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SCANNING ELECTRON MICROSCOPY OF MONOGASTRIC AND RUMINANT DIGESTION OF SORGHUM GRAIN

by

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B.S., Oregon State University, 1969

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science and Industry

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Manhattan, Kansas

1973

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Table of Contents

Introduction and Literature Review

The development of the scanning electron microscope in the latter half of the nineteen sixties has given scientists a method for observing and interpreting objects and phenomena which were previously difficult due to small size and difficulty of interpretation affecting light and transmission electron microscopy respectively.

Scanning electron microscopy involves a high energy electron beam (5 to 50 Kv. accelerating voltage) scanning the object and producing, among other things, secondary electrons which are collected via a bias voltage into a scintillation counter. Signal from the counter is suitably amplified and modulates the intensity of an electron beam which reproduces the original scan on a cathode ray tube. Circuitry is designed to modify contrast and installation of a camera over a viewing cathode ray tube allows permanent recording of images (Kimoto, 1972). Observation is limited to surface irregularities but Hall and Sayre (1973) note that this is at times useful in determining which structures observed by other means, such as light microscopy, are on the surface and which are internal.

Scientists are now using scanning and transmission electron microscopy as a primary tool in characterization and definition of cereal grains in general and starches in particular. Pomeranz and Sachs (1972) have outlined the structure of oats, barley, and buckwheat kernels using scanning electron microscopy. Hoseney et al. (1973) determined structural differences among twelve sorghum grain varieties and related them to amino acid analysis based on the presence of small protein bodies within the endosperm. Isolated starches from a number of sources have

been studied both in unmodified form and following various treatments intended to clarify structure or simulate natural degredation processes.

Alsbert (1938) found that anhydrous starch had no x-ray diffraction pattern while hydrated starch developed a characteristic pattern indicating a crystalline type structure. He proposed radially arranged starch chains which collapsed on drying and stretched when wet to give the pattern noted. Numerous authors have noted the major structural feature of unmodified starch granules to be a series of concentric shells or, when seen in section, rings. Under the light and transmission electron microscope these rings have been reported by Sanstedt (1946), Alsberg (1938), Buttrose (1963), Hall and Sayre (1973), and Gallant et al. (1972).

Gallant (1972) ascribed the shell structure as alternating layers of amylose and amylopectin which varied in susceptability to attack by periodic acid and alpha amylase treatments. Badenhuizen (1960) determined that all layers were alike and that within each layer longer amylose chains were located on the internal edge with pregressively shorter chains and more branching (amylopectin) towards the outer surface. Whistler and Young (1960) found that labeled carbon entered wheat starch within four hours during daylight and much more slowly at night suggesting a mechanism for shell formation. They determined that labeled carbon showed up more rapidly in amylose than in amylopectin but that the amylose: amylopectin ratio was unchanged from day to night. Buttrose (1960, 1962) demonstrated that shell structure in cereal grains was dependent on diurnal variation and growth in total light eliminated shells from wheat, corn, and barley. Potato starch, however, produced typical shell structure

when grown either in normal day-night rhythm or in total light. Whistler and Young (1960) and Buttrose (1963) both suggest that the observed shell structure in starch granules is primarily a function of molecular packing density due to some rhythm in formation of amylose and amylopectin molecules rather than a difference in molecular composition of layers.

The major enzyme responsible for natural degredation of starch is alpha amylase which occurs widely in nature. Its principle action is hydrolysis of alpha 1:4 glucosidic bonds which unite glucose units into starch molecules. All known alpha amylases contain at least one atom of calcium per mole of enzyme. There are differences in alpha amylase molecular weights and ion requirements for activation, depending on source. Amylose is split rapidly to maltose and maltotriose, then slowly to maltose and glucose. Amylopectin is broken to maltose and maltotriose except where 1:6 glucosidic linkages, themselves immune to attack, render some neighboring 1:4 links impervious to hydrolysis resulting in formation of limit dextrins (Whelan, 1960). Robyt and French (1967) found that porcine pancreatic alpha amylase has a multiple form of attack as opposed to a single- or multi-chain type. This attack accounts for the enzyme acting as an endo enzyme to produce, initially, a number of different end products which, following a time period, become identical.

The effect of amylases on starch granules has been observed by a number of workers. Dronzek et al. (1972) used scanning electron microscopy to observe native alpha amylase attack in sprouted wheat. They noted concentric shell structure and holes from enzyme attack and divided starch granules into type A, which were larger and irregular in shape, and type B, which were smaller and generally spherical. Type A granules were preferentially attacked by the enzyme. In a similar experiment Evers and

McDermott (1970) noted that some granules were attacked while others were not, but made no distinction between them based on structure. They observed that enzymatic attack was seemingly random and bore no relationship to visible structure of the granule. Leach and Schoch (1961) found extensive erosion and fragmentation of starch granules after alpha amylase treatment. They also determined normal sorghum starch to be more resistant to attack than waxy sorghum starch.

Starch makes up 68 to 73%, by weight, of sorghum grain (Edwards and Curtis, 1943), 90% of which is in the endosperm fraction (Hubbard et al., 1950). Normal sorghum starch contains 23 to 28% amylose with the remainder as amylopectin (Leartherage, MacMasters, and Rist, 1955). Sorghum grain structure has been reviewed by McCollough (1973). Chemical composition was found to be very similar to corn (Rooney and Clark, 1968). Utilization studies comparing corn and sorghum grain for various animal diets have given mixed results. Weber et al. (1951) found no differences among three sorghums and corn for fattening steers. Net energy values calculated for corn and sorghum grain were found to be similar (Hall et al., 1968). Richardson (1956, 1967) and Totusek et al. (1963) found significant differences, from 10.5 to 18.9%, in feed efficiency, all in favor of corn. Additional trials have shown feed efficiency differences among varieties of grain sorghum (Riewe and Brewer, 1970; Hale et al., 1964; McCollough, 1973; Sherrod et al., 1969; Brethour and Duitsman, 1965) with waxy endosperm varieties generally best followed by yellow and white types. Bird resistant varieties, typified by brown pericarps, have been found to contain greater amounts of polyphenolic compounds classed as tannins, and these have been implicated in the frequently lowered feed utilization

associated with bird resistant grain sorghums (Chang and Fuller, 1964; Vohra et al., 1966; Fox et al., 1970; Hale et al., 1969). Other workers have found no difference between bird resistant and other sorghum varieties in broiler rations (Damron et al., 1968). Tannins are generally located in the pericarp and their presence is credited with making the grain unpalatable to birds (McGinty, 1968; Blessin et al., 1963). They have been found to inhibit various enzymes including cellulase (Mandles et al., 1961), alpha amylase, trypsin and lipase (Tamir and Alumot, 1969), in addition to precipitating proteins, especially in acid pH conditions as encountered in the monogastric stomach (McGinty, 1968). Although it seems likely that tannins are at least partly responsible for lowered utilization of bird resistant sorghum grains and that they are located in the pericarp, Jambunathan and Mertz (1972) found that dehulling bird resistant sorghum grain did little to improve weight gains in rats over the intact grain. Sanstedt and Beckord (1946) postulated an alpha amylase inhibitor located in the endosperm of wheat kernels. Cummins (1971) found that ensiling high and low tannin forage sorghums resulted in loss of tannins from the high tannin variety until it had nearly the same content as the low tannin variety which ensiling had not affected. These results were reflected in measurements of in vitro dry matter disappearance which were in favor of the low tannin variety before ensiling and nearly equal after.

A review of the literature indicates general agreement that large differences do occur in utilization of sorghum grain varieties. Some of the differences seem dependent on amylose content but the major differences noted relate to lowered use of bird resistant types. These differences seem related to tannin content but addition of tannic acid to normal diets,

while simulating the lowered utilization of bird resistant grains, does not duplicate it in that the natural inhibition may be largely overcome by addition of methionine and choline where the simulated inhibition is unaffected by additional amino acids (Chang and Fuller, 1964). Saba et al. (1972) found that addition of tannic acid would duplicate the reduction of gas production in in vitro fermentation but the amounts required were in excess of those available from natural high tannin sorghum grains.

This study was undertaken to determine if scanning electron microscopy would show differences in mode of enzyme attack on three varieties of sorghum which recent cattle feeding trials had shown to be substantially different in degree of utilization. In addition, isolated starches from each variety were treated with purified alpha amylase to determine if in vivo and in vitro attack patterns were similar and if possible some portion of the inhibiting factor in the bird resistant grain might reside in the endosperm starch.

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HYDROLYSIS OF SORGHUM GRAIN STARCH BY RUMEN MICROORGANISMS AND PURIFIED PORCINE α-AMYLASE AS OBSERVED BY SCANNING ELECTRON MICROSCOPY [1]

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Summary

Three grain sorghum types (waxy, yellow, bird resistant) were subjected to either rumen digestion for 75 min. to 8 hr. or hydrolysis by purified α-amylase for 48 hr. and observed by scanning electron microscopy.

Starch granules in soft endosperm of yellow and bird-resistant types were smooth and spherical; those in a waxy variety were irregular in shape.

Split kernels immersed in rumen fluid for as little as 75 min. showed differential hydrolysis: point-surface attack on the starch from the waxy variety, linear-track hydrolysis on that from the yellow variety, and minor-point hydrolysis on that from the bird-resistant variety. Photomicrographs of split kernels suspended inside ruminally fistulated steers indicated different modes of starch attack. Surface attack on the waxy starch was mainly point hydrolysis with alternate layers digested inside the starch granule. Yellow endosperm starch was digested without structural resistance. The bird resistant variety showed a mixture of both types of hydrolysis on the starch surface, with preferential layer digestion internally.

1/ Contribution No. 455, Department of Animal Science and Industry, Kansas Agricultural Experiment Station.

2/ Part of a thesis submitted as partial requirements of an M.S. degree.

Purified starch from each variety was hydrolyzed by porcine α-amylase at different rates only when the substrate was greater than one mg. starch/I.U. enzyme. The waxy variety then hydrolyzed more rapidly than did the yellow endosperm type. The bird-resistant variety showed substrate inhibition. Differentiation of starch damage by the enzyme could be readily detected by scanning electron microscopy. Hydrolysis of starch as observed on the scanning electron microscope, was similar to that of rumen microorganisms.

Introduction

Ruminant response to different varieties of sorghum grain is economically important to the livestock industry (Hale, et al., 1964; Sherrod, Albin and Furr, 1969; Rieme and Brewer, 1970; Hinders and Eng., 1970). Differences in digestibility among varieties have been shown to depend on both variety (McGinty and Riggs, 1968; Nishimuta, Sherrod and Furr, 1969; McCollough, 1973) and feed processing (Buchanan-Smith, Totusek and Tillman, 1968; Hale and Theurer, 1972). In general, waxy varieties are superior and bird-resistant varieties comparatively inferior for ruminants.

Gallant, Aumaitre, and Guilbot (1973), using transmission and scanning electron microscopy to study starch granules subjected to hydrolysis by pig pancreatic juice, concluded that kind of attack is related to plant species. These workers found that hydrolysis ranged from mainly surface erosion in potato and cassava to random endocorrosion in waxy corn. Scanning electron microscopy is a unique way to observe amylase activity because it allows excellent depth of field at magnifications permitting detailed observation of individual starch granules.

To further evaluate varietal differences in sorghum grain, we studied, by scanning electron microscopy, types that recently have been shown to differ in feedlot performance and digestibility (McCollough, 1973). This report presents visual indications of differential enzymatic attack by rumen microorganisms and purified porcine pancreatic α-amylase on starch granules from three grain sorghum varieties.

Materials and Methods

Rumen Studies. Individual sorghum grains of three endosperm varieties (CP 622-waxy, C42Y-yellow and Acco 1023-bird resistant) were split in half with a sharp blade and placed in cheesecloth packets. Each packet (three layers of cheesecloth tied at both ends) contained approximately 25 half grains of one variety; five packets for each variety were prepared.

Rumen fluid for in vitro trials was collected from fistulated steers fed a high concentrate ration: 80% sorghum grain, 15% corn silage plus 5% of 32% protein supplement. Rumen fluid was strained through three layers of cheesecloth, then placed in three warmed quart vaccum bottles. Packets were suspended in the bottles (one bottle for each variety) and bottles sealed. Each packet was weighted with a short glass rod to insure rapid immersion. After 75 min. incubation packets were removed, opened, and the half grains washed briefly in distilled water. Samples were dried overnight at room temperature, then mounted on aluminum stubs with Delco No. 93 colloidal silver.

Packets of grain samples for in vivo studies (prepared similarly) were suspended on 50 cm. strings within the rumen of a fistulated steer

(2 hr. postprandial) that two days earlier had donated rumen fluid for in vitro trials. Samples removed at 2, 4, and 8 hr. were treated in the same manner as those from in vitro studies.

α-Amylase Experiments. Starch was isolated from each variety of sorghum grain by a simple wet-milling method. Samples of each grain (500 g) were soaked overnight in water at 5°C., then coarsely ground at low speed in blender and sieved through a 100-mesh screen. Material remaining on the screen was resuspended in water, which allowed most of the germ fraction to be removed as it floated on the surface. The remainder, after germ seperation, was reground at high speed three times with a screening step each time. That material was suspended in water and centrifuged at 450 x g. for 15 min., followed by removal of the protein layer with a spatula. This procedure was repeated at least four times or until starch appeared pure. Purity was confirmed later by observation under the scanning electron microscope. Isolated starches were dried overnight at room temperature and bottled for later use.

The enzyme used, twice-crystallized porcine pancreatic α-amylase (alpha-1, 4 glucan, 4 glucanohydrolase) 3.2.1.1.[1], was diluted to 2.97 I.U. activity per ml. in 0.02 M phosphate buffer (pH 6.9) containing 0.006 M NaCl. (One I.U. releases one micromole of reducing units, calculated as maltose, per min. at 25°C.).

Isolated starch samples (0.5-50.0 mg) and 4.0 ml of enzyme were placed in 25-ml Erlenmeyer flasks, then incubated 48 hr. at 25°C in a Dubanoff metabolic shaker. The reaction was stopped by adding the color reagent: 3,5-dinitrosalicylic acid. One ml of supernatant was removed

according to the Worthington method[1/]. Residual starch was dried overnight at room temperature, then mounted on double-stick tape.

All samples were coated with 150 Å of gold palladium and observed with an Etec Autoscan scanning electron microscope at an accelerating voltage of 20 Kv. Polaroid PN55 film was used to photograph the scanned image.

Results and Discussion

Figures 1 to 3, photomicrographs of the cut surface of grains soaked in phosphate buffer for 48 hr., show intact starch granules of soft endosperm from each grain type as they appeared in situ. Yellow (Figure 1) and bird-resistant (Figure 2) varieties contained smooth, spherical starch granules (approximately 15 microns in diameter), and starch granules in the waxy variety (Figure 3) were irregular in shape. In the photomicrographs thin sheets of protein and protein bodies can be observed between starch granules, each group of which is enclosed within a layer of hemicellulose, partially visible. Figure 4 shows starch granules that were split when the kernel was cut. That type of break was common in the hard endosperm of all varieties, but the bird-resistant grain had less hard endosperm than did the other types.

Samples treated 75 min. in rumen fluid showed differential attack (Figures 5, 6, 7). The waxy variety (Figure 5) showed a general point attack and also hydrolysis of apparent hilum zones similar to that of cassava starch granules subjected to porcine pancreatic juice (Gallant et al., 1973). Bird-resistant starch showed no attack beyond a few pinholes (Figure 7).

1/ Worthington Biochemical Corporation, Freehold, N. J. 007728

Because some starch granules may fall out of the kernel during incubation, it is impossible to determine the exact time any one granule has been digested. As noted, some granules on the original surface fracture when the kernel is split; those areas assure that the exposure time is known. Figures 8 and 9 show areas in waxy and yellow kernels after 4 hr. in the rumen; Figure 10, the same type of area in bird-resistant grain after 8 hr. in the rumen. The concentric shell structure suggested by Evers and McDermott (1970) can be seen clearly in the waxy starch where it was accentuated by digestion of alternate layers. It is also visible, though not so clearly, in the bird resistant sample, perhaps because of the longer treatment period, although attack seemed to concentrate on groups of layers (not on alternate layers as it did on waxy sorghum). Yellow endosperm starch was attacked in a strikingly different way; in Figure 8, the concentric shell structure is faintly visible, the major form of attack is indicated by large, irregularly shaped holes formed without pattern relative to the starch structure.

Figures 11, 12, and 13 show soft endosperm of each variety after 8 hr. in the rumen. As already noted, it is impossible to be certain that exposure time for the starch granules in all three samples was identical, but the photomicrographs show obvious differences in mode of attack between the yellow (Figure 11) and the other (Figures 12 and 13) endosperms. Differences between the waxy and bird-resistant types are less apparent, but repeated observation showed more erosion of specific areas in the bird resistant type. Such resistant areas also have been observed in wheat and potato starch granules (Gallant et al., 1973).

Purified starches from the three sorghum grain types were hydrolized at similar rates when the substrate was equal to or less than one mg starch/

I.U. porcine α-amylase (Figure 14). At higher substrate/enzyme ratios, waxy sorghum grain starch released more maltose equivalents than did the yellow endosperm type, and bird-resistant starch was depressed (Characteristic of substrate inhibition). Photomicrographs of starch granules incubated 48 hr. with α-amylase (50 mg starch/sample) confirmed the enzymatic hydrolysis data. Waxy-variety starch exhibited considerable point hydrolysis (Figure 15), similar to that produced by rumen microorganisms (Figures 5 and 12); split starch granule showed the characteristic alternate layer hydrolysis for this variety. Yellow-type starch (Figure 16) eroded in a way similar to that of rumen microorganisms. The stage of hydrolysis was intermediate between that of starch in the rumen digested 75 min. and 8 hr. Linear-tract hydrolysis (tangentral erosion) of the yellow variety was present in starch digested in rumen 48 hr. but not so evident when digested for 75 min. (Figure 6); radial erosion occurred concurrently as it did when exposed to rumen contents 4 hr. (Figure 8). Only slight point erosion was evident in purified starch from the bird-resistant variety (Figure 17), as was observed in starch in rumen fluid 75 min. (Figure 7). The bird-resistant starch granules showed preferential attacks; some were quite resistant, but those having indentations from protein bodies appeared to be vulnerable at those locations.

These experiments show that starch hydrolysis from different sorghum grain varieties is as physically variable as previously shown for isolated starches from other botanical species. Mode of hydrolysis by rumen microorganisms and purified porcine α-amylase were similar for each variety of starch, even though enzymatic conditions (substrate and enzyme

purity and concentrations, temperature, reaction time) differed drastically. Differential hydrolysis rates were evident, however, when the substrate concentration exceeded one mg starch/I.U. of enzyme. Though lack of accurate estimates of in vivo conditions precludes direct interpretations of these findings for practical nutrition, it is evident that limited enzyme concentration may be partially responsible for decreased nutrient utilization of grain when intake is increased.

Using scanning electron microscopy to differentiate starch hydrolysis may aid in developing better sorghum grain varieties for livestock.

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Fig. 1. Scanning electron photomicrograph of soft endosperm of a yellow variety of sorghum grain (C42-Y) treated with phosphate buffer (1600x).

Fig. 2. Scanning electron photomicrograph of soft endosperm of a bird resistant sorghum grain (Acco 1023) treated with phosphate buffer (1560x).

Fig. 3. Scanning electron photomicrograph of soft endosperm of a waxy variety (CP-622) of sorghum grain treated with phosphate buffer (1440x).

Fig. 4. Scanning electron photomicrograph of split starch kernels in hard endosperm of a bird resistant sorghum grain (1520x).

Fig. 5. Scanning electron photomicrograph of soft endosperm starch (waxy variety) following 75 min. digestion in rumen fluid (2000x).

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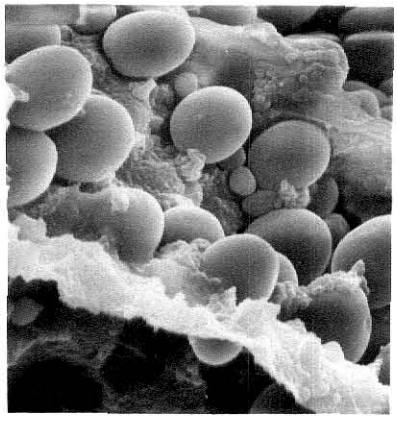

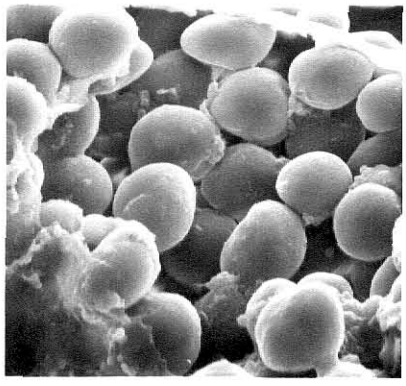

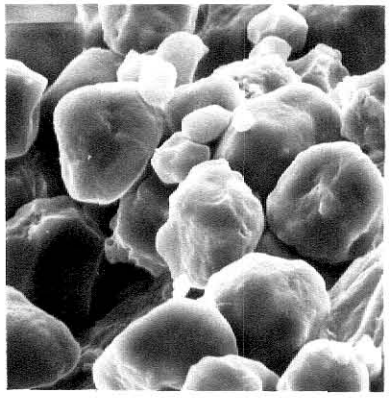

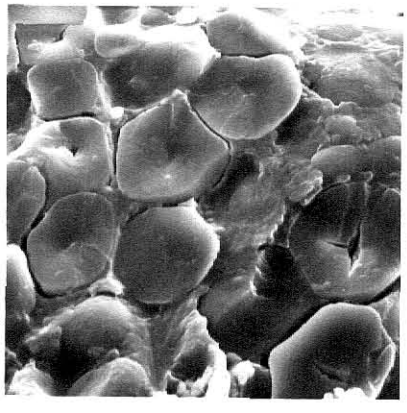

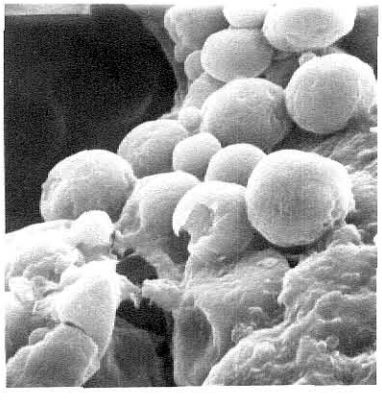

Fig. 6. Scanning electron photomicrograph of soft endosperm starch (yellow variety) following 75 min. digestion in rumen fluid (2000x).

Fig. 7. Scanning electron photomicrograph of soft endosperm starch (bird resistant variety) following 75 min. digestion in rumen fluid (1600x).

Fig. 8. Scanning electron photomicrograph of split starch granules of hard endosperm from yellow sorghum grain following 4 hr. suspension in rumen of fistulated steer (2000x).

Fig. 9. Scanning electron photomicrograph of split starch granules of hard endosperm from waxy sorghum grain following 4 hr. suspension in rumen of fistulated steer (1680x).

Fig. 10. Scanning electron photomicrograph of split starch granules of hard endosperm from bird resistant sorghum following 8 hr. suspension in rumen of fistulated steer (1600x).

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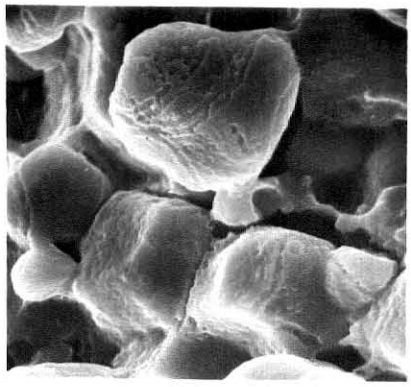

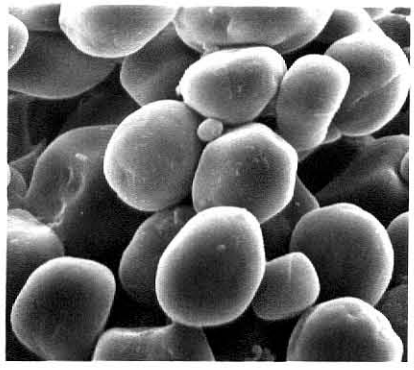

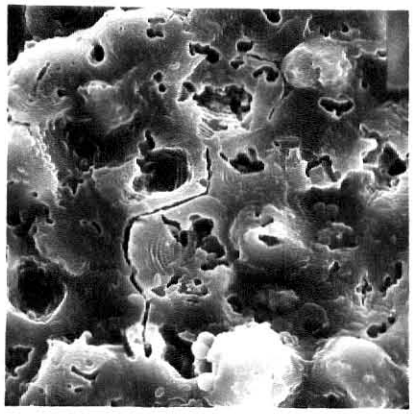

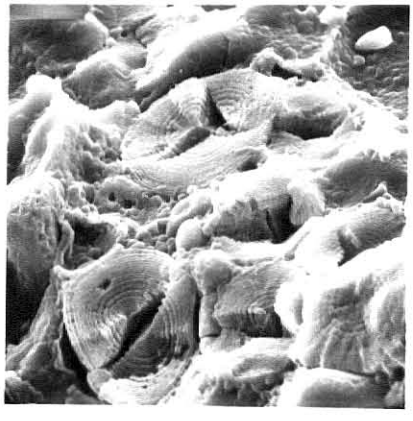

Fig. 11. Scanning electron photomicrograph of soft endosperm starch granules (yellow type) following 8 hr. suspension in rumen of fistulated steer (2000x).

Fig. 12. Scanning electron photomicrograph of soft endosperm starch granules (waxy type) following 8 hr. suspension in rumen of fistulated steer (1600x).

Fig. 13. Scanning electron photomicrograph of soft endosperm starch granules (bird resistant type) following 8 hr. suspension in rumen of fistulated steer (1600x).

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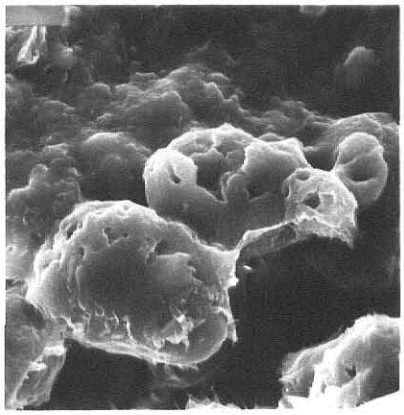

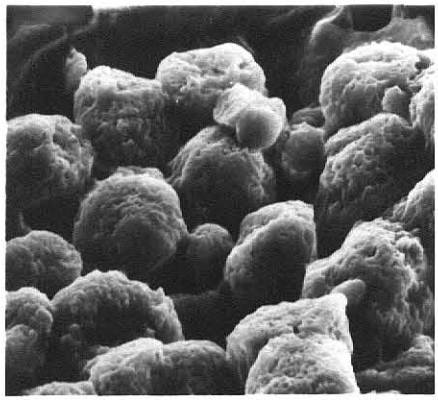

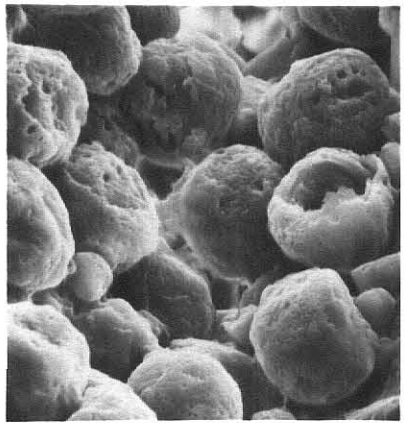

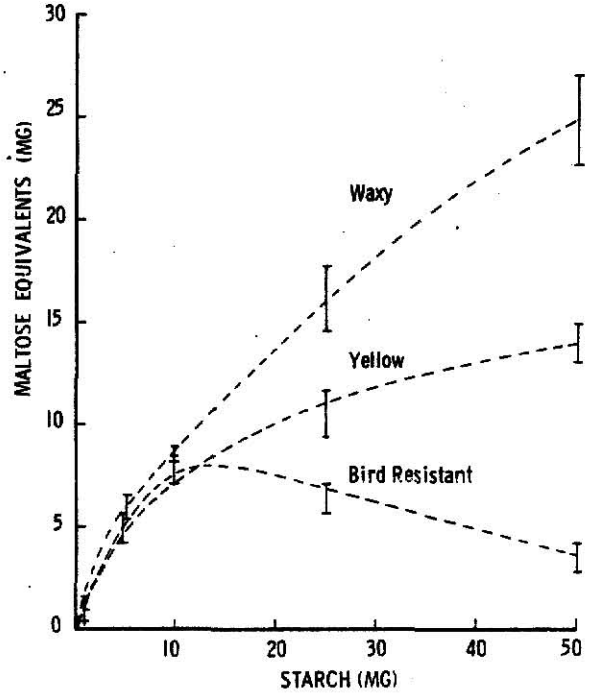

Fig. 14. Plot of maltose production from purified starches of three sorghum grain types following 48-hr. incubation with porcine pancreatic α-amylase (mean with range indicated).

Fig. 15. Scanning electron photomicrograph of amylase treated starch isolated from a waxy endosperm sorghum (2000x).

Fig. 16. Scanning electron photomicrograph of amylase treated starch isolated from a yellow endosperm sorghum (2000x).

Fig. 17. Scanning electron photomicrograph of amylase treated starch isolated from a bird resistant sorghum (2000x).

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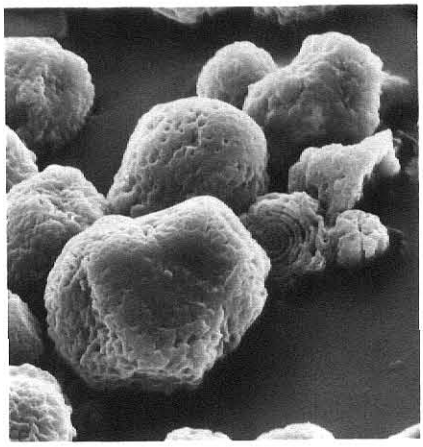

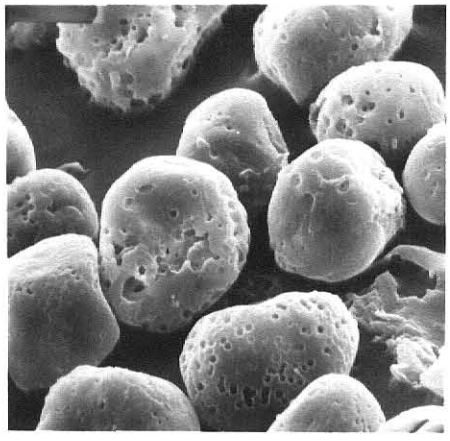

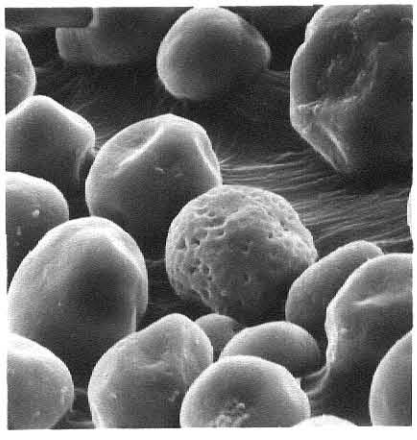

SCANNING ELECTRON MICROSCOPIC OBSERVATION OF STARCH GRANULE DIGESTION IN THREE VARIETIES OF SORGHUM GRAIN BY THE RAT AND PIG

Summary

Split kernels of three sorghum grain varieties were fed to adult rats and recovered by dissection at one, three, and six hour periods after dosing. A bird resistant type (Acco 1023) passed through the stomach much slower than a waxy endosperm (CP 622) or a yellow endosperm (C 42 Y). Following recovery kernels were mounted and observed on a scanning electron microscope. Photomicrographs are presented of various stages of digestion which appear quite similar to previous work using purified porcine pancreatic alpha-amylase and isolated starch. Different attack patterns and amounts of attack depending on grain variety are visible and are discussed in light of feeding trials conducted with the same varieties.

Introduction

Differential responses have been found in digestion and utilization of several sorghum grain varieties fed to various animal species. McCollough (1973) found large differences between a bird resistant variety and all others in feedlot steer performance. He also found smaller differences between yellow and waxy endosperm types. Axtel et al. (1972) showed bird resistant (high tannin content) sorghums produced greatly reduced weight gains in rats and at times resulted in weight loss.

Other sorghum grain varieties gave relatively normal gains especially when supplemented with lysine. Lysine supplementation of high tannin grain did little to improve its utilization.

Davis and Harbers (1973) used the scanning electron microscope to observe differences between sorghum varieties exposed to in vivo and in vitro rumen digestion. Enzymatic attack on the starch granules was different for different types and similar differences were noted using isolated starch from the same sorghum varieties digested with purified alpha amylase.

The present microscopic trials were undertaken to observe monogastric starch digestion patterns of some sorghum grain varieties in the gastrointestinal tract of rats and pigs. We were also interested in possible differences between ruminant and non-ruminant digestion.

Materials and Methods

Adult Holtzman rats (three per treatment) were force fed, to prevent mastication, four to six kernels each of one of three varieties of sorghum grain. The varieties used were CP 622 (waxy endosperm), C 42 Y (yellow endosperm, and Acco 1023 (bird resistant). At one, three, and six hours following feeding, one rat from each variety was sacrificed and the kernels recovered by dissection.

Fecal samples of split kernels were obtained from another group of similarly dosed adult Holtzman rats (two per treatment). Feces were removed from collecting trays every four hours until kernels were recovered.

Samples from the swime digestive system were obtained from 100 kg. pigs dosed 12, 24, and 36 hours prior to slaughter. Collection and preparation were identical to those methods outlined above for rat samples. Limitations in animal numbers and slaughter procedures prevented complete sampling of the digestive tract possible in the rat trials.

The half kernels were then washed briefly in distilled water, dried overnight at room temperature, and mounted on aluminum stubs with Delco No. 93 collodial silver. To view starch damage of underlying layer some samples were split following digestion at right angles to the original cut then mounted so that both sides were visible.

All samples were coated with 150 Å of gold palladium prior to scanning electron microscope observations made on an Etec Autoscan scanning electron microscope at 20 Kv accelerating voltage. Images were recorded on Polaroid PN 55 film.

Results and Discussion

Samples recovered from the stomach of the rat show little damage other than possible removal of a portion of the matrix (sheet) protein. Figure 1 is an overall view of a yellow endosperm sample recovered from the stomach after one hour. Some loss of starch granules, especially in the soft endosperm area, is visible but this could be due to wetting as well as enzymatic attack. Figure 2 is a view of bird resistant grain recovered from the stomach after one hour. Both intact starch granules and some split when the kernel was fractured are visible. Split granules show faint concentric rings which are typical of sorghum starch.

Differences in rates of passage through the stomach for different varieties of grain were large. Waxy endosperm was recovered from the stomach only at one hour, yellow at one and three hours, and bird resistant remained for up to six hours. No bird resistant grain was recovered from the small intestine until three hours after feeding where both yellow and waxy kernels were found in that area after one hour.

Samples recovered from the small intestine show increasing loss of starch granules from the kernel and obvious signs of enzymatic attack on those granules remaining attached. Waxy endosperm starch was most readily attacked and most rapidly removed from the kernel. Figure 3 shows a pair of starch granules (waxy endosperm) which are still attached to the kernel recovered from the medial small intestine. The overall attack pattern is typical of previously observed (Davis and Harbers, 1973) alpha-amylase action on isolated waxy endosperm starch granules. The cell wall has been shrunk and starch granules underneath the layer appear to have been attacked. Figures 4 and 5 are yellow endosperm and bird resistant varieties, respectively, both recovered from the small intestine. The attack patterns are different in type and amount from the waxy endosperm and from each other. While this might be attributed to individual differences between rats or some other external factor it seems likely to reflect some inherent variation within the starches because the results obtained are very similar to those from controlled experiments with isolated starches digested by purified alpha amylase (Davis and Harbers, 1973). The differences might be related to structure based on variation in amylose content. This would separate

the waxy and yellow endosperms where the difference is less in the amount of attack and more in the pattern. However, differences between yellow endosperms where the difference is less in the amount of attack and more in the pattern. However, differences between yellow and bird resistant starches tend to be more in amount rather than pattern. Other workers (McCollough, 1973; McGinty, 1968; Axtell et al., 1972; Chang and Fuller, 1964) have determined that bird resistant sorghums are inferior for steers, chicks, and rats when weight gains and efficiency are calculated. They suggest some factor, probably poly phenolic in character, which reduces utilization of nutrients from sorghum grains of bird resistant varieties.

Towards the end of the small intestine all, or nearly all, of the starch is removed down to the first continuous cell wall leaving the bare surface as seen in Fig. 6 of a waxy kernel taken from the distal small intestine. Higher magnification (Fig. 7) shows a few remnants of starch granules on the surface of cell wall structure. A typical observation after splitting the kernel following its recovery from the distal small intestine at 90^{o} to the original cut is shown in Fig. 8. A section of bare cell wall is visible, and underneath it starch granules. Those granules have undergone a considerable amount of attack which would suggest either a crack in the cell wall layer or diffusion of enzyme through the wall to starch below. The degree to which this result was repeatable makes the enzyme diffusion hypothesis rather attractive. The enzyme did not diffuse through subsequent cell wall layers.

Kernels recovered from the cecum demonstrate that little additional action occurs in this section of the gut except some smoothing of the kernel surface. In the large intestine the covering cell wall structures

are removed in some manner, possible by bacterial hydrolysis, and underlying starch undergoes enzymatic attack which appears very similar to that occurring in the small intestine. Figure 9 is an example of yellow endosperm grain from the large intestine of the rat. Fecal samples from the rat were not noticeably different from large intestine samples.

There were no obvious differences between samples from the rat and the pig. Figure 10 shows a sample from the small intestine of the pig. Typical emptying of starch from cells is visible and closer observation (Fig. 11) shows attack patterns associated with alpha-amylase and yellow endosperm starch granules. The large intestine samples showed gradual errosion of the cell walls followed by attack on the starch granules as was seen in the rat.

From these trials it appears that exposed sorghum grain starch is rapidly attacked in the small intestine of the monogastric. Starch which remains covered by cell wall material may be digested by enzyme movement across the wall but this, if it occurs, is a slow process. These findings support the large amount of data showing improved feed utilization from particle size reduction in processing.

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Fig. 1. Scanning electron photomicrograph of a split yellow endosperm kernel recovered from rat stomach (76x).

Fig. 2. Scanning electron photomicrograph of a split bird resistant kernel recovered from rat stomach (800x).

Fig. 3. Scanning electron photomicrograph of a waxy kernel recovered from rat small intestine (2200x).

Fig. 4. Scanning electron photomicrograph of a yellow kernel recovered from rat small intestine (1600x).

Fig. 5. Scanning electron photomicrograph of a bird resistant kernel recovered from rat small intestine (1900x).

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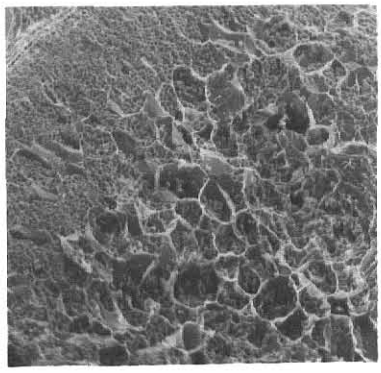

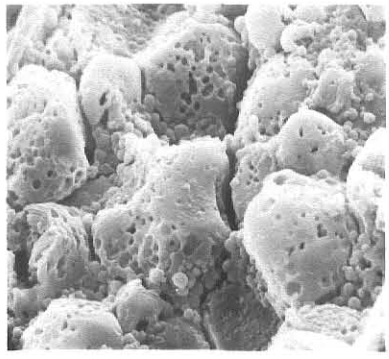

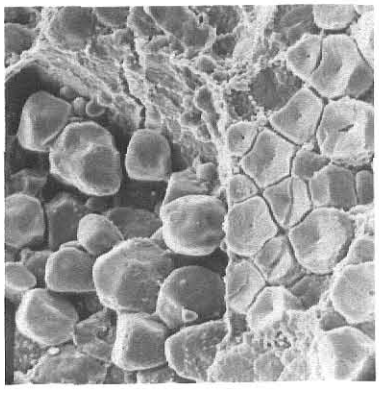

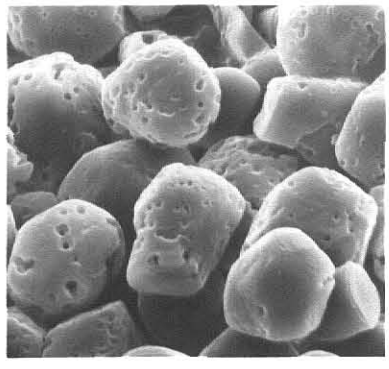

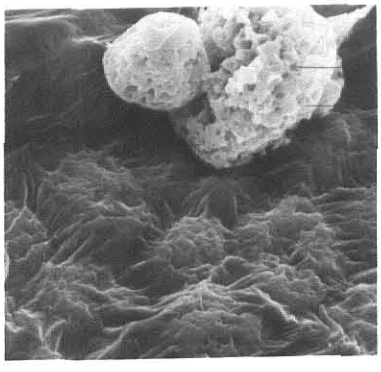

Fig. 6. Scanning electron photomicrograph of a waxy kernel recovered from rat small intestine (76x).

Fig. 7. Scanning electron photomicrograph of a waxy kernel recovered from rat small intestine (1600x).

Fig. 8. Scanning electron photomicrograph of a waxy kernel recovered from rat small intestine and split at right angles to original cut (2000x).

Fig. 9. Scanning electron photomicrograph of a yellow kernel recovered from rat large intestine (1600x).

Fig. 10. Scanning electron photomicrograph of a yellow kernel recovered from pig small intestine (80x).

Fig. 11. Scanning electron photomicrograph of a yellow kernel recovered from pig small intestine (1600x).

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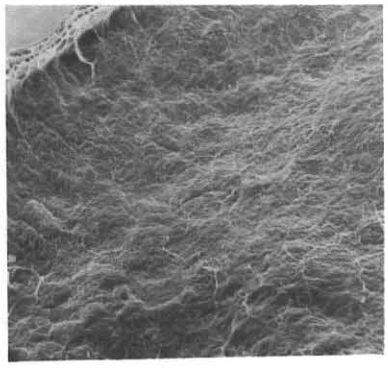

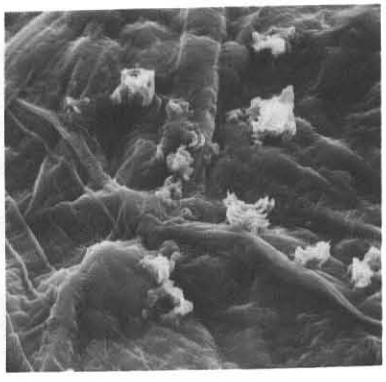

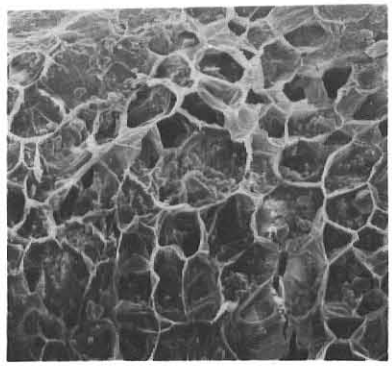

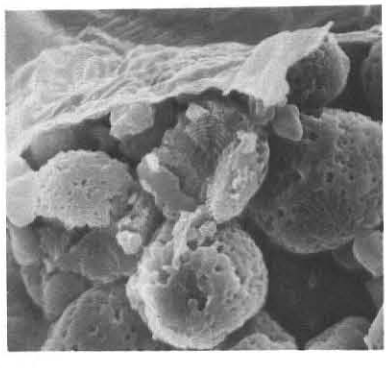

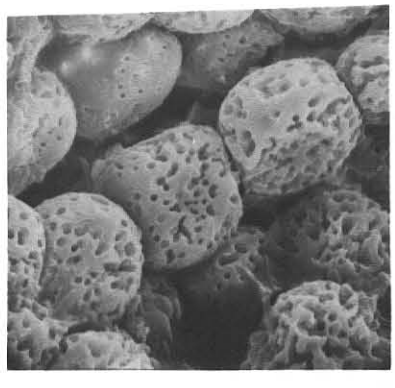

SCANNING ELECTRON MICROSCOPY OF MONOGASTRIC AND RUMINANT DIGESTION OF SORGHUM GRAIN

by

ARTHUR BENGT DAVIS
B.S., Oregon State University, 1969

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science and Industry

Kansas State University

Manhattan, Kansas

1973

Abstract

Visual images produced by scanning electron microscopy and maltose production from enzymatic attack were used to determine relative amounts of starch digestion among three types of sorghum grain. Sorghums used were a waxy endosperm (CP 622), yellow endosperm (C42 Y), and a bird resistant variety (Acco 1023). Split kernels of each type were in the rumen for periods of up to eight hours before recovery and observation. Split kernels were introduced into the digestive system of the rat and pig and recovered by dissection from various locations along the tract. Isolated starch from each variety was subjected to attack by purified porcine alpha-amylase, then observed on the scanning electron microscope. Visual results were correlated with maltose production.

Results indicated differences in both pattern and amount of attack dependent on starch source. Waxy endosperm starch was extensively degraded with a point type hydrolysis. Yellow endosperm showed somewhat less attack. Photomicrographs show a different pattern of attack, larger and more irregular erosion, indicating greater amounts of tangential hydrolysis compared with waxy endosperm. Bird resistant starch was much more resistant to hydrolysis than either of the other two varieties and showed some of both types of attack in addition to occasional large circular areas of surface erosion. Analysis of supernatant for maltose production confirmed differential rates of enzymatic hydrolysis. Such studies suggest that starch from the bird resistant variety will produce substrate inhibition.