IVDMD		IVGP	
	d.f.	EMS	d.f.	EMS
6-hour				
Year 1	18	6.7772	18	15.2008
Year 2	27	11.1802	27	10.0474
Year 3	21	3.0562	28	7.0139
12 or 24 hour				
Year 1	18	23.7171	18	21.6822
Year 2	27	17.4050	27	31.5561
Year 3	21	11.6854	21	10.8060

TABLE XXXVII

ERROR MEAN SQUARE (EMS) AND DEGREES OF FREEDOM (d.f.) FOR IVDMD AND ENZYMATIC DIGESTIONS FOR YEARS 1-3

	IVDMD			
	6 hour		24 hour	
	d.f.	EMS	d.f.	EMS
Year 1	30	14.3236	30	24.6899
Year 2	36	51.0579	36	35.4313
Year 3	31	4.4548	21	18.2415

	Enzymatic Digestion			
	IVGP		α-amylase	
	d.f.	EMS	d.f.	EMS
Year 1	18	17.4099	6	0.000092
Year 2	45	46.9100	9	0.000717
Year 3	28	28.0824	7	0.002333

TABLE XXXVIII

ERROR MEAN SQUARE (EMS) AND RESPECTIVE DEGREES OF FREEDOM (d.f.) FOR PROTEIN FRACTIONS (FI - FV) IN YEARS 2 AND 3

	Year 2		Year 3	
	d.f.	EMS	d.f.	EMS
F I	8	0.000350	9	0.000211
F II	8	0.000389	9	0.000425
F III	8	0.000359	9	0.001091
F IV	8	0.000025	9	0.000058
F V	8	0.000586	9	0.000696

TABLE XXXIX

ERROR MEAN SQUARES (EMS) AND RESPECTIVE DEGREES OF FREEDOM (d.f.) FOR WET-MILLING COMPOSITION IN YEARS 1-3

	Year 1		Year 2		Year 3	
	d.f.	EMS	d.f.	EMS	d.f.	EMS
Bran and Germ	7	1.9100	10	0.0745	8	0.2931
PEC	7	0.2171	10	0.0195	8	0.0719
Gluten	7	0.1107	10	0.3960	8	0.2094
Starch	7	1.4085	10	0.5515	8	0.5744

TABLE XXXX

CORRELATION COEFFICIENTS OF IVDMD AND PROTEIN FRACTIONS (n=15)

	FI	FII	FIII	FIV	FV
IVDMD	.6229[a]	-.6476	.5519	-.4475	-.3501
	.0131[b]	.0091	.0329	.0944	.2008
FI	----	-.5373	.2710	-.7090	-.2978
	----	.0389	.3286	.0031	.2811
FII	----	----	-.4530	.2202	-.0189
	----	----	.0900	.4303	.9467
FIII	----	----	----	-.4860	-.6172
	----	----	----	.0663	.0142
FIV	----	----	----	----	.5098
	----	----	----	----	.0522

[a]Correlation coefficient.

[b]Significance level.

TABLE XXXXI

BERRY SIZE OF YEAR 3 SORGHUMS

Variety	Wt. of 100 Kernels (g)
Waxy	
Dwarf Redlan	2.61
1122	3.25
1126	2.55
Waxy-BR	
1133	2.67
Normal	
Redlan	2.90
Floury-BR	
Soft Endo	1.65
Normal-BR	
Darset	1.96
ROKY 78	2.60

TABLE XXXXII

GAS PRODUCTION OF UNTREATED SORGHUMS
IN YEAR 3 BY HOUR[1]

	Hour Number							
	1	2	3	4	5	6	Total 6-hr	Total 12-hr
Waxy								
Dwarf Redlan	17.6	14.4	12.1	11.9	10.0	9.5	75.5	105.8
1122	17.9	13.5	12.1	11.4	10.7	9.8	75.5	104.7
1126	13.8	10.5	9.0	8.9	8.3	8.0	58.6	87.8
Waxy-BR								
1133	16.0	10.8	9.5	8.8	8.6	8.6	62.2	96.9
Normal								
Redlan	14.4	10.5	9.4	8.7	8.1	7.8	58.9	87.3
Floury-BR								
Soft Endo	13.8	12.7	11.9	11.0	9.9	8.7	68.1	101.6
Normal-BR								
Darset	10.3	6.2	5.4	5.0	5.4	5.2	37.6	61.7
ROKY 78	11.0	8.7	7.9	7.5	7.0	6.7	48.9	77.2

[1]Values are an average of 5 determinations.

TABLE XXXXIII

GAS PRODUCTION OF RECONSTITUTED SORGHUMS IN YEAR 3 BY HOUR[1]

	Hour Number							
	1	2	3	4	5	6	Total 6-hr	Total 12-hr
Waxy								
Dwarf Redlan	12.5	17.4	16.2	14.2	13.4	11.1	84.8	120.3
1122	14.3	17.8	16.6	13.7	13.7	11.3	87.3	124.2
1126	15.3	16.2	15.3	12.7	12.1	10.6	82.3	115.4
Waxy-BR								
1133	13.8	18.4	16.8	14.7	13.8	11.3	88.8	122.1
Normal								
Redlan	15.0	14.9	14.1	11.8	11.5	10.2	77.4	116.2
Floury-BR								
Soft Endo	18.2	18.1	15.7	13.1	12.5	10.5	88.0	120.4
Normal-BR								
Darset	14.7	14.3	13.6	11.6	11.5	10.0	75.6	115.4
ROKY 78	12.5	14.2	13.1	11.5	11.5	9.6	72.4	111.5

[1]Values are an average of 5 determinations.

TABLE XXXXIV

GAS PRODUCTION OF PURIFIED STARCH
IN YEAR 3 BY HOUR[1]

	Hour Number						
	1	2	3	4	5	6	Total 6-hr
Waxy							
Dwarf Redlan	22.9	21.7	19.6	17.3	16.0	14.9	112.5
1122	23.5	23.3	19.4	17.7	16.6	14.3	114.7
1126	23.7	23.0	18.6	16.4	14.7	12.6	109.1
Waxy-BR							
1133	20.2	21.4	17.4	16.0	14.2	12.6	101.9
Normal							
Redlan	16.0	17.4	15.3	13.7	12.7	11.7	86.8
Floury-BR							
Soft Endo	21.6	20.8	17.6	15.8	14.2	12.4	102.4
Normal-BR							
Darset	16.4	16.1	15.4	13.2	12.2	10.6	83.9
ROKY 78	17.2	18.8	16.3	14.8	13.5	11.4	92.0

[1]Values are an average of 5 determinations.

TABLE XXXXV

ALPHA-AMYLASE DIGESTION OF SOLUBLE STARCH (YEAR 3)[1]

Variety	μ moles glucose equivalents/ml/min.
Waxy	
Dwarf Redlan	.272
1122	.252
1126	.211
Waxy-BR	
1133	.185
Normal	
Redlan	.027
Floury-BR	
Soft Endo	.026
Normal-BR	
Darset	.024
ROKY 78	.026

[1]Values are an average of 4 determinations.

TABLE XXXXVI

ALPHA-AMYLASE DIGESTION OF WHOLE STARCH IN YEAR 3 (μ MOLES GLUCOSE EQUIVALENTS/ml/HR)[1]

	100 mg[2]	200 mg[2]	400 mg[2]	800 mg[2]
Waxy				
Dwarf Redlan	.102	.299	.440	.642
1122	.106	.236	.472	.701
1126	.144	.312	.628	1.040
Waxy-BR				
1133	.108	.265	.553	.744
Normal				
Redlan	.118	.255	.430	.702
Floury-BR				
Soft Endo	.108	.217	.426	.560
Normal-BR				
Darset	.086	.210	.381	.510
ROKY 78	.087	.180	.388	.526

[1]Values are an average of 2 determinations.

[2]mg starch/50 ml acetate buffer.

TABLE XXXXVII

STARCH CONTENT OF WET-MILLING FRACTIONS IN YEAR 3[1]

	Percent Starch in		
	Bran & Germ	PEC	Gluten
Waxy			
Dwarf Redlan	30.4	52.4	41.0
1122	41.2	46.5	32.6
1126	48.5	33.1	30.3
Waxy-BR			
1133	30.3	53.2	41.6
Normal			
Redlan	42.0	64.8	43.7
Floury-BR			
Soft Endo	18.1	38.8	30.2
Normal-BR			
Darset	41.7	57.9	40.7
ROKY 79	39.8	59.2	50.0

[1]Values are an average of 2 determinations on composite wet-milling fractions.

VITA[2]

Charles Alan Hibberd

Candidate for the Degree of

Master of Science

Thesis: VARIATION IN STARCH AND PROTEIN CHARACTERISTICS OF GRAIN SORGHUM AS INFLUENCED BY VARIETY AND THEIR RELATIONSHIP TO DIGESTIBILITY CHARACTERISTICS

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