DIGESTION OF LETTUCE AND BRAN IN THE RAT

by

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INTRODUCTION

Over the years the direction of civilized man's diet has been a continual drift towards more refined foods. In the last decade, however, partially due to the African studies of Burkitt (1-4), there has been a reoccurrence of interest in dietary fiber as a necessary ingredient in our diets. Burkitt found a great difference in dietary fiber intake between African and western civilizations and attributed the lack of western diseases in African countries to a high intake of dietary fiber. Since this rekindling of interest, fiber has been investigated for its hypothesized effects in dealing with diseases as diverse as constipation, diverticulosis, appendicitis, varicose veins, hemorrhoids, hiatal hernias, colon cancer, obesity, diabetes and coronary heart disease. Most of the effects attributed to fiber are based on its physical properties; however, much of the evidence is not conclusive and more scientific research is needed before the actual role of fiber in the diet is known (5-20).

Questions have arisen about the very definition of fiber, how it is (or is not) metabolized, and the observable effects of fiber in the diet. Physiologically, the accepted benefits of fiber include softer, bulkier stool; water absorption; sequestering properties which allow fiber to attach such agents as bile, cholesterol and fats to its surface; and enhanced bacterial action which aids in elimination (5, 21-28). Because fiber is now of principal interest in a wide number of fields, it has prompted a great deal of new research into many disease oriented areas. The sales potential for fiber as a low-cost bulk ingredients in packaged food products has stimulated commercial interests to

re-examine fiber. The physiological and biochemical changes in the gastrointestinal tract require further study.

One of the long standing problems in fiber research has been an absence of a definitive description of fiber. Originally, fiber was identified as an acid and alkali-resistant residue, defined as being nondigestible, and called crude fiber. Crude fiber was defined by the Weende procedure to be the difference observed between the residue weight of fiber treated with an acid (sulfhuric) and then a base (sodium hydroxide), and the residue's ash weight (29). This technique tends to underestimate cell wall constituents, and there is variation in the measurement methodology upon which the definition depends. As generally used in the literature, dietary fiber consists of, or constitutes, "the structural polysaccharides of the cell wall, lignin, plant lipids, nitrogen, trace elements, and other unidentified substances" or the plant cell wall or plant residues resistant to the digestive enzymes (30-32). According to Cummings (5), the definition of fiber "depends on one's point of view", and could include all or part of the whole plant cell-wall structure. He further states, "not all fiber is in fact fibrous, nor is it totally indigestible." Since the term fiber failed to present a sufficiently restrictive definition to be of specific use in this research fiber was defined as the whole plant cell structure. This definition of fiber includes starch, soluble proteins, lipids, sugars, pectin, hemicellulose, cellulose, lignin, lignified nitrogenous compounds, cutin, ash and fiber-bound proteins, with dietary fiber and crude fiber included as fractional components (33, 34, 35).

The initial aim of this research was to examine the various changes that fiber undergoes within the gastrointestinal tract (GIT) by means of the scanning electron microscope (SEM). A direct observation of the microanatomical processes of fiber digestion at various sites in the GIT would promote understanding of the physiological and perhaps bacterial changes ascribed to fiber. Since different fibers were expected to react differently under identical conditions, two types of fibers were used; lettuce as an example of vegetable fiber and wheat bran as a cereal fiber.

Following the SEM studies, the characteristics of fiber digestion were examined to provide insight into the biochemical functions of digestion. Ther term "digestibility was not rigidly defined. To enable measurements for this experimentation, digestibility was operationally defined as the total diet dry matter percentage digestion. The formulation for this measurement was:

Digestion = (dry feed wt. - dry fecal wt.) x 100/(dry feed wt.) (36). The belief that various components of fiber would be digested at different rates required examination of the digestibility of the several components of fiber. The laboratory techniques available for these analyses suggested measurements of 1) dry matter digestibility (DM), 2) acid detergent digestibility (ADF), 3) neutral detergent digestibility (NDF), and 4) hemicellulose digestibility (H).

The purpose of this research was to visually observe the fate of lettuce and bran during digestion and obtain digestibility coefficients of these fiber sources at varying fiber levels and grind sizes in the rat.

LITERATURE REVIEW

FIBER ANALYSIS

The increased awareness of the role of fiber in digestion as a possible cure for many common gastrointestinal disorders and diseases has led to new methods for analyzing the components and properties of fiber. Fiber has been a concern for quite some time from the ruminant nutrition standpoint, and researchers have used chemical forage analysis for a hundred years. Crude fiber determination as defined by the "Weende system" of "proximate analysis", developed in the mid 1800's, is one of the most common chemical methods of forage fiber analysis (29, 36). Researchers have recently begun to utilize the more comprehensive Van Soest fiber analysis technique (33, 34, 35, 37-39). This technique is preferred over the Weende proximate crude fiber analysis which has inherent disadvantages. In the Weende procedure, a major portion of the lignin within a sample is solubilized by sodium hydroxide digestion and as a result, lignin is calculated as a component of the nitrogen-free extract (29, 37). The Van Soest neutral detergent fiber (NDF) technique is, presently, the preferred method of estimating plant cell wall constituents. The Van Soest method, however, does require protracted time for analysis and presents problems with the lignin determination resulting from high temperatures (33, 40, 41).

Alternative methods to chemical analysis have recently been utilized to provide better interpretations of forage and fiber quality and digestibility. The light microscope was used to determine those plant cellular components which passed through the gastrointestinal tract virtually

intact (42). Development of the scanning electron microscope (SEM) in the latter half of the nineteen sixties has provided a more specific method for visually observing and interpreting structures. The SEM allows the researcher to analyze structures in situ, due to the high magnification, extended depth of field and high resolution power (43). The SEM theory and operational techniques are described in detail by Kimoto (44), Weakley (45), and Meek (46). High quality SEM pictures serve as a valuable tool for observing and identifying complex structures. The concept of stereoviewing SEM pictures is a relatively new technique and is a valuable asset in determining internal structural arrangements. The procedure for obtaining stereo pictures and subsequently observing the photographed specimen in three dimension detail is described by Howell (47). Another additional development to the SEM is x-ray analysis described by Kimoto (44). The x-ray procedure has aided in expanding the SEM application and analytical capabilities allowing definition of mineral element composition.

In a relatively short period of time the SEM has proved to be a useful technique in investigative research. Akin (48) used the SEM to study the microanatomical differences of warm season grasses. Researchers have been able to examine forage digestion and factors which influence the rate of digestion by rumen microbes (49-51). The inherent structural characteristics of plant tissues have been found to influence bacteria interaction with the plant tissue which effects the digestibility of forage (52). The SEM has been extensively used for studying cereal grain starch (53-58). A new application for the SEM was determined by Brazle (59), Brazle and Harbers (60) to describe the in vivo

digestion of alfalfa hay, brome and tall fescue.

Fiber studies utilizing the SEM are limited. The SEM has been widely used in textile and wood fiber studies (61-64) however, very little work has been done using the SEM in relation to the monogastric digestion of fiber, except for the work by Davis and Harbers (53), and Hoseney et al. (57) on sorghum grain starch. The scope of work dealing with lettuce and bran is even more limited. Several SEM investigations have been performed on lettuce diseases, but only the disease itself is discussed (65-68). At this time no published data exists on the structure or structural changes that lettuce of bran undergoes within the gastrointestinal tract. The thickness of wheat bran has been studied by SEM corroborating light microscope digestion studies (69-71). A similar human study on the digestibility of bran performed by Booth and Moran (72) also showed that the digestion of bran consisted essentially of the absorption of the aleurone cell contents.

FIBER UTILIZATION

A review of the literature on fiber digestion suggests that as the fiber content of the diet is increased, total digestibility decreases. Increases in dietary crude fiber content reduce the digestibility of selected nutrients in the diet. This can be explained by the fact that higher crude fiber decreases the action of the enzymes, adds bulk, thus reducing total ration intake, and elevates the rate of passage, resulting in less time for digestive bacterial action (73, 74). Digestibility increases with longer transit times and s fairly constant for diets containing fiber up to about 5% by weight (5, 73, 75, 76). Observed digestion declines as fiber content increases above this level (36, 73, 74).

Although fiber quantity is a significant factor, fiber particle size also affects digestibility (73, 75, 76, 77-80). Some researchers found that finer grind size increased the digestibility of the diet while the opposite effect, that larger grind sizes decreased transit time, has been observed by others (79). Ewing, Smith and Derreckson (73, 75) however, found a reduced transit time associated with fine grind. The observed differences by investigators may be due to the following:

1. Results are different for different fiber types.
2. Various findings, though not quantified, indicate different digestion rates for different particle sizes.
3. Confusion exists due to the various parameters used in digestion research, pointing out the necessity for rigorously defined terminology.
4. There may be a significant interaction between fiber content and particle size affecting digestibility.

The findings of Kornegay (81) were of particular interest. He found that feeding about 6% fiber to hogs tended to maximize weight gain. Kornegay made no digestion trials nor did he hypothesize regarding the cause for the observation, however, his findings are significant because the hog is a monogastric animal whose GI system is usually considered most comparable to the human system.

A computer literature search indicated a lack of available data on visual fiber analysis utilizing the SEM. This limited information confirmed the desirability of the proposed study.

MATERIALS AND METHODS

SCANNING ELECTON MICROSCOPE STUDIES

Lettuce SEM Mineral Scan (X-ray Analysis)

A mineral scan was run on fresh lettuce samples following the procedure described by Kimoto (44). Lettuce samples (5 mm) were air dried and mounted on stubs with low resistance contact cement (E. F. Fullan Co., Schenectady, N.Y.). Air drying minimized ionic translocation and the carbon cement aided in eliminating a large silver peak from normal colloidal silver cement used for secondary images (82). The samples were coated with 1-2 nm evaporated carbon and viewed in the SEM equipped with an energy-dispersive x-ray analyzer (EDAX Model 711). The energy-dispersive x-ray spectrograph (210 and above) was photographed on Polaroid film (Polaroid Land Co., Inc., Rochester, New York).

Lettuce and Bran SEM trials

Twenty adult, male, Sprague-Dawley rats weighing 150-175 gm were each housed in individual digestion cages with raised wire floors at controlled temperature (22° C). Ten rats were fed ad libitum a diet of lettuce and water and ten were fed bran and water (ad libitum) for three days after which they were sacrificed using ether. Gastrointestinal contents (including feces) were obtained from the stomach, duodenum, jejunum, ileum, cecum and large intestine. Lettuce and bran control samples, and those obtained from each section, were placed in separate sealed test tubes and, for chemical fixation, treated with a 4% gluteraldehyde-phosphate buffer consisting of 7 ml of .07 M $Na_2HPO_4 \cdot 2H_2O$ and 3 ml of .07 M KH_2PO_4, pH 7.168 (83). Specimens were refrige-

rated 24 hours, then dehydrated through a graded ethanol) (84). Small representative samples from each container were critical point dried with carbon dioxide and mounted on aluminum stubs with Delco No. 93 colloidal silver conducting cement (Ted Pella Co., #1603-2) (85, 86). Specimens were then coated by vacuum evaporation (Kenny Vacuum Co., Model KSE-2A-M evaporator) with carbon followed by 10-20 nm of gold-palladium (87). Samples were then viewed at 10 Kv. acceleration voltage for observation with an ETEC Autoscan scanning electron microscope and images were recorded on Polaroid® film.

Observable changes in the stomach samples were expected to be due to the action of HCl, chewing, and mechanical abrasion in the stomach. Protein content of lettuce is only 1.2% so pepsin action was expected to be minimal. Control samples for comparison with the in vivo stomach samples were run in vitro. Fresh lettuce was cut into ten 5 mm square samples; five samples were placed in a test tube of 1 N HCl solution (86.0 ml concentrated HCl diluted to one liter). The other five samples were placed in a test tube of 1 N HCl with 5.7×10^{-5} molar pepsin (2.0 g of 1:10,000 pepsin dissolved in 850 ml H_2O, added to 100 ml 1 N HCl and diluted to 1 liter) (88). After 12 hours, the samples were removed from the solutions, and prepared for SEM viewing and photographing as indicated for in vivo trials.

Scanning Electron Microscope (SEM)

The ETEC Autoscan scanning electron microscope used in these studies has resolution capabilities up to 200 A^{o}, and an acceleration voltage of 5-50 Kv. Objects were scanned at 10 Kv acceleration voltage,

visually observed on the cathode ray tube and then recorded on Polaroid ® 55 P/N film with a camera mounted over the viewing tube. For the x-ray spectrograph, the SEM was equipped with an EDAX Model 711 energy dispersion x-ray analyzer. The theory and operational techniques of the SEM are described in detail by Kimoto (44), Weakley (45), and Meek (46).

DIGESTION STUDIES

Pilot Study

A pilot study was run on fiber digestibility based on percent fiber. Twelve adult, male, Sprague-Dawley rats weighing 150-175 grams each were housed in individual digestion cages with raised wire floors at controlled temperature (22°C). Three experimental diets were used: 0%, 5%, and 10% fiber. The fiber source, iceberg lettuce (Lactuca sativa), was dried for 24 hours at 54°C and ground to 400μ. The fiber was added to a basal diet of Purina rat chow (Ralston Purina Co., St. Louis Mo.) ground to the same particle size.

Each diet was fed to four randomly selected rats for three days prior to the onset of the experiment in order for the rats to adjust to the diet. The rats were then starved for 24 hours before starting the trial, after which they were fed the experimental diets and water ad libitum for 5 days. Fecal collections began at the onset of the trial period. At the end of the 5 day feeding period all feed was removed but fecal collections continued for another 24 hours. The total 6 day fecal collection was dried for 12 hours at 54°C and total diet digestibility percentages were determined:

Digestion = (dry feed wt. - dry fecal wt.) x 100/(dry feed wt.) (36).

The results of the pilot study revealed a quadratic relationship between digestibility and percent fiber which is in conflict with the accepted position that fiber levels produce no significant digestion differences up to the 5% level (36, 73, 74). This suggested that if the observations were true, a maximum level might exist for digestibility as a function of fiber content. With the prospect of two possibly non-linear variables, fiber level and grind size, interacting to produce different digestion measures, statistical analysis techniques that would yield predictive equation were in order. Response Surface Methodology (RSM) was used to analyze the effects of both percent fiber and particle size on digestibility using lettuce and bran as fiber added sources. There existed no guidelines to assist in determining the parameters for these variables and their interaction. Observations taken in the pilot study suggested that if the digestibility optimum existed it would probably be located in the 4-6% added fiber range. Fiber percent was chosen to vary from 1.2 to 6.8% added fiber. Particle size was selected to range from 208 μ to 1190 μ due to the physical size constraints inherent in the sources.

Lettuce and Bran Digestion Trials

Adult male, Sprague-Dawley rats were randomly assigned to preselected diets. The composition of the diets conformed the requirements of a quadratic two factor RSM design. This statistical technique provides an analysis of effects and interactions of grind size and percent fiber (89). Thirteen experimental diets were established (Table 1) and three replications were performed for both lettuce and bran (i.e. 39 observations for each added fiber source).

TABLE 1

Diet composition for a two factor RSM design

Diet #	Diet % Fiber[1]	Grind[2]	Diet Grind[3]	% Fiber[4]
1	2	-1	414 μ	-1
2	6	-1	414 μ	1
3	2	1	833 μ	-1
4	6	1	833 μ	1
5	1.2	0	590 μ	-1.414
6	6.8	0	590 μ	1.414
7	4	-1.414	208 μ	0
8	4	1.414	1190 μ	0
9	4	0	590 μ	0
10	4	0	590 μ	0
11	4	0	590 μ	0
12	4	0	590 μ	0
13	4	0	590 μ	0

[1]Percent added fiber
[2]Coded value for percent added fiber
[3]Grind size in microns
[4]Coded value for particle size

Five fiber percentages and five particle sizes were used to fit the statistical model required for the RSM analysis (90) (Table 2).

TABLE 2

Coded values, fiber levels and particle sizes for the RSM design

Percent (%) Fiber[1]	Particle Size (Grind)[2]	Coded Values[3]
1.2 %	208 μ	-1.414
2.0 %	414 μ	1.0
4.0 %	590 μ	0.0
6.0 %	833 μ	1.0
6.8 %	1190 μ	1.414

[1]Percent added fiber [2]Grind size in microns
[3]Coded Values for relative particle and grind size

The fiber sources were iceberg lettuce and bran. The lettuce was dried at 54°C for 24 hours and then ground to the specified particle sizes. Fiber was added to a ground basal diet of Purina rat chow® of the same grind size. Fiber quantities were calculated to produce the required fiber percentages based on total diet weight.

The rats, housed in individual digestion cages as indicated in the pilot study, were fed the specified lettuce and bran experimental diets for three days prior to the onset of the experiment to allow them to adjust. The rats were then starved for 24 hours to empty the gastrointestinal tract. They were then fed the experimental diets and water ad libitum for five days. The total weight of feed consumed by each rat during the five day feeding period was recorded. Fecal collections for each rat began at the onset of the trial period. At the conclusion of the five day feeding period all rations were removed but feces were collected for an additional 24 hour period. During the six day collection period the feces were kept under refrigeration. At the end of the collection period the feces were dried for 12 hours at 54°C, weighed and ground to 400 μ. Dry matter percent values were determined in duplicate for all 13 diets and 39 fecal collections (36). The ADF, NDF and H values were determined in duplicate for all 13 diets and 39 fecal collections by the Van Soest fiber analysis technique (33, 34, 35, 37, 39). The DM, ADF, NDF and H digestibilities were then calculated and used to generate the RSM models using the least squares criterion by means of the stepwise selection and backward elimination procedures. Since the nature of the investigation was exploratory the regression coefficients, the β values, were retained

at the 10% significance level ($\alpha = 0.10$). Once generated, the models were tested for lack of fit, and significance was assessed for each term retained. Upon final selection of the appropriate model, an in-file program was used to generate a surface plot for each model (90, 91).

Dry Matter Determination

Dry matter was determined for the samples by a standard analytical procedure wherein diet and feces were dried to determine a dry matter index. Dry matter digestibilities were calculated using the standard formula (36).

Van Soest Techniques (ADF, NDF and H)

Plant composition is very complex, consisting of many different components which have vastly different levels of digestibility (5, 13, 25-27, 29-31, 92-94). For example, carbohydrates are almost totally digested by the monogastric, while compounds such as lignin act as digestion inhibitors by physically coating available carbohydrates, thus limiting access of digestive enzymes (5, 75, 92, 95-97). The most reliable technique for the measurement of fractional fiber digestibility is the set of analytical techniques developed by P. J. Van Soest (28, 33, 34, 35, 37, 39). These techniques provide valid and reliable measurements which are used for calculating the digestion of various components of fiber. The neutral detergent fiber (NDF) residue analysis is used to quantify the extent to which starch, soluble protein, lipids, non-protein nitrogenous compounds, sugars and pectin are digested. The acid detergent fiber (ADF) residue analysis is used to

quantify hemicellulose (H) and fiber-bound proteins in addition to the other components quantified by NDF. Since quantity of fiber-bound proteins is functionally negligible, the difference in ADF and NDF digestibility is a reliable measurement of hemicellulose digestibility (34, 35).

Response Surface Methodology (RSM)

Response surface methodology (RSM) has been used extensively as an analytical tool in the area of food science, nutrition, and animal sciences (98-101). RSM was selected for this project because it generated predictive models for the dependent variable (digestibility) as a function of the independent variables (percent fiber and particle size) and would generate a "surface" descriptive of the interactions of the independent variables. In this particular case, the variables would interact in a non-linear fashion; therefore, a full quadratic model was selected (89).

Response Surface Methodology (RSM) was used to analyze the test results of the digestibility trials. A full quadratic regression model was selected to approximate the hypothesized model. The quadratic model is always symmetrical with the type of symmetry determined by the coefficients retained within the model. When a maxima is observed, the surface plot will be a series of concentric ellipses (Fig. 27). When the observations fail to produce an optimum, several types of plots can be generated. The interpretation becomes difficult because the quadratic model does not adequately describe the hypothesized bivariate bell curve. When the data generates a three dimensional

hyperbolic surface (e.g. Fig. 1) there exists no physiological or biological interpretation which is appropriate. The total data set indicates that when the hyperbolic models are encountered, the range of observations failed to include the digestion maxima. If such a maximum exists, it must be located outside the boundaries of the plot. This optimum, is located by extending a line from the intersection of the asymptotes (equilibrium point) through the centers of the ascending regions of the plot (Fig. 1). The location of the maximum is believed to be along this line and beyond the boundaries of the plot. Thus, in Fig. 1 a digestion maximum would be obtained for a combination of either high percent fiber and small grind size or low percent fiber and large grind size. See the conclusion and Fig. 35 for additional discussion.

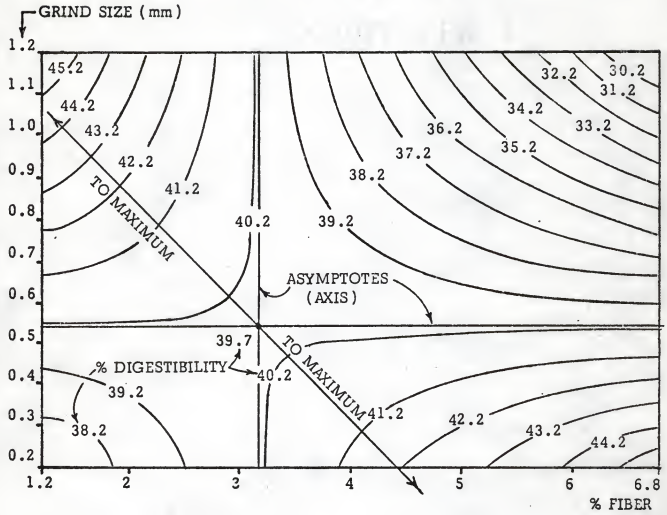


Fig.1 Equilibrium point.

RSM full quadratic regression model.

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1^2 + b_{22} X_2^2$$

RESULTS AND DISCUSSION

SCANNING ELECTRON MICROSCOPY STUDIES

Lettuce SEM Mineral Scan (X-ray Analysis)

A composite mineral scan of the adaxial and abaxial lettuce leaf (Fig. 2) revealed the epidermis to consist of four major peaks; sodium (Na), phosphorus (P), chlorine (Cl), and potassium (K). There are three minor peaks; silica (Si), silver (Ag) (from mounting cement), and calcium (Ca). Only qualitative measurements were made. Due to the inherent characteristics of the instrument and geometry of the plant specimens, quantitative measurements were prevented.

This study indicated that there is very little silica present that could act as a structural inhibitor to digestion, a problem in ruminants (60).

Lettuce, SEM Trial

In an examination of an untreated fresh lettuce sample, the adaxial (upper) leaf surface (Fig. 3a) showed irregularly shaped cells with very few, small, randomly oriented stomata (a characteristic of dicotyledons). The abaxial (lower) surface (Fig. 3b) showed similar cell structure but a larger occurrence of stomata. A cross section revealed random dispersions of dense vascular bundles (phloem and xylem) and undifferentiated mesophyll and fibrous material connecting the bundles to the cuticle (Fig. 3c).

Fresh lettuce *in vitro* samples were treated with HCl and HCl/pepsin to furnish a comparison with the *in vivo* stomach samples. The *in vitro* samples treated with HCl revealed excessive wrinkling and

lifting of the cuticular surface (Fig. 4a) and a separation of phloem and xylem in the vascular bundle, probably a result of HCl hydrolysis of the pectic substances beneath and between fibrous strands (Fig. 4b). The in vitro samples treated with HCl/pepsin revealed the same cuticular lifting and wrinkling (Fig. 5a) and the same vascular bundle separation as with HCl alone (Fig. 5b). The cuticular lifting and wrinkling and vascular bundle separation were probably the result of HCl hydrolysis.

Samples were obtained from two sections of the stomach, the forestomach (Pars cariaca) and the glandular stomach (Pars pylorica). The forestomach samples showed wrinkling and lifting of the cuticular surface as a result of HCl hydrolysis (Fig. 6a), similar to the in vitro trial, plus collapsing of the mesophyll due to mechanical action (Fig. 6b). Samples from the glandular stomach revealed continued mesophyll compaction caused by mechanical action (Fig. 7a), plus further lifting and separation of the cuticular layers away from the mesophyll as a result of HCl hydrolysis (Fig. 7b). In comparing the stomach samples with the in vitro trials the observable changes in the stomach appeared to result from HCl hydrolysis and mechanical abrasions.

Samples from the duodenum revealed separation of the abaxial surfaces with continued exposure of the compacted mesophyll (Fig. 8a). The appearance of globular stringy masses was probable evidence for the beginning of either protoplasmic digestion or artifacts of intestinal excretion (Fig. 8b).

Samples from the jejunum showed continued evidence of protoplasmic digestion with cuticular sluffing and a 1-2 cell thickness of mesophyll still attached to its underneath side (Fig. 9).

Samples from the ileum revealed crevices within the partially sluffed cuticle (Fig. 10a), probably as a result of mechanical action in the intestine, and the partially separated cuticle layers started folding over on themselves (Fig. 10b). Bacteria began to appear along the rough edges of the compacted mesophyll, however, little surface digestion is evident (Fig. 10c).

Samples from the cecum showed the cuticular surfaces totally separated from the mesophyll, leaving the isolated mesophyll available for further compaction and degradation (Fig. 11 a, b). Bacteria were increasingly evident on mesophyll surfaces, resulting in digestion of the exposed vascular bundles and leaving highly lignified tracheal tubes (Fig. 11c).

In the large intestine, there was evidence of continued folding and compaction of sluffed cuticle and isolated mesophyll (Fig. 12 a, b). The compacted mesophyll was separated from an exposed the vascular bundles to possible further bacterial degradation (Fig. 12c). Bacteria continued to attack the primary cell walls within the vascular bundle resulting in an unwinding of the tracheal tubes (Fig. 12d), and continued separation of the phloem and xylem within the vascular bundle (Fig. 12e).

As indicated by the feces, the upper surface of the totally sluffed cuticle was untouched by bacteria (Fig. 13); however, the sluffed cuticle undersurface that maintained a 1-2 cell thickness of mesophyll was extensively attacked by bacteria (Fig. 13 b, c). There was also extensive bacterial population on the isolated mesophyll, indicating some bacterial digestion of exposed mesophyll (Fig. 13 d, e). The

vascular bundle appeared to be totally digested except for the unwound tracheal tubes (Fig. 13f).

Fig. 2 Lettuce SEM Mineral Scan (X-Ray Analysis)
A composite mineral scan of a fresh lettuce leaf revealed the epidermis to consist of four major peaks (left to right) sodium (Na), phosphorus (P), chlorine (Cl), and potassium (K). There are two minor peaks; silica (Si), and calcium (Ca).

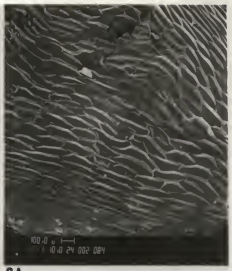
Fig. 3 Fresh Lettuce Leaf Samples

a) The adaxial leaf surface (ad) of fresh lettuce showed irregularly shaped cells with very few small randomly oriented stomata (s).

b) The abaxial surface (ab) of fresh lettuce leaves showed similar cell structure to the adaxial side but a larger occurrence of stomat (s).

c) Fresh lettuce leaf cross section revealed random dispersions of dense vascular bundles, phloem (p) and xylem (x) and undifferentiated parenchyma or mesophyll (m), and fibrous material (f) connecting bundles to cuticle.

2



3A

3B

3C

Fig. 4 Lettuce Treated With HCl

a) Lettuce sample treated in vitro with HCl revealed excessive wrinkling and lifting of the cuticular surface (c) as a result of HCl hydrolysis of the pectic substance beneath the cuticular surface.

b) Lettuce sample cross section treated in vitro with HCl showed the separation of the phloem (p) and xylem (x) in the vascular bundle probably as a result of HCl hydrolysis of the pectic substances between fibrous strands.

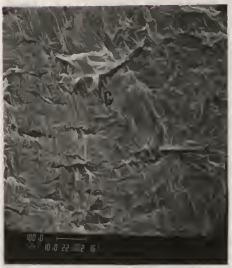
Fig. 5 Lettuce Treated With HCl And Pepsin

a) Lettuce sample treated in vitro with HCl and pepsin demonstrated the same cuticular (c) lifting and wrinkling as with the HCl alone.

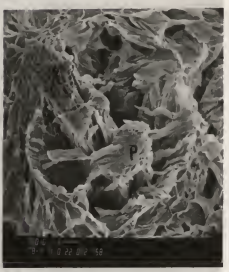
b) Lettuce sample cross section treated in vitro with HCl and pepsin revealed the same vascular bundle separation as with HCl alone.

4A

4B



5A



5B

Fig. 6 Lettuce Samples From The Forestomach (Pars cardiaca)

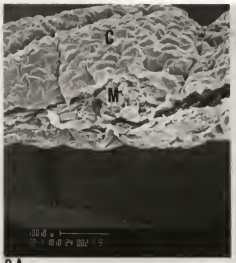
a) A cross section of lettuce from the forestomach showed wrinkling and lifting of the cuticular surface (c) as a result of HCl hydrolysis similar to the in vitro trial.

b) A second cross section from the forestomach indicated a collapsing of the mesophyll (m) as a result of mechanical action.

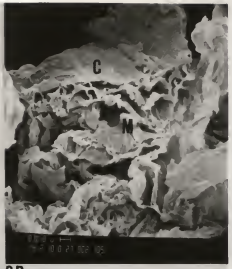
Fig. 7 Lettuce Samples From The Glandular Stomach (Pars pylorica)

a) An example from the glandular stomach revealed continued mesophyll (m) compaction caused by mechanical action.

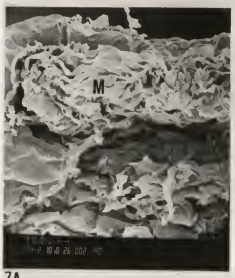
b) A sample from the glandular stomach showed further lifting and separation of the cuticular layers (c) away from the mesophyll (m) as a result of HCl hydrolysis.



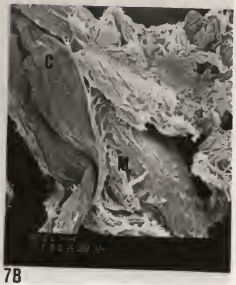
6A



6B



7A



7B

Fig. 8 Lettuce Samples From The Duodenum

a) A sample from the duodenum revealed separation of the abaxial and adaxial surfaces with continued exposure of the compacted mesophyll.

b) There appeared globular stringy masses within the duodenum probable evidence of beginning protoplasmic digestion or artifacts of intestinal secretions (d).

Fig. 9 Lettuce Sample From The Jejunum

A sample from the jejunum showed continued evidence of protoplasmic digestion along with cuticular sluffing. There appears to be a 1-2 cell thickness of mesophyll (m) still attached to the underneath side of the cuticle (c).

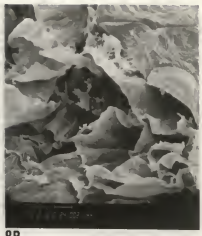
Fig. 10 Lettuce Sample From The Ileum

a) A sample from the ileum revealed crevices within the partially sluffed cutical, probably as a result of mechanical action within the intestine.

b) A second example from the ileum showed the partially separated cutical layer folding over on itself.

c) Bacteria (b) began to appear along the rough edges of the compacted mesophyll, however, little digestion was evident. These bacteria were more apparent at a higher magnification.

8A



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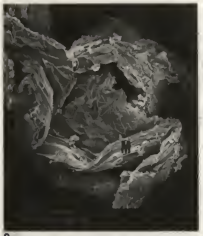


9

10A

10B

10C

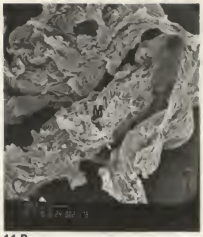
Fig. 11 Lettuce Samples From The Cecum

a) In a sample from the cecum the cuticular surfaces (c) appeared to be totally separated from the interior mesophyll.

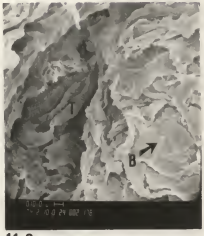
b) Another cecum sample showed that the separated cuticle leaves the isolated mesophyll (m) available for further compaction and degradation.

c) Bacteria (b) were increasingly evident on mesophyll surfaces within the cecum, resulting in digestion of the exposed vascular bundles leaving highly lignified tracheal tubes (t).

11 A



11 B



11 C

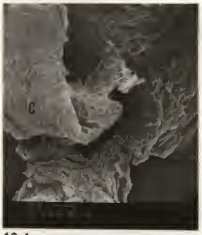
Fig. 12 Lettuce Samples From The Large Intestine

a) and b) In the large intestine there was evidence of continued folding and compaction of sluffed cuticle (c) and isolated mesophyll (m).

c) Another large intestine sample revealed the compacted mesophyll separated from the vascular bundles (v) exposing them to further bacterial degradation.

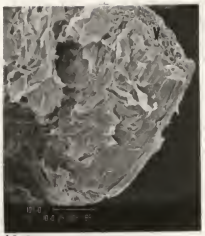
d) A cross section sample from the large intestine showed bacteria continue to attack the primary cell walls within the exposed vascular bundle resulting in an unwinding of the tracheal tubes (t).

e) Another large intestine example indicated continued separation of the phloem (p) and xylem (x) within the vascular bundle.



12 A

12 B



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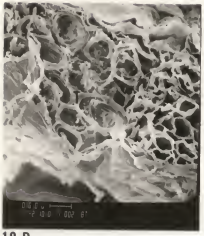

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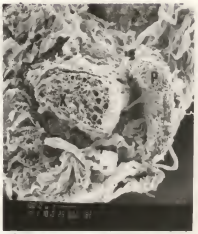


12 E

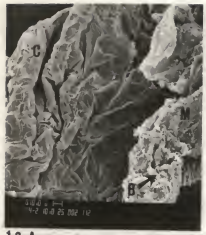
Fig. 13 Lettuce Fecal Samples

a) A sample of feces showed the upper surface of the sluffed cuticle (c) untouched by bacteria through the digestive process, however, the under-surface that maintained 1-2 cell thickness of mesophyll (m) was attacked by bacteria (b).

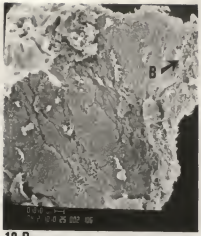
b) and c) Bacterial (b) population on the underneath surface of the sluffed cuticle was quite extensive.

d) and e) Extensive bacteria (b) were present on the isolated mesophyll (m), indicating some bacterial digestion of exposed mesophyll.

f) The vascular bundle appeared to be totally digested except for the unwound tracheal tubes (t).



13 A



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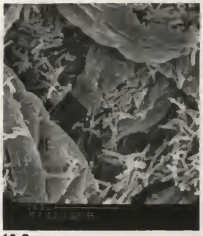
13 C



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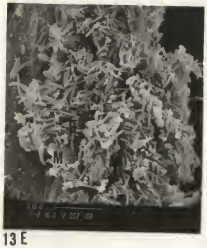
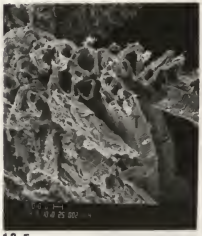


13 E



13 F

Bran SEM Trial

The epidermis of untreated fresh wheat bran revealed irregularly shaped cells and, as a result of milling, some starch granule contaminants on the epidermal surface plus breaking of the epidermis which exposed the underlying cross cells (Fig. 14 a, b). The inner surface of the epidermis indicated a layer of exposed endosperm still attached, along with some starch granules (Fig. 14c). A cross section showed a separation of the epidermis and tube cells from the aleuron cells and endosperm (Fig. 14d).

Bran samples were obtained from each section of the stomach, as for the lettuce trials. The forestomach samples indicated cracking or splitting of the epidermis, probably due to a combination of the milling process and mechanical action within the stomach (Fig. 15a). Sluffing of the epidermis along with swelling of the aleuron cells was probably due to hydration (Fig. 15b). Samples from the glandular stomach revealed continued separation of the epidermis and aleuron cells. Loosening of the aleuron cells around and within was due to the hydrolysis of the protein matrix (Fig. 16).

Within the duodenum, extensive sluffing of the epidermis was apparent along with separation between the exposed cross and aleuron cells due to the hydrolysis of protein substances between the cross cells (Fig. 17a). A disruption of the cellular integrity of both the aleuron and cross cells was also evident (Fig. 17b).

The disruption of the aleuron cells was a result of the hydrolysis of the aleuron contents (protein lipid matrix) as stated by Saunders (71).

In the jejunum, hydrolysis between and within the endosperm cells began to occur along with continued disruption of the aleuron cell wall integrity and compaction of the cross cells and epidermis (Fig. 18a). Beginning signs of endosperm hydration was also evident (Fig. 18b).

Within the ileum, continued epidermal cracking occured and, as expected, the first signs of bacteria were present on the surface breaks which exposed the interior surface (Fig. 19a). A cross section revealed continued cross and aleuron cell degradation along with separation of the epidermis from the cross cells and continued evidence of endosperm hydrolysis due to bacterial action (Fig. 19b). Bacteria were not evident on the endosperm at 200X but were very evident at a higher magnification (800X) (Fig. 19c).

In the cecum, the bacterial attack was much more evident, especially along the breaks on the epidermal surface (Fig. 20a). Extensive dpidermal distortion and lifting occured due to bacterial attack beneath the surface, and the bacterial population on interior tissues appeared uniform over the entire surface (Fig. 20b).

Samples removed from the large intestine showed evidence of the effect of bacterial attack and continued separation of the epidermal surfaces in an elongated fashion (Fig. 21a). There was also evidence of concentrated bacterial attack on the aleuron and endosperm (Fig. 21b). At a high magnification (Fig. 21c, 1000X; Fig. 21d, 2000X) the bacterial concentration within the aleuron cells and endosperm was clearly indicated. The bacteria appeared to attack along the endosperm cell walls until the cells eventually loosened enough so that they either fell

out or were totally digested (Fig. 21e).

Fecal samples indicated a continued bacterial degradation and extensive desegmentation and separation of endosperm tissues (Fig. 22 a, b). The SEM pictures showed the underlying partially digested aleuron and tube cells and associated desegmentation of the cross cells. At a high magnification, the concentrated bacterial attack between endosperm cell walls and within the cell walls of absent cells was evident (Fig. 22 c, d).

Fig. 14 Fresh Bran Samples

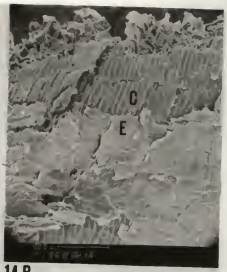
a) A sample of untreated fresh wheat bran epidermis (e) revealed irregular shaped cells along with some starch granule (s) contaminants on the epidermal surface due to milling.

b) Another wheat bran epidermis (e) sample showed breaking of the epidermis, exposing the underlying cross cell (c), again as a result of milling.

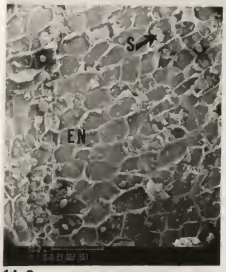
c) A sample of the inner epidermis surface showed a layer of endesperm (en) still attached along with some starch granules (s) present.

d) A cross section of wheat bran showed separation of the epidermis (e) and tube cells (t) from the aleuron cells (a) and endosperm (en).

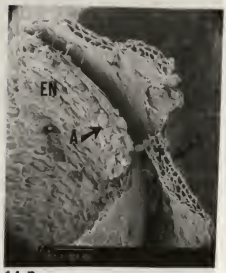
14 A



14 B



14 C



14 D

Fig. 15 Bran Samples From The Forestomach (Pars cardiaca)

a) Within the forestomach, cracking or splitting of the epidermis (e) occurred, probably due to a combination of mechanical action and the milling process.

b) A second sample from the forestomach revealed continued sluffing of the epidermis along with swelling of the aleuron cells (a) probably due to hydration.

Fig. 16 Bran Samples From The Glandular Stomach (Pars pylorica)

A sample from the glandular stomach revealed continued separation of the epidermis (e) and aleuron cells (a). Loosening of the aleuron cells occurs as a result of hydrolysis of the protein matrix.

15 A

15 B

16

Fig. 17 Bran Samples From The Duodenum

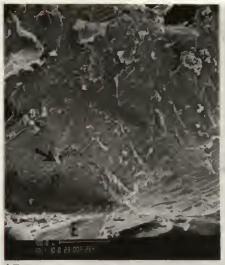
a) Extensive sluffing of the epidermis (e) and separation between the exposed cross cells (c) occurred with the duodenum due to hydrolysis of protein substances between the cross cells (arrow).

b) Separation of the cross cells (c) and aleuron cells (a) was observed within the duodenum and disruption of the cellular integrity of both the aleuron and cross cells.

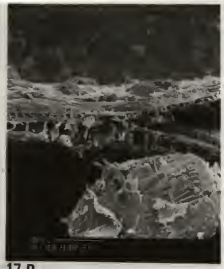
Fig. 18 Bran Samples From The Jejunum

a) Hydrolysis between and within the endosperm cells (en) began to occur within the jejunum (arrow).

b) A sample from the jejunum demonstrated continued disruption of the aleuron cell (a) wall integrity along with compaction of the cross cells (c) and epidermis. Beginning signs of endosperm (en) hydration were also evident.

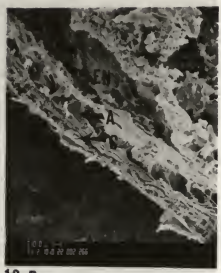


17 A



17 B

18 A



18 B

Fig. 19 Bran Samples From The Ileum

a) Continued epidermal cracking and the first signs of bacteria (b) were present on epidermal surfaces (e) of ileal samples. The bacteria were concentrated along the surface breaks which expose the interior surface.

b) An ileal sample cross section revealed continued cross cell (c) and aleuron cell (a) degradation and separation of the epidermils (e) from the cross cells. There was also continued evidence of endosperm hydrolysis (en).

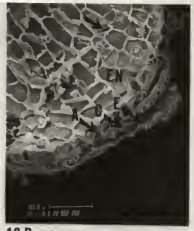
c) Bacteria (b) attached to the endosperm of ileal samples was evident at higher magnification.

Fig. 20 Bran Samples From The Cecum

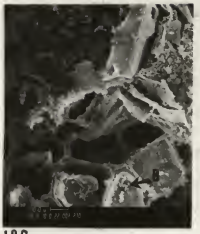
a) In the cecum the bacterial (b) attack was extremely evident especially along the breaks on the epidermal surface (e). Extensive epidermal distortion and lifting occured due to bacterial attack beneath the surface.

b) A cross section view of another sample from the cecum again demonstrated the concentrated bacterial (b) population on interior tissues.

19 A



19 B



19 C

20 A

20 B

Fig. 21 Bran Samples From The Large Intestine

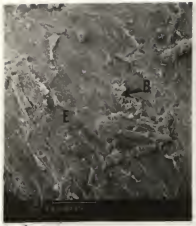
a) Epidermal (e) deterioration within the large intestine was evidence of the effect of bacterial (b) attack.

b) Continued separation of the epidermal surfaces (e) in an elongated fashion, and evidence of the concentrated bacterial (b) attack on the aleuron (a) and endosperm (en) was evident within the large intestine.

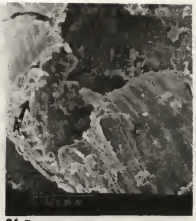
c) The bacterial (b) concentration within the aleuron cells (a) and on endosperm surfaces on samples removed from the large intestine were evident at higher magnification.

d) The extent of bacterial(b) attack on the endosperm was amplified at a higher magnification.

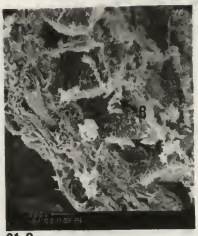
e) In the lower section of the large intestine the bacteria (b) attacked first along the endosperm cell walls until eventually the cells loosened to the point where they either fell out or were totally digested.



21 A



21 B



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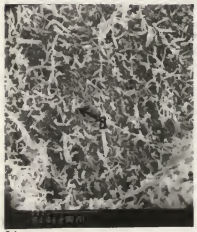
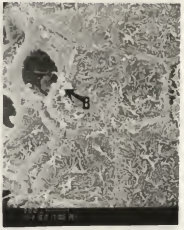


21 D



21 E

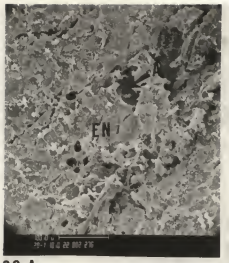
Fig. 22 Bran Fecal Samples

a) A sample from the feces demonstrated the extent of bacterial degradation of endosperm tissues (en). There was extensive desegmentation and separation evident along with endosperm digestion and rupture of aleuron cells (a).

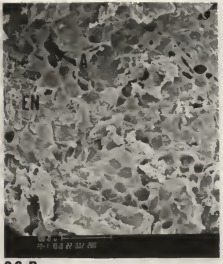
b) A second sample from the feces demonstrated the digestion of the endosperm cells (en) and exposure of the underlying aleuron cells (a).

c) As shown by this higher magnification the concentrated bacterial attack between endosperm cell walls and within the cell walls of absent cells indicated the pattern of digestion.

d) Destruction of the aleuron (a) and tube cells (t), and desegmentation of the cross cells (c) was revealed in a sample removed from the feces.



22 A



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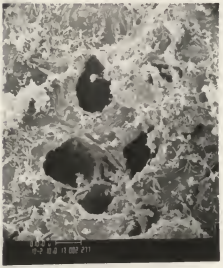

22 C

22 D

DIGESTION STUDIES

A diagram representing the pilot digestion study results and the "classic" digestibility distribution (Fig. 23), indicates that digestibility is fairly constant for diets containing fiber up to about 5% by weight, with digestion declining as fiber content increases above this level. The pilot study indicated that this is not the case and revealed that lower levels of fiber bring about lower levels of total digestion. The pilot study indicated that the relationship of percent fiber to digestibility was quadratic. This condition is represented by the sloping solid line to the right and the sloping dashed line to the left of the 4-5% fiber line. The conclusion drawn from the pilot study was that a maximal digestibility point existed at some percent fiber, rather than a level condition followed by a digestibility reduction as represented by the classic hypothesis.

A possible explanation of the quadratic effect of percent fiber on digestibility is graphically presented in Figure 24. Position #1 on the diagram indicates a low fiber percent accompanied by a relatively high non-digested percent and a relatively low digested. Position #2 on the diagram indicates a higher fiber percent accompanied by a relatively low non-digested percent and a relatively high digested percent. This position represents the optimum condition on the digestibility curve. Position #3 on the diagram indicates the highest fiber percent accompanied by a relatively low non-digested percent and a relatively low digested percent. The general relationship of the percent digested bar on the bar graph is an indication of the bell digestibility curve at the top of the page.

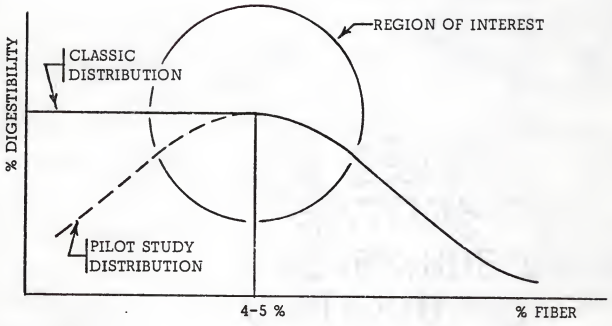

Fig. 23 Pilot study versus classic digestibility distribution as a function of percent fiber.

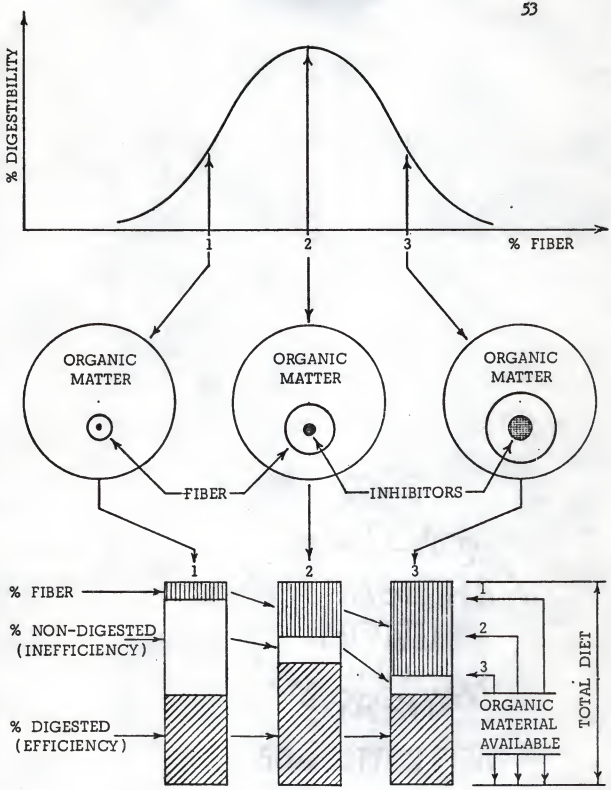

Fig. 24 Digestibility distribution as a function of percent fiber.

The significant factor indicated by the illustration is that percent digestibility goes down as fiber percent increases or decreases on either side of the optimum point on the digestibility curve.

A schematic representation of the quadratic effect of grind size on digestibility is represented in Fig. 25. This figure graphically indicates how digestibility may be a function of grind size. The SEM photographs reveal that the physical structure of a plant is highly porous and has a diverse composition. Up to a point, a reduction of particle size increases the surfaces available for chemical and bacterial action in the digestive process and increases digestibility, however, as confirmed by SEM observation, a reduction of particle size below some level tends to physically collapse the structure. This condition of compaction ultimately reduces the available surface for either chemical or bacterial action (79).

Position #3 of Fig. 25 represents a large particle with large porous openings. Position #2 represents an optimum particle size, with the maximum surface area available for digestion. Position #1 represents the collapsed or compacted situation discussed above.

The discussion indicates that digestibility may be a quadratic function of grind size, and that a maximum digestibility exists.

A three-dimensional representation of digestibility as a function of percent fiber and grind size is shown in Figure 26. The results of the response surface methodology (RSM) analysis indicate that for any given level of fiber content, digestibility will maximize at some specific particle size. Conversely, for any given particle size, digestibility will maximize for some specific fiber content.

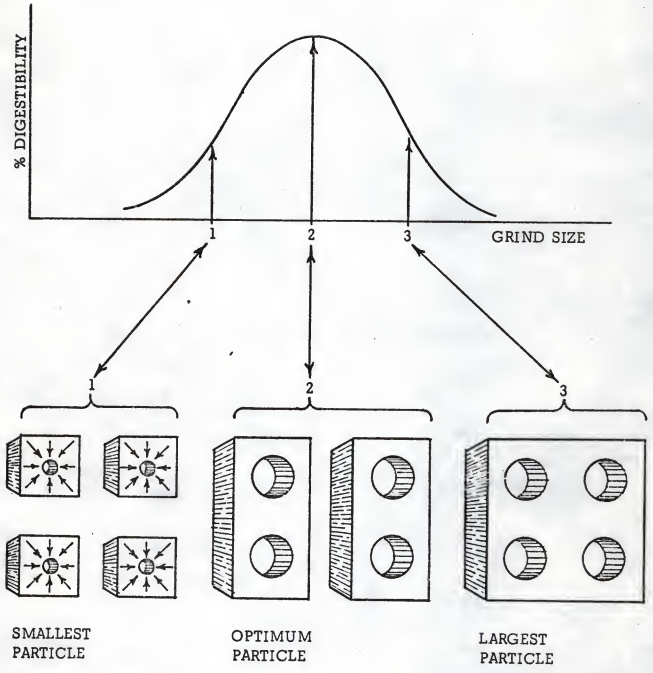

Fig.25 Digestibility distribution as a function of grind size.

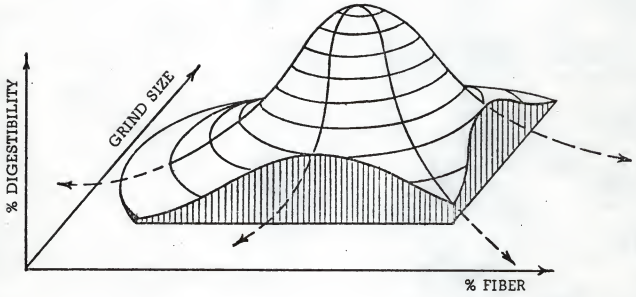

Fig.26 Three dimensional representation of digestibility as a function of percent fiber and grind size.

The dry matter (DM) digestibility of lettuce (Fig. 27) was found to obtain a digestion model of the form:

$$DM = G + F^2 + G^2 \quad [F(3, 35) = 12.81;\ p = 0.0001] .$$

The RSM plot for the above model yielded a family of concentric circles, indicating increasing digestibility maximizing at a fiber content of 4.0% and a particle size of 0.970 mm. A regression analysis of varience (AOV) indicates an inherent error term of 46% of the total variability. A 'best' model should account for up to 54% of the total variability. The model selected had an $r^2 = 0.52$, implying that the model accounts for 52% of the variation. A lack of fit test indicated the model was adequate $[F(5, 30) = 0.2549;\ p = 0.9345]$.

The results indicate that optimal levels exist for total lettuce diet DM digestibility of 82.6% at 4.0% fiber and 0.970 mm. grind size. Digestibility would decrease with either fiber or grind size on either side of these parameters. Previous investigations indicate that percent fiber and particle size in the diet are factors influencing digestibility (36, 73-80).

The acid detergent fiber (ADF) digestibility of lettuce (Fig. 28) was found to obtain a digestion model of the form:

$$ADF = F + G + FG \quad [F(3,35) = 13.07;\ p = 0.0001] .$$

The RSM plot for the above model yielded a family of right rectangular hyperbolae. This surface is very similar to bran NDF (Fig. 33) and bran H (Fig. 34). A regression AOV indicates an inherent error term of 16% of the observed variability. A 'best' model should account for up to 84% of the total variability. The model selected had an

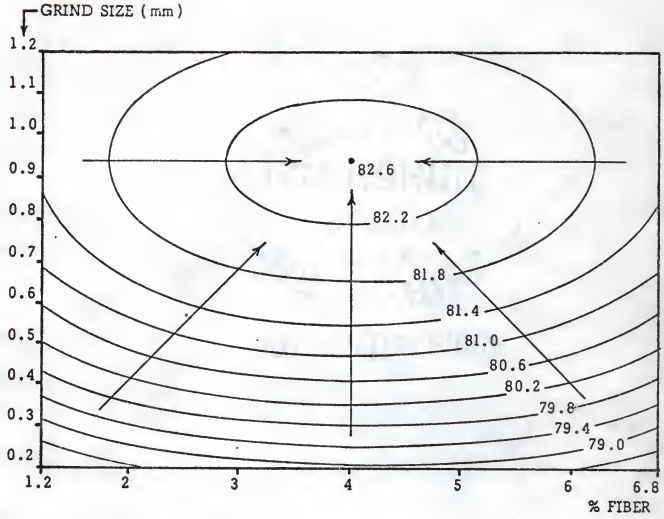

Fig. 27 RSM plot of the effect of percent fiber and grind size on lettuce dry matter (DM) digestibility.

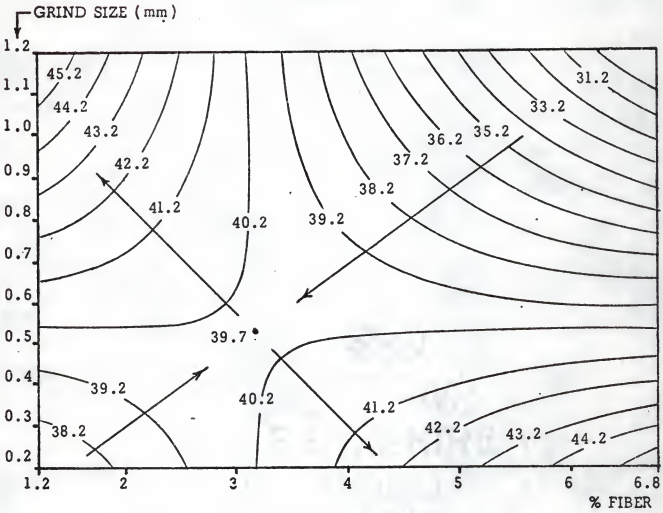

Fig. 28 RSM plot of the effect of percent fiber and grind size on lettuce acid detergent fiber (ADF) digestibility.

$r^2 = 0.528$, implying that the model accounts for 52.8% of the variation. A lack of fit test indicated the model was inadequate [$F(5, 30) = 4.6452$; $p = 0.0023$] .

The results indicate that the range of observations failed to cover the area which may include an optimal level. The optimal lettuce ADF digestibility points, if they exist, will be at a low grind and high % fiber or a high grind and low % fiber beyond the range of the observations. See the conclusion (pg.70) and Figure 35 (pg.72) for further discussion.

The neutral detergent fiber (NDF) digestibility (Fig. 29) was found to obtain a digestion model of the form:

$$NDF = F + G + F^2 + G^2 \quad [F(4, 34) = 15.90;\ p = 0.0001] .$$

The RSM plot for the above model yielded a family of concentric circles, indicating increasing digestibility maximizing at a fiber content of 3.7% and a particle size of 0.800 mm. A regression AOV indicates an inherent error term of 32% of the observed variability. A 'best' model should account for up to 68% of the total variability. The model selected had an $r^2 = 0.65$, implying that the model accounts for 65% of the variation. A lack of fit test indicated the model was adequate [$F(4, 30) = 0.7303$; $p = 0.5776$] .

The results indicate that optimal levels exist for total lettuce diet NDF digestibility of 77.4% at 3.7% fiber and 0.800 mm grind size. Digestibility would decrease on either side of these parameters as discussed in Figure 27.

The hemicellulose (H) digestibility of lettuce (Fig. 30) was found to obtain a digestion model of the form:

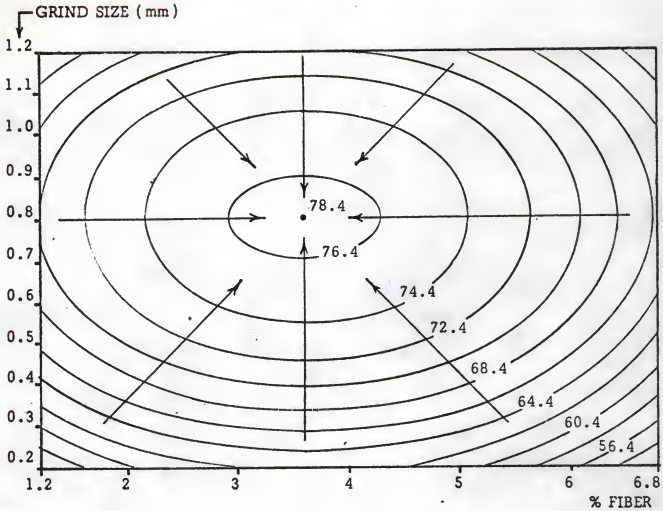

Fig. 29 RSM plot of the effect of percent fiber and grind size on lettuce neutral detergent fiber (NDF) digestibility.

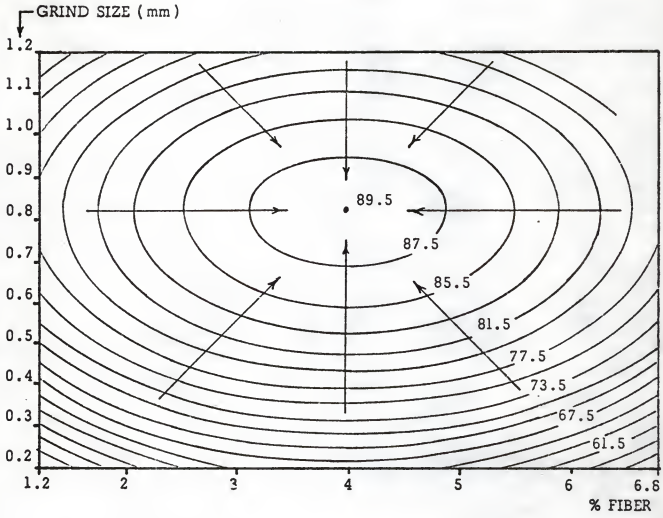

Fig. 30 RSM plot of the effect of percent fiber and grind size on lettuce hemicellulose (H) digestibility.

$H = G + F^2 + G^2$ $[F(3, 35) = 26.90;\ p = 0.0001]$.

The RSM plot for the above model yielded a family of concentric circles, indicating increasing digestibility maximizing at a fiber content of 4.0% and a particle size of 0.800 mm. A regression AOV indicates an inherent error term of 27% of the total variability. A 'best' model should account for up to 73% of the total variability. The model selected had an $r^2 = 0.698$, implying that the model accounts for 69.8% of the variation. A lack of fit test indicated the model was adequate $[F(5, 30) = 0.7603;\ p = 0.5845]$.

The results indicate that optimal levels exist for total lettuce diet H digestibility of 86.5% at 4.0% fiber and 0.800 mm grind size. Digestibility would decrease on either side of these parameters as discussed in Figure 27.

The dry matter (DM) digestibility of bran (Fig. 31) was found to obtain a digestion model of the form:

$DM = F + G + G^2$ $[F(3, 35) = 6.96;\ p = 0.0009]$.

The RSM plot for the above model yielded a family of parabolas indicating increasing digestibility but there is no optimal combination of fiber and grind within the area investigated. If there is an optimum, it would be located in a region with a grind size of approximately 0.880 and a fiber content below 1.2%. A regression AOV indicates an inherent error term of 38% of the observed varia lity. A 'best' model should account for up to 62% of the total variability. The model selected had an $r^2 = 0.374$, implying that the model accounts for 37.4% of the variation. A lack of fit test indicated that the model was inadequate $[F(5, 30) = 2.7389;\ p = .0344]$.

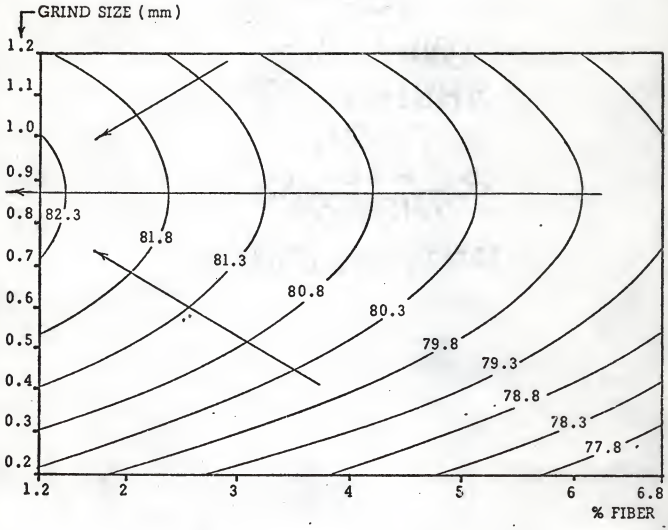

Fig. 31 RSM plot of the effect of percent fiber and grind size on bran dry matter (DM) digestibility.

The results indicate that the range of observations failed to cover the area which may include an optimal level. The optimal bran DM digestibility point, if it exists, would be slightly above 82.1% at less than 1.2% fiber and approximately 0.880 mm grind size. Digestibility would decrease at grind sizes on either side of 0.880 mm and % fiber greater than 82.1%.

The acid detergent fiber (ADF) digestibility of bran (Fig. 32) was found to obtain a digestion model of the form:

$$ADF = F + F^2 + G^2 \quad [F(3, 35) = 8.15; \ p = 0.0003] .$$

The RSM plot for the above model yielded a family of concentric circles, indicating increasing digestibility maximizing at a fiber content of 3.1% and a particle size of 0.700 mm. A regression AOV indicates an inherent error term of 47% of the observed variability. A 'best' model should account for up to 53% of the total variability. The model selected had an $r^2 = 0.41$, implying that the model accounts for 41% of the variation. A lack of fit test indicated the model was adequate $[F(5, 30) = 1.4225; \ p = 0.2404]$.

The results indicate that optimal levels exist for total bran diet ADF digestibility of 51.6% at 3.1% fiber and 0.700 mm grind size. Digestibility would decrease on either side of these parameters as discussed in Fig. 27.

The neutral detergent fiber (NDF) digestibility (Fig. 33) was found to obtain a digestion model of the form:

$$NDF = F + G + FG \quad [F(3, 35) = 11.48; \ p = 0.0001] .$$

The RSM plot for the above model yielded a family of right rectangular hyperbolae. This surface is very similar to the lettuce ADF model

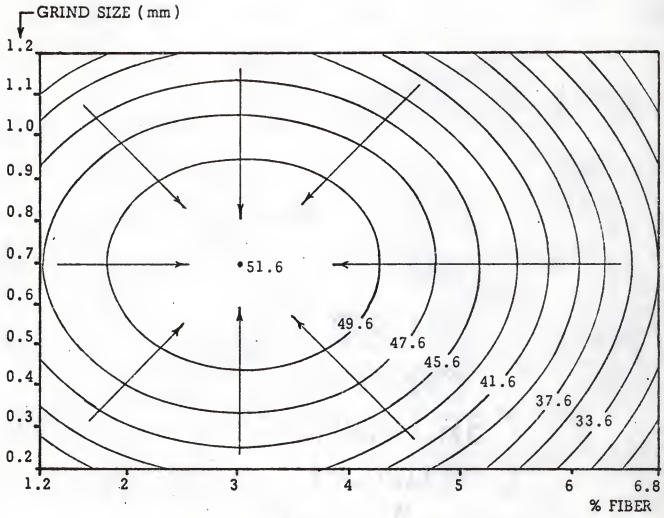

Fig. 32 RSM plot of the effect of percent fiber and grind size on bran acid detergent fiber (ADF) digestibility.

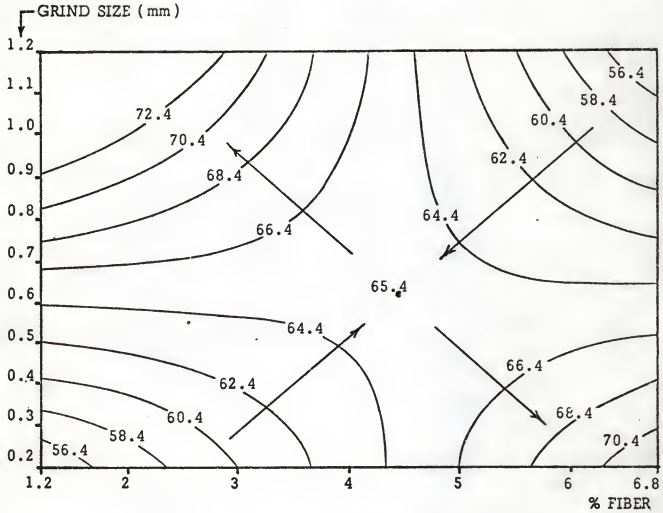

Fig. 33 RSM plot of the effect of percent fiber and grind size on bran neutral detergent fiber (NDF) digestibility.

(Fig. 28) and bran H (Fig. 34). A regression AOV indicates an inherent error term of 15% of the observed variability. A 'best' model should account for up to 85% of the total variability. The model selected had an $r^2 = 0.496$, implying that the model accounts for 49.6% of the variation. A lack of fit test indicated the model was inadequate $[F(5, 30) = 4.9794;\ p = 0.0015]$.

The results indicate that the range of observations failed to cover the area which may include an optimal level. The optimal bran NDF digestibility points, if they exist, will do so under the same conditions described for Fig. 28.

The hemicellulose (H) digestibility of bran (Fig. 34) was found to obtain a digestion model of the form:

$H = FG$ $[F(1, 37) = 14.48;\ p = 0.0005]$.

The RSM plot for the above model yielded a family of right rectangular hyperbolae. This surface is very similar to the lettuce ADF (Fig. 28) and bran NDF (Fig. 33). A regression AOV indicates an inherent error term of 25% of the observed variability. A 'best' model should account for up to 75% of the total variability. The model selected had an $r^2 = .281$, implying that the model accounts for 28.1% of the variation. A lack of fit test indicated the model was inadequate $[F(7, 30) = 5.2712;\ p = .0021]$.

The results indicate that the range of observations failed to cover the area which may include an optimal level. The optimal bran H digestibility points, if they exist, will do so under the same conditions described for Fig. 28.

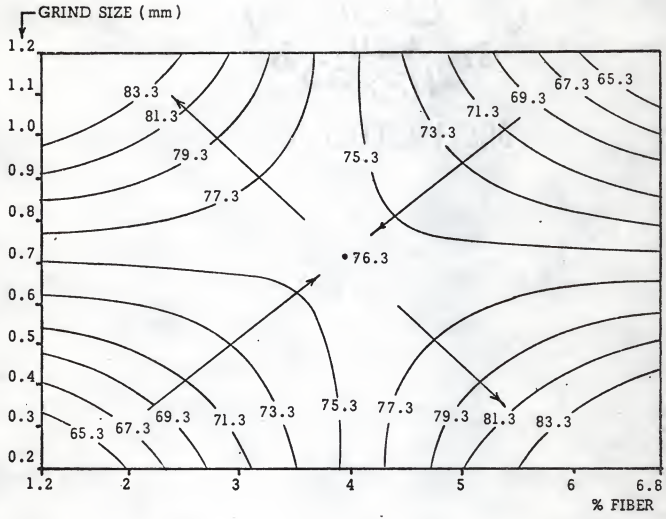

Fig. 34 RSM plot of the effect of percent fiber and grind size on bran hemicellulose (H) digestibility.

In conclusion the SEM served as a valuable tool for understanding the digestive process of fiber. As expected, the denser bran fiber was observed to be more resistant to compaction than the more delicate fibrous lettuce structure. Bran was capable of maintaining its structural integrity at smaller particle sizes where the lettuce compacted. This resistance to compaction may be one explanation of why bran was observed by the SEM to have a higher bacterial activity than lettuce. The visual observation of the mode of bacterial attack on fiber digestion aided in understanding the structural and chemical composition of the ingested tissue. Bacterial action began in the ileum for both lettuce and bran.

The results of the investigation of lettuce indicated that the action in the stomach included cuticular separation, probably due to HCl hydrolysis of the pectic substances under the cuticular surface along with collapsing mesophyll as a result of mechanical action. Protoplasmic digestion became evident in the duodenum and jejunum. Bacterial action on the mesophyll became evident in the ileum, and digestion of the primary cell walls of exposed tracheal tubes occurred in the cecum and large intestine. The gross structure of lettuce was altered during its passage through the gastrointestinal tract of the rat but little digestion appeared to occur.

The results of the investigation of bran indicate that the action in the stomach included epidermal splitting due to mechanical action and loosening of the aleuron cells as a result of protein hydrolysis. There was continued disruption of the epidermis and interior cross, tube, and aleuron cells within the duodenum. As a result of continued

mechanical action and protein hydrolysis, beginning signs of endosperm hydration occurred within the jejunum. Bacteria became evident on epidermal surfaces within the ileum, beginning to extensively attack the interior endosperm in the cecum and increased in activity in the large intestine where the endosperm cells were either totally digested or fall out. It was evident that the structural integrity of bran was altered, however, except for the extensive bacterial attack on the endosperm very little digestion appeared to occur.

Interpretation of some of the (RSM) digestibility plots required extrapolation beyond the area of observation. The observed and hypothesized maximum digestibility points for both lettuce and bran have been plotted on Fig. 35.

The plots generated by the RSM bran studies yielded no observed maxima for DM, NDF, or H, but did yield a maximum of 51.6% digestibility for ADF at approximately 3% fiber and 0.7 mm grind size (Fig. 35). The DM plot specifically indicated that if a maximum exists, it is beyond the range of this study, below 1% added fiber and above 0.8 mm grind size (Fig. 31). Comparing the plots of both bran and lettuce, a reasonable conclusion would be that if an optima exist for bran NDF and H, they would be located beyond the range of observation in the vicinity of the hypothesized bran DM maximum (upper left of Fig. 35). This indicates that the ADF maximum would be located at a relatively higher fiber level and smaller grind size, than the other criterion measures.

The three criteria measurements DM, NDF and H, observed for lettuce, maximized in about the same location (0.8-0.96 mm grind size

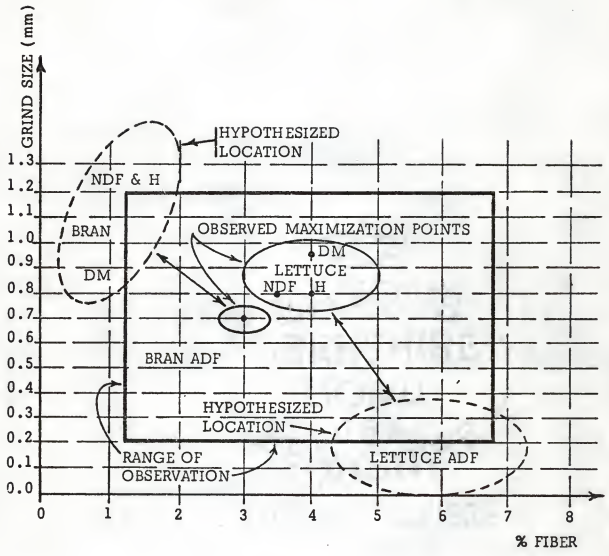

Fig. 35 Hypothesized location of maximum digestibility points beyond the range of observation.

and 3.5-4.0% fiber level). The ADF measurement indicated no maximum in the range covered in this investigation. If an ADF maximum does exist, it should occur at either higher grind size and lower fiber levels or at lower grind size and higher fiber levels. Comparing the bran and lettuce plots and from the foregoing conclusions on bran, a justifiable assumption is that if a lettuce ADF maximum does exist, it is located beyond the range of observation at the lower grind size and higher fiber level (lower right on Fig. 35).

The difference between the NDF and the ADF residues is the absence of hemicellulose (H) in the ADF residue. Since H is a major component of the NDF residual, the ADF fraction is available in the diet in small quantities. The low ADF dietary content probably accounts for the higher gross fiber levels required to achieve an observable maximum digestion for this component.

The difference in the observed results of bran and lettuce in the RSM analysis appears to be a combination of the differences in physical structure (observed in the SEM studies) and in the quantity of the various fiber components. Lettuce is more porous than bran, thus providing greater surface area for chemical and bacterial action. This same characteristic, however, causes lettuce to be less resistant to compaction than bran. A comparison of the chemical structure of lettuce and bran shows that lettuce has a lower percent of hemicellulose (readily digestible), a greater percent of cellulose (lower digestibility) and a lower percent of lignin (inhibitor). The combination of the physical and chemical makeup, plus differences in water binding and transit time are probably all contributing factors. Bran

digestibility appears to optimize at a lower percent fiber and larger particle size than lettuce. A conclusion that any factor alone causes the differences in the lettuce and bran digestibilities would be in error.

In conclusion, this study showed that in a rat diet of Purina Rat Chow® using added fiber sources of wheat bran or lettuce, dry matter digestibility (DM) varies as a function of both particle size and percent fiber. For either fiber source there exists an optimal combination of particle size and percent fiber which maximizes digestibility. The optimal digestibilities for fiber sources occurred at different combinations of particle size and percent fiber, and the relationship of digestibility to particle size or percent fiber was not linear but was more closely approximated by a bivariate normal distribution.

Conclusions beyond those stated above would be speculative. However, such speculation may be of heuristic value. The data tends to support the belief that:

(1) For any given fiber source, there exists an optimum combination of particle size and percent fiber which will maximize dry matter digestibility (DM).

(2) For any given fiber source and any given particle size there will be a corresponding percent fiber at which DM will maximize and the converse is true. This optimum point will normally not be the same as in (1) above.

(3) For any given fiber source DM, NDF and H will optimize at a similar point while ADF will optimize at a higher percent

fiber and smaller particle size.

(4) Numerous investigators suggest that fiber particle size is a factor affecting digestibility (74, 76-81). Contradictory results are reported regarding the effect of particle size on digestion (74, 76, 80). The observation that fiber content interacts with particle size may help reconcile these findings. Thus, a study of either particle size or of fiber content may depend on the covariate. If all fibers have an optimum particle size and if digestibility can be realistically represented by the bell curve, the results of any digestibility study will depend on the relationship of the study area to the optimum point of digestion.

SUMMARY

The scanning electron microscope (SEM) was used to observe the digestion sequence of lettuce (*lactuca sativa*) and wheat bran in the rat. Sprague Dawley rats were fed a diet of lettuce or bran and water *ad libitum* for three days. Samples were removed from various sections of the gastrointestinal tract and prepared for SEM viewing.

Observations of fresh lettuce leaf surfaces showed irregular shaped cells with small randomly oriented stomata. Cross sections revealed random dispersions of vascular tissue and undifferentiated mesophyll. Cuticular separation began in the stomach, probably due to HCl hydrolysis of pectin localized under the cuticular surface. Collapsing of the mesophyll by mechanical action was evident. Protoplasmic digestion became evident in the duodenum and increased in the jejunum along with continued separation of the cuticle. Bacteria were observed on the mesophyll surfaces of ileal samples. In the cecum, the mesophyll was separated from the vascular tissue along with further compaction and some degradation of the primary cell walls of exposed tracheal tubes. Continued digestion of these primary walls occurred in the large intestine along with phloem separation and an increased bacterial population. Sluffed cuticle, disrupted tracheal tubes, and compressed mesophyll were present in the feces. These results indicated that the gross structure of lettuce was altered but little digestion occurred.

Observation of control wheat bran samples revealed irregularly shaped epidermal cells and a layer of endosperm attached to the

interior surface with some separation of the aleuron and cross cells. Epidermal sluffing and swelling of the aleuron cells occurred in the stomach. Sluffing of the epidermis along with distortion and separation of the aleuron and cross cells occurred within the duodenum. Intercellular and intracellular hydrolysis of the endosperm along with tissue compaction began in the jejunum. Bacteria were first observed on the epidermis and endosperm of ileal samples. Continued structural distortion and increased bacterial population occurred on fecal samples. Bacterial attack was pronounced between endosperm cell walls from large intestinal samples. Continued bacterial attack and digestion was observed on fecal samples. These results indicated epidermal disruption, interior cross, tube, and aleuron cell distortion and hydrolysis of protein substances between internal tissues, but little other digestion except for the bacterial attack on the endosperm remnants.

Response surface methodology (RSM) was used to analyze the effects of particle size and percent fiber on total dry matter (DM) and partitioned fiber components (ADF, NDF, and H) digestibilities in the rat. Sprague Dawley rats were fed thirteen experimental diets consisting of combinations of five fiber percentages and five particle sizes in two trials, one with lettuce and one with bran. The rats were fed the experimental diets and water ad libitum for five days. The total weight of feed consumed by each rat and the total feces for each rat were recorded over the trial period. At the end of the trial period the feces were dried for 12 hours at 54°C and ground through a 400 micron (μ) mesh screen. DM, ADF, NDF, and H digestibilities were calculated

for both lettuce and bran and RSM digestion plots were developed for each. The RSM results indicated that total diet digestibility varies as a function of both particle size and percent fiber. For either fiber source there exists an optimal combination of particle size and percent fiber which maximized DM, ADF, NDF and H digestibility. However, the optimal digestibility for the two representative fiber samples does not occur at the same combinations of particle size and percent fiber. The relationship of total diet digestibility to particle size or percent fiber is not linear but is more closely approximated by a bivariate normal distribution.

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APPENDIX

TABLE 1

Beta coefficients[1] intercepts, and probabilities of the RSM digestibility models

LETTUCE

Model	Fiber	Grind	Fiber * Grind	Fiber^2	Grind^2	Intercept
DM	-	0.9751 p .0001	-	-0.4533 p .0404	-0.7140 p .0019	82.005
ADF	-3.4574 p .0017	-2.7490 p .0106	-6.4842 p .0001	-	-	46.6851
NDF	-1.6214 p .0424	2.4521 p .0031	-	-4.2903 p .0001	-4.4001 p .0001	76.3569
H	-	4.3982 p .0001	-	-5.4871 p .0001	-6.9307 p .0001	87.8687

BRAN

Model	Fiber	Grind	Fiber * Grind	Fiber^2	Grind^2	Intercept
DM	-1.0767 p .0008	0.5803 p .0555	-	-	-0.5800 p .0719	80.7556
ADF	-5.0072 p .0018	-	-	-5.0120 p .0034	-3.4603 p .0370	50.2589
NDF	p .0505	1.2859 p .0736	-5.1092 p .0001	-	-	65.2808
H	-	-	-6.9358 p .0005	-	-	75.8018

[1] P = the levels of significance for calibrated coefficients.

TABLE 2

Digestibility coefficients[1] of dry matter, and detergent fiber, neutral detergent fiber, and hemicellulose from rats fed lettuce and bran at various fiber levels and grind sizes.

Diet Number	Coded Values[2] Fiber	Grind	Digestibility Coefficients % DM[3]	ADF[4]	NDF[5]	H[6]
Lettuce						
1	-1.0	-1.0	80.02	45.73	68.81	73.71
2	1.0	-1.0	79.77	45.60	71.77	73.67
3	-1.0	1.0	82.07	53.32	71.65	80.77
4	1.0	1.0	82.04	27.35	67.35	79.72
5	-1.4	0.0	21.70	49.72	68.98	76.04
6	1.4	0.0	21.93	48.61	65.65	74.83
7	0.0	-1.4	79.22	55.71	62.05	64.77
8	0.0	1.4	81.67	47.78	72.16	80.35
9	0.0	0.0	82.42	46.90	83.47	84.21
10	0.0	0.0	81.77	46.69	75.63	86.68
11	0.0	0.0	81.93	47.35	77.66	90.39
12	0.0	0.0	81.93	47.58	79.07	90.78
13	0.0	0.0	81.98	44.48	72.80	84.04
Bran						
1	-1.0	-1.0	79.44	50.71	54.23	56.40
2	1.0	-1.0	77.46	33.56	63.04	77.96
3	-1.0	1.0	81.94	48.56	71.58	80.56
4	1.0	1.0	77.87	30.73	61.29	74.37
5	-1.4	0.0	81.91	42.96	69.85	83.21
6	1.4	0.0	80.11	39.44	61.97	75.94
7	0.0	-1.4	80.00	44.16	69.31	82.28
8	0.0	1.4	81.22	44.40	66.48	78.28
9	0.0	0.0	81.01	48.53	64.14	74.99
10	0.0	0.0	80.90	33.98	65.71	78.45
11	0.0	0.0	81.67	56.93	67.14	75.45
12	0.0	0.0	80.95	50.17	66.88	78.52
13	0.0	0.0	80.72	61.67	65.70	69.00

[1]Values are means of three replications. [2]Computer input codes for percent fiber and grind size. [3]Dry matter digestibility. [4]Acid detergent fiber digestibility. [5]Neutral detergent fiber digestibility. [6]Hemicellulose digestibility.

TABLE 3

Stepwise regression analysis of digestibility coefficients for rats fed lettuce and bran

Digestibility	Source of Variance	DF	Sum of Squares	Mean Square	F	Prob.
Lettuce						
Dry Matter	Regression	3	36.059	12.020	12.81	.0001
	Error	35	32.842	.938		
	Total	38	68.901			
Acid Detergent Fiber	Regression	3	971.398	323.799	13.07	.0001
	Error	35	866.941	24.770		
	Total	38	1838.339			
Neutral Detergent Fiber	Regression	4	899.943	224.986	15.90	.0001
	Error	34	480.979	14.146		
	Total	38	1380.913			
Hemicellulose	Regression	3	1902.572	634.191	26.90	.0001
	Error	35	825.230	23.578		
	Total	38	2727.802			

Contd.

Table 3 continued.

Digestibility	Source of Variance	DF	Sum of Squares	Mean Square	F	Prob.
Bran						
Dry Matter	Regression	3	42.872	14.291	6.96	.0009
	Error	35	71.870	2.053		
	Total	38	114.742			
Acid Detergent Fiber	Regression	3	1287.148	429.049	8.15	.0003
	Error	35	1843.586	52.674		
	Total	38	3130.734			
Neutral Detergent Fiber	Regression	3	400.547	133.516	11.48	.0001
	Error	35	407.122	11.632		
	Total	38	807.669			
Hemicellulose	Regression	1	577.269	577.269	14.48	.0005
	Error	37	1474.895	39.862		
	Total	38	2052.164			

DIGESTION OF LETTUCE AND BRAN IN THE RAT

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ABSTRACT OF A MASTER'S THESIS

The scanning electron microscope (SEM) was used to observe the digestion sequence of lettuce (Lactuca sativa) and wheat bran in the rat. Sprague Dawley rats were fed a diet of lettuce or bran and water ad libitum for three days. Samples were removed from various sections of the gastrointestinal tract and prepared for SEM viewing.

Observations of fresh lettuce leaf surfaces showed irregular shaped cells with small randomly oriented stomata. Cross sections revealed random dispersions of vascular tissue and undifferentiated mesophyll. Cuticular separation began in the stomach, probably due to HCl hydrolysis of pectin localized under the cuticular surface. Collapsing of the mesophyll by mechanical action was evident. Protoplasmic digestion became evident in the duodenum and increased in the jejunum along with continued separation of the cuticle. Bacteria were observed on the mesophyll surfaces of ileal samples. In the cecum, the mesophyll was separated from the vascular tissue along with further compaction and some degradation of the primary cell walls of exposed tracheal tubes. Continued digestion of these primary walls occurred in the large intestine along with phloem separation and an increased bacterial population. Sluffed cuticle, disrupted tracheal tubes, and compressed mesophyll were present in the feces. These results indicate that the gross structure of lettuce is altered but little digestion occurs.

Observation of control wheat bran samples revealed irregularly shaped epidermal cells and a layer of endosperm attached to the interior surface with some separation of the aleuron and cross cells.

Epidermal sluffing and swelling of the aleuron cells occurred in the stomach. Sluffing of the epidermis along with distortion and separation of the aleuron and cross cells occurred within the duodenum. Intercellular and intracellular hydrolysis of the endosperm along with tissue compaction began in the jejunum. Bacteria were first observed on the epidermis and endosperm of ileal samples. Continued structural distortion and increased bacterial population occurred on fecal samples. Bacterial attack was pronounced between endosperm cell walls from large intestinal samples. Continued bacterial attack and digestion was observed on fecal samples. These results indicate epidermal disruption, interior cross, tube, and aleuron cell distortion and hydrolysis of protein substances between internal tissues, but little other digestion except for the bacterial attack on the endosperm remnants.

Response surface methodology (RSM) was used to analyze the effects of particle size and percent fiber on total dry matter (DM) and partitioned fiber component (ADF, NDF and H) digestibilities in the rat. Sprague Dawley rats were fed thirteen experimental diets consisting of combinations of five fiber percentages and five particle sizes in two trials, one with lettuce and one with bran. The rats were fed the experimental diets and water ad libitum for five days. The total weight of feed consumed by each rat and the total feces for each rat were recorded over the trial period. At the end of the trial period, the feces were dried for 12 hours at 54°C and ground through a 400 micron (μ) mesh screen. DM, ADF, NDF, and H digestibilities were calculated for both lettuce and bran and RSM digestion plots were

developed for each. The RSM results indicate that total diet digestibility varies as a function of both particle size and percent fiber, and for either fiber source there exists an optimal combination of particle size and percent fiber which maximizes total diet digestibility for the various fiber components; however, the optimal digestibility for the two representative fiber samples does not occur at the same combinations of particle size and percent fiber. The relationship of total diet digestibility to particle size or percent fiber is not linear but is more closely approximated by a bell curve.