

STARCH DIGESTION IN THE BOVINE AS INFLUENCED BY LEVEL
AND PROCESSING OF SORGHUM GRAIN

by

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TO MY WIFE AND DAUGHTER

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ABSTRACT

Laboratory analyses were run on a variety of substrates to compare glucose recovery, as measured by the glucose oxidase procedure, resulting from hydrolysis in 0.76 N HCl or with "Agidex," an amyloglucosidase. Higher recoveries were obtained with acid hydrolysis on high cellulose substrates but not on high starch substrates. Coefficients of variation ranged from 0.0 to 19.2%, with lowest coefficients generally corresponding to the high starch substrates.

Two digestion trials were conducted to determine the effects of level of intake, full-fed vs. restricted, of a high milo ration on starch digestion. The second of the two trials also included a steam processed flaked vs. dry rolled milo comparison. The animals from trial 2 were slaughtered at 3 hr post-prandial, and digesta were collected from different segments of the tract.

Level of starch intake did not alter either ruminal or total tract starch digestion. Steam processing and flaking, as compared to dry rolling, increased ($P < 0.05$) starch digestion in the total tract but not in the rumen. No differences in dry matter digestion were found between treatments in either trial. No conclusions could be drawn from feed efficiency data due to inconsistencies.

Problems in estimating ruminal digestion using chromic oxide powder as an indigestible marker were experienced, with the chromic oxide apparently moving more rapidly than the digesta.

INTRODUCTION

Historically, the ruminant animal has been primarily a consumer of roughages. Although this is still the case in most situations, the trend for feeding cattle in the feedlot has been to increase the levels of concentrates (largely grains) in the diet in order to maximize intake of readily available energy for rapid finishing. Whereas on a roughage diet almost all of the energy is derived through microbial breakdown of cellulose, high grain rations contain relatively high percentages of starch which constitutes the major source of energy. This starch (alpha-linked glucose polymers) is digestible not only in the rumen but also in the small intestine and follows two very different methods of digestion.

The theoretically greater energetic efficiency of production and utilization of glucose from intestinal digestion over the volatile fatty acids from ruminal fermentation (Walker 1965) has given rise to speculation that efficiency in feedlot animals could be enhanced by increasing the proportion of dietary starch which is ingested in the small intestine.

The attempt by Karr, Little, and Mitchell (1966) to determine the quantities of starch escaping ruminal digestion with diets containing varying amounts of corn sparked considerable interest in elucidating the degree and location of starch digestion in the ruminant under different experimental conditions. Although a variety of grains and

processing methods have been utilized, the studies of this type involving sorghum grains (the primary grain utilized in southwestern feedlots) are limited. In addition, studies in which different starch intake levels were involved were concerned primarily with the percentage of starch in the ration, not with total intake. The levels of starch intake which have been reported in the literature are considerably lower than levels which would be consumed by an animal in the feedlot on a typical high concentrate finishing ration under normal conditions.

The objectives of this study were to determine the quantity and site of digestion of the starch in high grain (milo) rations by steers as affected by starch intake level (ad libitum vs. restricted) and method of grain processing (steam processing and flaking vs. dry rolling).

LITERATURE REVIEW

The study of starch digestion in the ruminant animal has been complicated by the use of a wide variety of analytical techniques, feed processing methods, starch sources, experimental designs, and markers for estimating digestion. As a result one is able to get only a generalized picture, at best, by looking at any one study or at several studies involving starch digestion.

Starch Analysis Techniques

Although several methods of starch analysis have been used in starch digestion studies, most of them fall into one of two categories. One involves acid or enzymatic hydrolysis of the starch and subsequent analysis for glucose or reducing sugars. The second method depends on the color reaction of various reagents with starch, the most common being the anthrone reagent (Hassid and Neufeld 1964). Phenol, a second reagent forming a color complex with starch, was used by Keating et al. (1965) and Saba et al. (1964) according to the method of Dubois et al. (1956).

Analysis for reducing sugars following acid hydrolysis has been shown by some workers (MacRae and Armstrong 1966, 1968) to result in high glucose recovery estimates with some substrates such as hay, digesta, and feces. However, Frederick (1968) found little difference in glucose estimation from grains subjected to acid hydrolysis followed by

analysis for either glucose or reducing sugars. Hassid, McCready, and Rosenfels (1940) utilized a salivary amylase as a starch-specific hydrolyzing medium followed by analysis for reducing sugars. However, a "limiting hydrolysis value" required the application of a correction factor to account for unhydrolyzed starch.

A method developed by MacRae and Armstrong (1968) makes use of amyloglucosidase (Agidex), an enzyme produced by Aspergillus niger. The advantages of using this enzyme for hydrolysis are that it is highly specific for alpha-linked glucose polymers and can hydrolyze starch completely to glucose since it will readily attack alpha 1:4, alpha 1:6, and alpha 1:3 linkages (Abdullah et al. 1963, Pazur and Ando 1959, Pazur and Kleppe 1962).

Although Agidex enzyme hydrolysis has been used by several workers recently, Topps and Kay (1969) have shown that considerable variation can occur among workers using this same technique. Four laboratories which had previously analyzed starch using Agidex in their experiments were asked to analyze starch contents of identical samples taken from the same experiment. Starch analysis for a pelleted hay ration varied from 43 to 57 mg of starch per gram of dry matter, whereas that for a concentrate diet ranged from 480 to 610 mg per gram of dry matter.

The anthrone method has been used extensively in starch determinations, although it has been shown to react with carbohydrates other than starches (McCready et al. 1950, Scott and Melvin 1953). Topps, Kay, and Goodall (1968) and Topps, Kay et al. (1968), following the

anthrone procedure of Clegg (1956), found considerably higher starch estimates (often as much as twofold) in hay diets when analyzed by the anthrone method as compared to the Agidex hydrolysis followed by glucose oxidase analysis of resulting glucose. For concentrate diets the anthrone method tended to give lower starch estimates than the Agidex method, although agreement between the methods was much better than for the hay diet comparisons. Duplicate samples of a concentrate ration analyzed for starch content by two different laboratories using the anthrone method gave estimates of 520 and 810 mg of starch per gram of dry matter (Topps and Kay 1969).

Starch Digestion Studies

Early work in the area of starch utilization came as a result of efforts to develop feeds to be used as a milk replacer for early weaned calves. Experimenters attempted to determine the ability of the calf to utilize glucose as a breakdown product of starch and the capabilities of the small intestine to digest starch.

Dollar and Porter (1957) found that the pre-ruminant calf could not utilize starch, although it could utilize glucose, suggesting an inability of the small intestine to break down starch and starch products such as dextrans and maltose. The ability to digest maltose was developed by the ninth week, however. These findings have been confirmed by Siddons et al. (1969).

It was reported by Larsen et al. (1956) that ruminating dairy calves of eight to nine months of age could readily utilize maltose and glucose post-uminally, but that little starch digestion occurred

with the introduction of purified starch or ground corn into the omaso-abomasal area.

Results obtained by Huber et al. (1961) indicated that the ability of the ruminant to absorb and utilize glucose and maltose decreases with the age of the animal. No appreciable digestion of starch, as measured by an increase in blood glucose concentration, was found in any of the test animals ranging in age from 11 days to over two years. It was suggested that starch digestion might have occurred too slowly to be detected by a rise in blood glucose.

This is not in agreement with Little, Mitchell, and Reitnour (1968) who found that direct measurement of intestinal starch showed as much as 290 g digested in the small intestine when 600 g of purified cornstarch were infused into the abomasum of steers. They did suggest, however, that the capacity of the small intestine for starch digestion might be limited.

Wright, Grainger, and Marco (1966) also found the small intestine to be capable of rapid degradation of starch and maltose when wethers were fed a high concentrate ration, although this was not detectable by increased blood glucose levels.

The ability of the ruminant to digest starch appears to be dependent to some degree upon the adaptation of the rumen and small intestine to the particular diet. For example, Clary et al. (1966) and Clary, Mitchell, and Little (1967) showed an increase in pancreatic amylase volume and activity as the percentage of corn in the diet was increased. These workers also reported increases in blood glucose

levels as amylase activity and corn levels were increased. These changes required an adaptation period to the particular diet, although this period was not accurately determined.

A similar adaptation period was required by the rumen micro-organisms in work reported by Templeton and Dyer (1965). It was shown that as the concentrate in the diet was increased from 0 to 50 to 80% the amylase activity of the rumen fluid also increased significantly.

Recent starch digestion studies have chiefly been concerned with the location and quantity of starch digestion as affected either by the level of starch in the ration or by the starch source and the method used to process that source. A third, related area is concerned with the choice and administration of a suitable indigestible marker for measuring digestion at various points along the gastro-intestinal tract.

Effects of Source and Processing on Starch Digestion

Processing of starch sources, which for the purposes of this study refer to grains unless otherwise stated, for feeding purposes is normally concerned with altering the physical or chemical structure of the grain to improve starch digestion or utilization or both. Several papers dealing with source of starch, grain processing, and levels of starch in the ration have been reviewed by Armstrong and Beever (1969). Methods of studying alterations in structure largely fall into one of three classifications: (1) the study of alterations in the starch molecule or granule (Florence, Riggs, and Potter 1968; Johnson,

Matsushima, and Knox 1968; Rooney 1970; Schoch and Maywald 1956); (2) the use of in vitro and in vivo techniques to measure such changes as rate of gas or volatile fatty acid production from fermentation (Loy-nachan 1970; Trei, Hale, and Theurer 1970), rate of enzymatic degradation (Frederick 1968), or loss of dry matter in the rumen using the nylon bag technique (Figroid 1967); and (3) the use of feeding trials to measure the influence of processing of grain on such factors as animal performance, total digestion, and digestion in the different segments of the gastro-intestinal tract.

Johnson et al. (1968) fed corn treated in four ways to steers and found steam flaked and flaked-cracked (steam flaking followed by rolling the dried flake) corn to have higher dry matter digestion coefficients than either cracked or steam-cracked (steaming followed by drying and cracking) corn.

Orskov, Fraser, and Kay (1969) found a lower ruminal starch digestion of cracked corn (86%) than for either ground corn (88%) or flaked corn (95%). However, total apparent digestibility coefficients were greater than 99% for all treatments.

Beever, Coehlo Da Silva, and Armstrong (1970) also found lower ruminal digestion of starch in corn when subjected to grinding as compared to flaking. Sheep fed flaked corn digested 96% in the rumen, whereas those fed ground corn digested only 78%. Total starch digestion exceeded 99% for both treatments.

Rolling or grinding barley does not appear to improve starch digestion over that of the whole grain. Orskov et al. (1969) found

ruminal digestion of the starch in barley which had been rolled or ground to vary from 93 to 96%, and MacRae and Armstrong (1969b) found 95% ruminal digestion with whole barley vs. 97% with rolled barley. Total starch digestion approximated 100% in the latter trial. Parrott et al. (1969) found no increase in dry matter or nitrogen free extract (NFE) digestion when steers were fed barley which had been dry rolled or steam flaked (regular or flat flake), suggesting little alteration by processing on starch digestion.

Differences in digestibility resulting from treating sorghum grain have been highly variable, in part probably due to the lack of definition and standardization of different processing methods (Hale 1965). Differences in analytical methods may also be a factor. For example, Keating et al. (1965) found starch digestibility coefficients of 79 and 80%, respectively, when steers were fed either dry rolled milo or cooked milo. The use of the starch analysis technique of Dubois et al. (1956) was brought into question when cooking the milo increased the digestibility of the NFE fraction (from 70 to 76%) but failed to show an increase in starch digestibility.

There have been several studies with sorghum grains involving in vitro and in vivo techniques as well as measurements of NFE and dry matter digestibility which parallel the actual starch digestion studies which have been conducted. The results from in vitro and in vivo digestibility studies generally correlate well with those from feeding trials. That is, increases in gas production, rate of hydrolysis, volatile fatty acid production, and loss of dry matter in the rumen

tend to correspond to those processing methods which are associated with improved performance and efficiency of cattle in the feedlot. They also tend to agree with studies involving the direct measurement of starch in digestion studies.

Osman et al. (1970) found an increased ($P < 0.05$) rate of digestion of steamed processed sorghum grain due to degree of flake flatness (flat flake > intermediate flake > poor flake) when incubated for 30 min with porcine pancreatin. Similar trends were reported by Frederick (1968). Theurer, Trei, and Hale (1967) found that total volatile fatty acid production increased with increasing flake flatness when steam processed sorghum grain was incubated for 3 hr with a mixed suspension of rumen microorganisms. Similar treatments also gave increased ($P < 0.05$) gas production with increased flake flatness (Trei et al. 1970).

Figroid (1967) found a consistently greater dry matter disappearance with steam processed flaked milo as compared to either dry rolled or steam processed poorly rolled milo in in vivo nylon bag studies.

Husted et al. (1968) found increased ($P < 0.05$) NFE digestion with steam processed flaked milo (84%) when compared to dry rolled (71%), steam cut (73%), or water soaked cut milo (75%). Likewise, Hale, Cuitun et al. (1966) found increased NFE digestion with steam processed flaked milo (78%) vs. dry rolled milo (69%).

Little difference was found in starch digestibility (93 to 94%) when Buchanan-Smith, Totusek, and Tillman (1968) fed equal amounts of sorghum grain treated in four different ways to sheep. Fine ground and

coarse ground grain tended to have slightly lower digestibilities than steam processed rolled or reconstituted rolled. A similar but more marked pattern of starch digestion was found when these rations were fed to cattle. Digestion coefficients were 91 and 92% for the coarse and fine ground treatments, respectively, and 94 and 95% for the steam processed and reconstituted treatments, respectively. Although none of the starch values were significantly different, nonprotein organic matter digestibility values were higher ($P < 0.05$) for the moisture treated grains when fed to cattle.

Ruminal and total starch digestions were approximately the same when steers were fed dry ground or micronized sorghum grain (42 and 43% for respective ruminal digestions and 97% for both total digestions) in an experiment conducted by McNeill, Potter, and Riggs (1971). Total digestion was higher ($P < 0.05$) for both reconstituted ground (99%) and steam processed flaked grain (99.8%). The reconstituted grain was more completely digested ($P < 0.05$) in the rumen (67%) than either the dry ground or micronized grain and the steam processed flaked was highest at 83%.

Holmes, Drennan, and Garrett (1970) found some difference ($P < 0.05$) in ruminal digestion but no difference in total digestion of starch in milo which had been steamed for 8 min at atmospheric pressure and rolled (8 AP) or steamed under pressure (3.5 kg/cm^2) for 1.5 min and rolled (3.5 KP). Ruminal digestion amounted to 90% in cattle and 89% in sheep for the 8 AP and 96% in cattle and 94% in sheep for the 3.5 KP. Total starch digestion for the four treatments ranged from 96 to 99%.

An evaluation of the effect of source of grain is difficult without a concurrent consideration of processing. For example, Orskov et al. (1969) found little effect on barley digestion arising from processing in either the rumen (93 to 96%) or the total tract (99%+), whereas corn processing greatly altered the ruminal digestion (from 86% for cracked corn to 95% for flaked corn) but showed no overall digestibility differences (over 99% for all treatments).

Corn and barley starches were found by MacRae and Armstrong (1969b) to be almost totally digested in sheep. The near total digestion of the starch in corn processed in different ways is in agreement with Orskov et al. (1969), Beever et al. (1970), and Waldo, Keys, and Gordon (1971), but does not agree with one trial of Tucker, Mitchell, and Little (1968).

In vitro work and digestibility studies generally indicate a greater digestibility of barley as compared to milo. Osman et al. (1970) found a faster ($P < 0.05$) rate of digestion of barley starch vs. milo starch by porcine pancreatin or lyophilized bovine pancreas. This was true for each processing method used. Likewise, Theurer et al. (1967) found barley to be more rapidly fermented than milo as measured by total volatile fatty acid production resulting from incubation for 3 hr in a mixed suspension of rumen microorganisms. Steam processed flaked barley also had a higher ($P < 0.05$) gas production from in vitro fermentation than did steam processed flaked milo (Trei et al. 1970).

Feeding trials were conducted by Saba et al. (1964) to determine relative feeding values of dry rolled milo or barley at two levels

of protein. Barley showed a higher starch digestibility (88 to 91%) than milo (81%), and gain and feed efficiency tended to favor the barley rations. Similar results were obtained by Keating et al. (1965). In contrast, Garrett (1965) found no difference in gain or feed efficiency when feeding cattle steam rolled or ground barley and milo.

Generally, where hay makes up a sizable portion of the diet, both ruminal and total tract digestibilities of starch are lowered. A total of 23 and 27 g of starch (66 and 82% of respective intakes) was digested in the rumen of sheep fed a hay diet in an experiment conducted by Topps, Kay, and Goodall (1968), with total digestion coefficients of 90 and 85%, respectively. In contrast, when fed a concentrate ration, 303 and 280 g were digested in the rumen, respectively amounting to 98 and 94% of the dietary starch. Total digestion exceeded 99% for both concentrate rations.

Similarly, Topps, Kay et al. (1968) found the starch in hay to be less digestible (66 to 70%) in the rumen than that in concentrate rations (95 to 96%), as well as overall (82 to 87% for hay vs. over 99% for concentrate). MacRae and Armstrong (1969b) found lower ruminal digestibilities for starch in hay but 100% total digestion, unlike the above workers. Orskov et al. (1969) also found near complete starch digestion (99.7% +) in a barley ration and a ration consisting of 60% barley and 40% hay. Ruminal starch digestion was slightly lower (87 to 92%) for the hay-barley ration than for the barley alone (93 to 96%).

Weller and Gray (1954) fed dehydrated potato chips and wheaten hay to sheep and found 7.8 g of starch per day (out of 140 g of starch fed) escaping ruminal digestion. When wheaten hay alone was fed,

supplying 20 to 40 g of starch daily, approximately one-tenth of the starch reached the abomasum in 24 hr. Lucerne hay provided only 2.9 g of starch per day, of which 1.1 g reached the abomasum.

Effects on Digestion of Different Levels of Starch in the Ration

Several experiments involving corn and barley levels have been reported in relation to starch digestibility. However, to date, none has been reported utilizing sorghum grains. Although there are several different treatments applied in experiments involving level differences, where possible, intakes will be expressed as gram of starch consumed per kilogram of metabolic weight ($\text{g/W}_{\text{kg}}^{0.75}$) to facilitate comparisons between experiments.

When rolled barley or rolled barley + grass was fed to sheep (Orskov et al. 1969), intakes restricted to 70% of ad libitum tended to have higher ruminal digestion of starch than when fed ad libitum (96 vs. 93%). Digestion for corresponding intakes of rolled barley + grass was 92 and 87%. There were no differences in total digestibility, all values exceeding 99%.

Topps, Kay et al. (1968) fed an 85% barley ration at four levels to steers providing starch intakes ranging from 27 to 44 $\text{g/W}_{\text{kg}}^{0.75}$. Ruminal digestion was about the same for all levels (95 to 96%), and total digestion was nearly complete (99.4 to 99.5%). Hay fed at two levels (2600 and 3500 g per day) showed similar starch digestibilities in the rumen (66 and 70%) and overall (87 and 82%, respectively).

Nicholson and Sutton (1969) fed hay, dairy cubes, and flaked corn at levels to meet 1x, 2x, and 3x maintenance requirements of sheep (with 45, 62, and 69% corn in the respective diets) but found essentially no differences in starch digestion in either the rumen (94 to 96%) or the total tract (99.6 to 99.8%).

Three separate experiments were conducted to study the effects of feeding equal quantities of rations containing 20, 40, 60, or 80% ground corn. Karr et al. (1966) fed steers at levels providing 12, 24, 30, and 32 g of starch/ $W_{kg}^{0.75}$ for the four corn concentrations, respectively. Respective ruminal digestibilities were 64, 73, 65, and 62%. Total digestion ranged from 98% for the 80% corn ration to 99% for the 40% ration.

In related work Tucker et al. (1968) fed similar rations to sheep and found respective ruminal digestions of 78, 67, 73, and 79% and total digestions of 87, 92, 95, and 96% for the 20, 40, 60, and 80% corn rations, respectively. Starch intakes were similar to corresponding treatments above at 11, 20, 29, and 36 g/ $W_{kg}^{0.75}$.

In an attempt to verify these results using a different starch analysis technique, Waldo et al. (1971) also fed 20, 40, 60, and 80% ground corn rations to bulls and heifers in two trials. Total starch digestion exceeded 99% for all treatments in both trials. Ruminal digestion varied from 76% for the 60% ration to 90% for the 20% corn ration in the first trial. In the second trial ruminal digestion ranged from 50% to 74% for the 60 and 80% corn rations, respectively.

Choice and Administration
of the Indigestible Marker

It has long been recognized that to accurately estimate nutrient digestibility in the gastro-intestinal tract it is necessary to have a marker which will not be destroyed or absorbed and which follows closely the nutrient which one desires to study. A number of markers have been used to measure passage and digestibility in ruminants. Among the most common are internal markers such as chromogens and lignins and external markers such as polyethylene glycol (PEG) and chromic oxide.

Radioactive isotopes have been shown to act very effectively as indigestible markers for estimation of digestion, but their use is limited in working with large animals due to the problem of excreta and animal segregation and disposal necessitated by radioactive contamination (Kane, Jacobson, and Damewood 1959; Mautz 1971).

Recently, rare earth elements, which are analyzed by neutron activation, have been tested as possible indigestible markers (Ellis 1968, Huston and Ellis 1968, Miller et al. 1967). Preliminary results indicate that these elements may prove very satisfactory, but to date there have been very few actual digestion trials conducted which would permit evaluation of this technique.

Lignin and chromic oxide are the most commonly used markers in ruminant digestion studies. Both have been studied extensively in a variety of experimental situations (Balch 1957; Johnson, Dinusson, and Bolin 1964; McCann 1967), but both have characteristics which can limit their use under certain conditions.

Low digestibility estimates were reported using lignin as compared to total collection by Elam et al. (1962) and by McCann (1967), possibly due to partial digestion of the lignin. Increases in measurable lignin have been reported both as a result of heat (Van Soest 1965) and entering the rumen (Lascano et al. 1970). The low concentrations of lignin in high grain rations would tend to exaggerate any errors in analysis as found by Waldo et al. (1971).

Johnson et al. (1964) and Corbett, Greenhalgh, and McDonald (1958) have reported rapid passage of chromic oxide powder from the rumen and abomasum, possibly leading the digesta. Langlands et al. (1963) reported a lack of uniformity in excretion patterns when ruminants were given chromic oxide powder. A method was devised by Corbett et al. (1958) to impregnate chromic oxide on paper, thus providing for a more even, sustained release of the marker as the cellulose in the paper was digested by the rumen microorganisms. It was suggested that this would reduce the problem of the rapid separation of the chromic oxide and digesta.

MacRae and Armstrong (1969a) studied the use of chromic oxide impregnated on paper as a marker for estimating ruminal starch digestion when sampling from a duodenal re-entrant cannula. It was concluded, due to the large sampling variation, that short collection periods could lead to large errors.

Drennan, Holmes, and Garrett (1970) suggested that the use of chromic oxide powder would result in low ruminal starch digestion estimates and offered this as an explanation of the apparent discrepancies

in estimates obtained by different workers for ruminal starch digestion. In comparing chromic oxide powder to lignin as a reference substance, it was concluded that the powder was unsuitable for estimating ruminal digestion.

In contrast, Waldo et al. (1971) found smaller errors when using chromic oxide as opposed to estimating by lignins. They also found theoretically possible dry matter digestibilities in the rumen using chromic oxide in seven out of eight treatments, whereas Drennan et al. (1970) found none.

In summary, there are numerous studies involving the effects of grain processing on starch digestion, although few of these include sorghum grains. Ruminal digestibility studies, though indicating trends, are not in agreement concerning magnitude of starch digestion, and further work is needed to elucidate this area. Starch intake level studies have generally been conducted using relatively low intake levels, and no studies of this nature have involved sorghum grains. Attempts should be made to study digestion patterns at levels comparable to those found in feedlot situations. Considerable work needs to be done with analysis of marker techniques, not only for future work, but also to evaluate past studies.

EXPERIMENTAL PROCEDURE

Laboratory tests were conducted to compare starch analysis techniques under a variety of experimental conditions. Subsequently these techniques were applied to the analysis of samples obtained in two feeding trials.

Total Starch Determination

Preliminary investigation was conducted to compare the A.O.A.C. (1960) acid hydrolysis, which had been used previously at The University of Arizona, with the more recently developed enzyme hydrolysis method of MacRae and Armstrong (1968), as a technique for starch analysis. The basis for each method is hydrolysis of the starch molecule (alpha-linked glucose polymers) to glucose, which is quantitatively analyzed.

Reflux Apparatus

Since both procedures for starch hydrolysis utilized in this study require refluxing, an apparatus suitable for both procedures was desired. A Labconco Crude Fiber Digester was tested, having been used successfully for acid hydrolysis by Osman (1966) and Frederick (1968). However, the beakers were large and unwieldy considering the small volume used in the enzymatic hydrolysis. The use of a Goldfish Fat Extractor apparatus appeared to work successfully for a period of time, but this resulted in corrosion of the metal in the thimble holder and

subsequent erratic glucose recoveries with acid hydrolysis, possibly due to a reaction of the glucose with copper in the holder.

An apparatus was then designed (Figure 1) using a 6-place heating element (Labconco Micro-Kjeldahl Digester) to provide heat for refluxing. Samples were contained in beakers of the type used on the Goldfish Fat Extractor, and 100-ml, round-bottomed boiling flasks were set up in series to act as the condensing units. Water circulation through each flask was provided by two 7-mm glass tubes mounted in a No. 4 rubber stopper, the inlet tube being long enough to carry water to the bottom of the flask and the outlet exiting near the top. To prevent expulsion of the rubber stopper due to water pressure within the flask, it was necessary to restrain the stopper by attaching a 4-inch length of "Scotch" brand strapping tape tightly over the stopper and anchoring the ends to the flask neck by wrapping with the same type of tape. A rack was constructed to permit the simultaneous raising or lowering of a series of six condenser units. When the condenser flasks were lowered onto the beakers, each flask settled independently of the others with only the weight of the individual condenser unit resting on each beaker.

Acid Hydrolysis

The hydrolysis of starch by acid was accomplished according to a modification of the A.O.A.C. (1960) method. A 0.5 g sample was refluxed for 2.5 hr in 90 ml of 0.76 N HCl using the reflux apparatus described above. The solution was cooled to room temperature and 90% of the acid was neutralized with 5.5 ml of 45% NaOH. The solution was



Figure 1. Reflux apparatus used in acid and enzyme hydrolysis procedures showing heating elements, beakers, and condenser units.

filtered and brought to final volume with distilled water in a 250-ml volumetric flask.

Enzyme Hydrolysis

The method of MacRae and Armstrong (1968) employing the amyloglucosidase Agidex was used initially for enzyme hydrolysis. By this method the volume of the hydrolytic solution used in calculating glucose concentration is indirectly determined by weight (gravimetric method). To prevent evaporation during incubation, which would result in a weight loss, the solution is covered by a layer of paraffin. A modification of this method was developed in which the volume is determined directly, and the use of paraffin is eliminated (volumetric method). Initial studies were conducted to compare the two methods, but all subsequent analyses utilizing enzyme hydrolysis were performed by the volumetric method.

A 0.5 g sample was refluxed for 4 hr in 50 ml of distilled water followed by the addition of 50 ml of 0.2 molar acetate buffer (0.2 M NaAc/HAc, pH 4.5). Approximately 0.5 g of Agidex enzyme was then added, the beaker was covered with a watch glass, and the sample was incubated in a constant temperature oven at 60°C for at least 24 hr. Following incubation, the sample was cooled to room temperature, and the solution was filtered through a 515 grade Eaton-Dikeman filter paper into a 250-ml volumetric flask and brought to final volume with distilled water.

Quantitative Determination of Glucose

The glucose produced by either enzymatic or acid hydrolysis was analyzed by the same procedure, a modification of the Worthington (1963) "Glucostat" method which utilizes the enzyme glucose oxidase. Two-tenths ml of filtrate from the hydrolyzing procedures was added to 4.0 ml of distilled water and deproteinized by the addition of 2.0 ml of 1.8% barium hydroxide and 2.0 ml of 2.0% zinc sulfate. The solution was centrifuged for 10 min at 3000 rpm, and 2.0 ml of the supernatant were transferred to a clean test tube. This was incubated for exactly 10 min with 2.0 ml of Glucostat reagent (diluted to 50 ml) followed by the inactivation of the glucose oxidase with the addition of a single drop of 4 N NCl. After 5 min the sample was read in a Beckman Model B Spectrophotometer set at 400 mu. A water blank and a glucose standard were included in each run and prepared as above. The blank was set at 100% transmission, and the standard was read as a reference. The percentage transmission was converted to optical density (absorbancy) and glucose concentration was calculated on a dry basis as follows:

$$\frac{A\text{-unknown}}{A\text{-standard}} \times C\text{-standard} = C\text{-unknown}$$

and

$$\frac{C\text{-unknown} \times \text{final volume} \times 10}{\text{sample weight} \times \text{glucose recovery}} = \text{mg glucose/g sample}$$

where A = absorbancy, C = concentration (mg/100 ml), and final volume is expressed in milliliters, sample weight in milligrams, and glucose recovery as a decimal. Glucose recovery is determined by running

glucose samples through the entire hydrolysis and glucose analysis procedures. For samples of equal weights which are brought to the same final volume these equations can be simplified to

$$\frac{A\text{-unknown}}{A\text{-standard}} \times K = \text{mg glucose/g sample}$$

where K is a constant equal to

$$\frac{C\text{-standard} \times \text{final volume} \times 10}{\text{sample weight} \times \text{glucose recovery}}$$

To convert glucose to starch it is necessary to multiply the glucose concentration by 0.90, since the weight of the glucose unit incorporated into a starch molecule is increased by approximately 10% upon hydrolysis due to the addition of water. Thus, theoretical glucose recoveries from 1000 mg of starch will vary from 1100 to 1111 mg, depending upon the average polymer length. In this case the constant, K_s , becomes equal to

$$\frac{C\text{-standard} \times \text{final volume} \times 9}{\text{sample weight} \times \text{glucose recovery}}$$

and starch is calculated as

$$\frac{A\text{-unknown}}{A\text{-standard}} \times K_s = \text{mg starch/g sample.}$$

This reduces the calculations for a large number of samples to a single division for each sample with all dividends being multiplied by a single constant.

Digestibility Studies

Two feeding trials were conducted to study the effects of intake level of a high concentrate ration on starch digestion in steers. The second of the two trials was designed to permit the examination of processing effects, in addition to intake level, on digestion. The steers involved in the second trial were slaughtered and digesta were collected from different segments of the gastro-intestinal tract to study the digestion of starch in different portions of the tract.

Trial 1

Twelve Hereford steers, initially averaging 338 kg, were confined to individual pens previously described by Mehen (1966) and were randomly assigned to one of two treatments. Six steers were fed an 80% concentrate ration (Table 1) ad libitum, and the other six were restricted to 80% of the mean ad libitum intake based on a metabolic weight basis ($W_{kg}^{0.75}$). Milo for the ration was subjected to steam processing and flaking as described by Hale, Cuitun et al. (1966). After an initial feeding period of eight weeks, during which time steers were weighed and restricted intakes adjusted weekly, a seven-day total collection digestion trial was conducted as outlined by Husted (1966).

Daily fecal samples were dried in a forced air oven at 45°C for 24 to 36 hr. Dry matter was determined at this time, and the dried samples were stored in plastic bags until the end of the digestion trial. Dry matter content of daily feed samples was determined in a similar manner. A small sample of feed from each steer at each feeding was collected and stored in a plastic bag. At the end of the digestion

Table 1. Composition of rations for feeding trials.*

Ingredient	Trial 1	Trial 2
	%	%
Alfalfa hay	5.00	5.00
Cottonseed hulls	15.00	5.00
Milo	63.30 ^a	79.50 ^b
Cottonseed meal	5.50	3.35
Molasses	5.00	5.00
Tallow	4.00	-
Urea	0.55	0.50
Dicalcium phosphate	0.50	0.20
Salt	0.50	0.50
Limestone	0.65	0.95

*. Vitamin A added at the rate of 2200 I.U. per kg of ration.

a. Steam processed flaked milo.

b. Steam processed flaked or dry rolled milo.

trial daily feed and fecal samples were ground in a Wiley Mill through a 1-mm mesh screen and seven-day composite feed and fecal samples for each steer were stored in sealed glass containers for chemical analysis.

Samples were analyzed for starch by volumetric enzyme hydrolysis and glucose oxidase as outlined in the experimental procedure for total starch determination. Analysis of variance was performed according to Steel and Torrie (1960), and differences between treatment means were tested for significance at the 1 and 5% levels, using Duncan's multiple range test (Duncan 1955). One steer was removed from the experiment before termination due to illness and was not included in the calculations for average daily gain and feed efficiency.

Trial 2

Twelve crossbred steers, initially averaging 311 kg, were penned individually as in trial 1 and randomly allotted to one of four treatments with three steers per treatment in a 2 x 2 factorial experiment. Six steers received a 90% concentrate ration containing steam processed flaked milo, and a similar ration containing dry rolled milo was fed to the other six steers (Table 1). Three steers receiving each ration were fed ad libitum, with the remaining steers restricted to 75% of the mean ad libitum intake level on a metabolic weight basis ($W_{kg}^{0.75}$).

Trial 2 was conducted in a similar manner as trial 1 except that there was a seven-week preliminary feeding period, restricted intakes were adjusted every two weeks, and chromic oxide powder (Cr_2O_3) was added to the feed ten days prior to the beginning of the digestion

trial at the rate of 0.2% to determine the recovery in the feces. The digestion trial and sample preparation were the same as for trial 1 with the exception that a second daily fecal sample from each steer was frozen at -15°C . At the end of the trial these samples were thawed, and a seven-day composite sample for each steer was freeze dried, ground, and stored in glass jars for analysis.

Samples were freeze dried on a Virtis "Unitrap" freeze dryer. Approximately 40 ml of the thawed digesta were placed in a 500-ml freeze drying flask, and a shell was formed around an aluminum beverage can which was held in place with string until the digesta had frozen. Warm water poured into the can facilitated the rapid removal of the can from the flask. The flask and contents were again frozen until solid. Samples were dried at pressures of less than 100 u of mercury for 24 to 48 hr, after which they were ground and stored in glass containers.

Starch analysis and analysis of variance were the same as for trial 1. Chromic oxide was determined by the method of Kimura and Miller (1957). Recovery of chromic oxide from fecal standards ranged from 97.5 to 99.0%.

Digesta Collection

In order to minimize body weight differences between treatments and to maximize feed intake, each steer was slaughtered when it weighed approximately 410 kg. At least 10 days prior to slaughter, or when the body weight ranged from 390 to 400 kg, chromic oxide was included in the ration of the steer to be slaughtered. Each steer was killed three hours after the morning feeding. Uneaten feed was

weighed, sampled, and ground for starch analysis. A sample of the ration fed was also collected and analyzed. Immediately after stunning and bleeding, the animal was eviscerated, and segments of the gastrointestinal tract were tied off with strings and the segments emptied of their contents. Segments isolated were the reticulo-rumen, abomasum, small intestine, large intestine and cecum, and rectum. The small intestine was divided into three equal segments for purposes of collection and analysis. The digesta from each segment was weighed and sampled, and the samples were rapidly cooled in a dry ice-acetone bath and kept frozen at -15°C until they could be freeze dried, ground, and stored in glass jars. A second sample was taken for dry matter determination by oven drying at 45°C . All samples were analyzed for starch and chromic oxide content. In addition, the feed and abomasal samples were analyzed for ash according to the method of A.O.A.C. (1960). Digestibility was estimated from the equations:

$$\% \text{ dry matter digestion} = 100 - \left[\frac{\% \text{ marker (feed)}}{\% \text{ marker (digesta)}} \times 100 \right] ;$$

$$\% \text{ starch digestion} = 100 - \left[\frac{\% \text{ marker (feed)}}{\% \text{ marker (digesta)}} \times \frac{\% \text{ starch (digesta)}}{\% \text{ starch (feed)}} \times 100 \right] .$$

Digestibility estimates were adjusted for 100% recovery of chromic oxide. Recovery by steer from the total fecal collection ranged from 83.4 to 103.3% with a mean of 92%. The concentrations of chromic oxide in the feces at the time of the digestion trial were similar to rectal concentrations at the time of slaughter. Total ruminal digestion was

estimated as the difference between starch intake and starch reaching the abomasum, and digestion in the small intestine as the difference between abomasal starch and that appearing in the colon. Total digestion was estimated as the starch loss from mouth to rectum.

RESULTS AND DISCUSSION

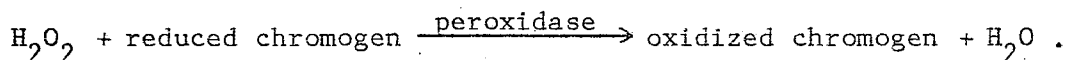
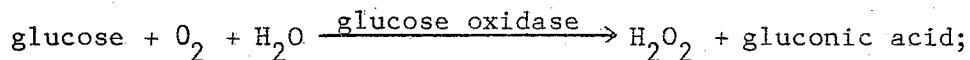
The collection of accurate data in studying starch digestion in the ruminant is dependent upon many factors. Starch analysis techniques, effects of grain processing, level of starch intake, and the selection of the indigestible marker were among the factors investigated in this study.

Starch Analysis Techniques

The starch in a variety of substrates was hydrolyzed by two methods to compare the glucose recovery patterns obtained for each method as influenced by the substrate being subjected to hydrolysis.

Glucose Recovery

A preliminary investigation was conducted to determine glucose recovery from acid and enzyme hydrolysis. The glucose analysis procedure utilized in these studies was a modification of the Glucostat method (Worthington Technical Bulletin 1963). This technique makes use of a coupled reaction as follows:



This method, first introduced by Keston (1956), provides a quantitative, colorimetric determination of glucose. It is sensitive enough

to measure glucose in very small concentrations, having been originally developed for general use in the analysis of glucose in biological fluids such as blood and urine.

Duplicate glucose samples were tested for recovery in four trials each for acid and volumetric enzyme hydrolysis and in three trials for gravimetric enzyme hydrolysis (Table 2). The 97.5% glucose recovery from the two enzymatic procedures compares favorably with the values given by Topps and Kay (1969) who reported recoveries of 98, 98, and 94% from three different laboratories using Agidex in their starch analysis procedures. The recovery for acid hydrolysis was only slightly lower than for enzyme hydrolysis at 96.3%. This is considerably higher than the values obtained by Osman (1966) or Frederick (1968) who reported recoveries of 91.3 and 90.1 to 91.3% with dilute HCl, respectively, but is similar to the approximate 96.5% reported by Pirt and Whelan (1951). MacRae and Armstrong (1968), although giving no recoveries of their own, utilized H_2SO_4 in their acid vs. enzyme hydrolysis comparisons, citing the findings of Pirt and Whelan (1951) that hydrolysis with H_2SO_4 resulted in less glucose loss than with HCl. The relatively rapid and easy to use method utilizing HCl, in addition to the relatively high glucose recoveries obtained and the resulting values for starch content reported here, seem to justify the use of HCl for starch hydrolysis as an alternative to the use of H_2SO_4 , which, as reported by MacRae and Armstrong (1968), would require a much longer and involved procedure.

Table 2. Recovery from glucose samples subjected to acid or enzyme hydrolysis.

Trial ^a	H y d r o l y s i s m e t h o d		
	Acid	Volumetric enzyme	Gravimetric enzyme
	<u>mg glucose/g</u>	<u>mg glucose/g</u>	<u>mg glucose/g</u>
1	964.3	991.9	977.6
	960.0	971.0	959.4
2	962.5	976.3	984.9
	953.4	980.4	980.8
3	967.9	966.4	991.5
	953.4	979.0	956.6
4	979.9	977.4	
	<u>965.8</u>	<u>959.2</u>	<u> </u>
Mean	963.4	975.2	975.1

a. Trials for different hydrolysis methods were run separately.

Enzymatic Hydrolysis of Starch

The term starch normally refers to a group of alpha-linked glucose polymers of plant origin with widely varying molecular weights. Starch exists in the straight chain form as amylose with only alpha-(1→4)-glycosidic bonds or in the branched chain form (amylopectin) with alpha-(1→6) bonds, the latter forming the branch points (Mahler and Cordes 1968, p. 251). Starch, as it is used in this study, refers to any alpha-linked glucose polymer which is subject to hydrolysis by amyloglucosidase. No attempt was made to separate dextrans or short chain alpha-linked glucose polymers from the larger "starch" molecule. Since the fate of these groups of polymers in the digestive tract of ruminants is essentially the same, it was felt unnecessary to distinguish between the different groups.

Amyloglucosidase, an enzyme produced by Aspergillus niger, was chosen as the standard for alpha-linked glucose polymer determination because it has been shown to be an enzyme which will completely hydrolyze alpha glycosidic linkages with a high degree of specificity (Abdullah et al. 1963, MacRae and Armstrong 1968, Pazur and Ando 1959, Pazur and Kleppe 1962). The enzyme has been shown to be an exo-glucosidase, splitting off single glucose units beginning from the reducing end of alpha-linked polymers (Abdullah et al. 1963, Pazur and Ando 1959).

A method was developed by MacRae and Armstrong (1968) in which amyloglucosidase (Agidex) could be used for the routine analysis of starch in feedstuffs and digesta. This procedure determines the volume

of the hydrolytic solution, which is used in calculating the glucose concentration, by weight (gravimetric method). A method of determining starch from enzyme hydrolysis based on a volumetric determination (volumetric method) was developed at this laboratory to provide the following advantages over the gravimetric method: (a) fewer weighings per sample, (b) elimination of the need for paraffin, and (c) simplified calculations.

As many as four weighings per sample may be necessary for starch determination by the gravimetric method. When volumetrically determined, only the sample needs to be weighed.

The paraffin used in the gravimetric method prevents any moisture losses from evaporation which would increase the concentration of the solution. However, small losses which might occur with a watch glass covering are not critical for the volumetric method. The need for paraffin to inhibit microbial growth, as suggested by MacRae and Armstrong (1968), did not appear to be a factor in these studies as indicated by glucose recoveries (Table 2).

Standardization of sample weights and final volume reduces calculations for a large number of dried samples to two steps--a single division for each sample with all quotients multiplied by a single, predetermined constant to give milligrams of glucose per grams of sample on a dry basis. Starch concentration can either be calculated from glucose content or determined directly by using a different constant.

Some difficulty with the volumetric method was encountered when the particle size of the substrate was fine enough to impede filtration.

The only substrates used in this study to give such problems were some finely ground fecal samples. For these it was necessary to use a filter flask and an aspirator. A rapid and convenient measure for the 0.5 g of Agidex used in this procedure was a level one-quarter teaspoon. It was found that considerable variations in the amount of Agidex used had only a small effect on the quantity of glucose produced from hydrolysis of sorghum grains (Table 3).

Analyses were made on a variety of substrates to compare the gravimetric and volumetric methods for glucose production and repeatability (Table 4). Statistical analysis indicated no differences between means for any of the substrates tested. All subsequent results reported in this study where enzyme hydrolysis was utilized were determined by the volumetric method.

Acid vs. Enzyme Hydrolysis of Starch

The question of acid vs. enzyme hydrolysis of starch arises due to the existence of other polysaccharides which, if hydrolyzed, could give erroneous starch values. When glucose is analyzed as a reducing sugar such as outlined by Oser (1965), there are many possible contributors to the reducing sugar pool, including many mono-, oligo-, and polysaccharides coming from both pentosans and hexosans. As shown by MacRae and Armstrong (1968), this can lead to gross overestimation of starch when feedstuffs or digesta are hydrolyzed by a nonspecific method such as acid hydrolysis. Therefore, when analyzing for reducing sugars, the starch must be hydrolyzed by an agent which is specific for the alpha-linked glycosidic bonds. These have generally fallen into

Table 3. Glucose recoveries from grain samples hydrolyzed with increasing levels of Agidex.*

Agidex	Glucose recovery
<u>g</u>	<u>mg Glucose/g sample</u>
0.1	710
0.2	714
0.3	710
0.4	723
0.5	714
0.6	727
0.7	723

*. Expressed on an "as is" basis.

Table 4. Mean glucose recoveries from four substrates using either volumetric or gravimetric enzyme hydrolysis.^{a,b}

Substrate	Hydrolysis method	
	Volumetric	Gravimetric
	<u>mg glucose/g ± SD</u>	<u>mg glucose/g ± SD</u>
Potato starch	1100.1 ± 6.0	1101.3 ± 4.9
Milo grain	773.2 ± 3.5	774.4 ± 4.6
Alfalfa hay	20.8 ± 2.1	23.0 ± 1.5
Feces ^c	15.1 ± 2.3	16.8 ± 2.0

- a. Means of 2 runs of 3 replications each (dry basis).
- b. Means tested for significance using Student's "t" test.
- c. Feces from steer fed ration for trial 1 (Table 1).

the category of plant enzymes such as beta amylase or animal enzymes such as salivary or pancreatic amylase. However, if the glucose analysis procedure is specific for glucose, a less specific hydrolytic method may be used. This would leave only substrates containing glucose or glucose polymers as possible contributors.

The major nonstarch glucose polymer in plant material is cellulose, a linear, beta-(1→4)-linked glycosidic chain and one of the principal structural components of plant cells (Mahler and Cordes 1968, pp. 248-49). Although cellulose can be hydrolyzed completely to glucose by strong acids, weak acids have only limited effects on it (Maynard and Loosli 1969, p. 75). In order to determine to what extent cellulose contributes to the glucose produced during starch hydrolysis by a weak acid, tests were run comparing weak acid hydrolysis to Agidex enzyme hydrolysis which is specific for alpha-linked polymers of glucose. The advantage of using acid hydrolysis is a shorter reflux period (2.5 hr vs. 4 hr for enzyme hydrolysis) followed by immediate glucose analysis, whereas enzyme hydrolysis requires a 24-hr incubation period. To avoid interference from other reducing sugars, glucose was analyzed using glucose oxidase, an enzyme specific for glucose.

A comparison of the two hydrolysis methods on various substrates indicates that when the starch was high in comparison to cellulose content acid and enzyme hydrolysis give similar results (Table 5). When cellulose was high compared to starch, a higher value ($P < 0.05$) was obtained for acid hydrolysis, indicating that some of the cellulose was being hydrolyzed. This was supported by the observation that

Table 5. Mean glucose recoveries from a variety of substrates using either acid or enzyme hydrolysis.^{a,b}

Substrate	Acid hydrolysis	Enzyme hydrolysis
	mg glucose/g \pm SD	mg glucose/g \pm SD
Red milo starch	1099.4 \pm 4.0	1106.3 \pm 10.4
Corn starch	1100.4 \pm 10.4	1101.9 \pm 2.6
Potato starch	1095.9 \pm 3.4	1100.1 \pm 6.0
Feces ^c	395.4 \pm 8.5	387.5 \pm 3.2
Feces ^d	30.7 ^e \pm 1.3	15.1 ^f \pm 2.3
Milo grain	772.2 \pm 3.9	773.2 \pm 3.5
Alfalfa hay	32.6 ^e \pm 4.0	20.8 ^f \pm 2.1
Cotton	11.0 ^e \pm 2.1	0.0 ^f \pm 0.0
Alfalfa : Milo		
4 : 1	179.7 ^e \pm 2.2 (180.5) ⁱ	169.8 ^f \pm 2.6 (171.2)
2 : 1	277.0 ^g \pm 5.7 (276.7)	269.4 ^h \pm 3.3 (271.3)
1 : 1	400.3 \pm 4.8 (402.4)	401.6 \pm 5.7 (397.0)
1 : 2	525.9 \pm 4.7 (528.1)	523.6 \pm 6.9 (522.6)
1 : 4	622.2 \pm 4.1 (624.3)	625.5 \pm 10.9 (622.8)

- a. Means of 2 runs of 3 replications each (dry basis).
- b. Means tested for significance with Student's "t" test.
- c. Feces from steer fed a whole corn, no roughage ration.
- d. Feces from steer fed ration for trial 1 (Table 1).
- e,f. Means on same line with different superscripts are significant ($P < 0.01$).
- g,h. Means on same line with unlike superscripts are significant ($P < 0.05$).
- i. Figures in parentheses are calculated concentrations based on individual hay and milo values shown above.

cotton, which is nearly pure cellulose, produced 11 mg of glucose per gram of sample, whereas the enzyme hydrolysis produced none. It was observed that the cotton fibers were greatly reduced in length by the acid hydrolysis procedure, another indication of some hydrolysis of the beta-linked bonds in cellulose by weak acid.

Two fecal samples were analyzed using acid and enzyme hydrolysis (Table 5). One sample came from a steer fed a ration containing 20% roughage and 63% steam processed flaked milo. Although the starch in the feces was low (3.1% for acid vs. 2.1% for enzyme hydrolysis), the nearly 50% higher value for acid hydrolysis was different from the enzyme hydrolysis value at the 1% level of significance. In contrast, a sample from a steer fed whole corn with no roughage showed a relatively high starch level (39.5 and 38.8%), but the differences were not significant, possibly due to the lower cellulose content and higher starch concentration.

To further test the idea that cellulose contributes to the glucose pool when using acid hydrolysis, alfalfa hay, which tested low in starch but has a high cellulose content, and milo, a grain relatively high in starch but low in cellulose, were mixed in varying ratios and tested for starch content. When alfalfa constituted 50% of the mixture or less, no differences were found between acid and enzyme hydrolyses (Table 5). When the alfalfa comprised 67%, a difference ($P < 0.05$) was found between methods and was highly significant ($P < 0.01$) when 80% of the mixture was composed of alfalfa hay. The experimental starch values for the mixtures closely approximated the theoretical concentrations

(based on experimental starch values for alfalfa and milo separately), indicating that the glucose arising from cellulose is proportional to the cellulose concentration.

The repeatability of glucose recovery from both methods of hydrolysis was relatively good in these comparisons with coefficients of variation ranging from 0.3 to 2.6% for the purified starches and 0.1 to 2.1% for the hay-grain mixtures. Other coefficients (enzyme hydrolysis and acid hydrolysis) were cotton, 0.0 and 19.2%; alfalfa hay, 10.1 and 12.3%; milo grain, 0.4 and 0.6%; feces (milo fed), 15.2 and 4.4%; and feces (whole corn fed), 3.2 and 2.1%.

Samples of feed, digesta, and feces from two steers were also analyzed comparatively using acid or enzyme hydrolysis (Table 6). Digesta samples were taken from the abomasum, the middle third of the small intestine, and the colon. Steer 1 had been fed ad libitum the 79.5% milo ration shown in Table 1 containing steam flaked milo, and steer 2 had received ad libitum a similar ration containing dry rolled milo. No differences were found in the analysis of feed or abomasal samples for either steer. However, samples from the small intestine, colon, and feces for both steers showed differences ($P < 0.01$) when hydrolyzed in acid or with Agidex.

These findings agree with the observations with alfalfa-milo mixtures. That is, as the cellulose content increases in comparison to starch, an ever increasing portion of the glucose pool resulting from acid hydrolysis comes from cellulose. In the feed and abomasal contents, glucose originating from cellulose hydrolysis would still

Table 6. Mean starch recoveries from feed, digesta, and feces using either acid or enzyme hydrolysis.

Substrate	Steer 1 ^a		Steer 2 ^a	
	Acid hydrolysis	Enzyme hydrolysis	Acid hydrolysis	Enzyme hydrolysis
	<u>mg starch/g ± SD</u>	<u>mg starch/g ± SD</u>	<u>mg starch/g ± SD</u>	<u>mg starch/g ± SD</u>
Feed ^b	678.4 ± 12.2	681.4 ± 11.1	673.9 ± 2.0	670.4 ± 11.4
Abomasum ^c	187.6 ± 3.3	184.6 ± 2.7	357.2 ± 7.0	357.7 ± 3.4
Small intestine ^c	84.8 ^d ± 2.1	71.9 ^e ± 8.6	116.3 ^d ± 3.1	107.4 ^e ± 2.7
Colon ^b	79.2 ^d ± 4.4	58.0 ^e ± 3.3	211.6 ^d ± 1.4	191.5 ^e ± 1.0
Feces ^b	46.4 ^d ± 4.1	12.1 ^e ± 0.4	200.9 ^d ± 4.7	180.5 ^e ± 1.4

- a. Steer 1 received the flaked milo ration and steer 2 the dry rolled milo ration fed in trial 2 (Table 1), both intakes being ad libitum.
- b. Means of 1 run with 3 replications each (dry basis).
- c. Means of 2 runs with 3 replications each (dry basis).
- d,e. Means on same line within steer with unlike superscripts are significant ($P < 0.01$) when tested with Student's "t" test.

form a relatively minor portion of the glucose pool. As the rapidly fermented and hydrolyzed starch was degraded and disappeared from the tract, the more slowly digested cellulose would form an increasing percentage of the total digesta dry matter, and the cellulose to starch ratio would grow larger. Therefore, when hydrolyzed by weak acid, the cellulose would contribute a significant and increasing portion of the glucose pool. It is not known what, if any, effect that passage through the digestive tract has upon cellulose and the ability of dilute HCl to hydrolyze it.

These findings suggest that with the acid hydrolysis technique cellulose can contribute a significant amount of "starch" where cellulose content is high compared to starch. To determine how this would affect total digestibility estimates, feed and fecal samples from trial 2 were analyzed by acid and enzyme hydrolysis and digestibility calculated for each method (Table 7).

No differences ($P > 0.05$) were found in the starch content of the feed resulting from hydrolysis method. Analysis of variance (Table 15, Appendix) indicates highly significant ($P < 0.01$) differences due to hydrolysis method for both fecal starch and digestibility, reflecting the consistently higher values obtained when feces were hydrolyzed with acid. The mean starch content of the feces as estimated from acid hydrolysis (9.4%) was approximately 50% higher than when hydrolyzed with Agidex (6.8%). However, when these values were used to compute digestibility, the difference between methods was less than one percentage unit (96.8% vs. 97.6%), indicating that when a high concentrate ration

Table 7. Effects of acid or enzyme hydrolysis on the estimation of feed and fecal starch content and starch digestibility.*

Item	Acid hydrolysis	Enzyme hydrolysis
Starch in feed, %	65.3 ^a	66.3
Starch in feces, %	9.4 ^b	6.8 ^c
Starch digestion, %	96.8 ^b	97.6 ^c

*. Composite data of 12 steers from trial 2 (dry basis).

a. See appendix Table 15 for analysis of variance.

b,c. Means on same line with unlike superscripts are different ($P < 0.01$).

is fed, acid hydrolysis can produce results comparable to those from enzyme hydrolysis with only small errors arising from nonstarch sources. Caution should be exercised when feeding a high roughage diet, however, since an increase in fecal starch estimation resulting from acid hydrolysis would tend to produce a more marked depression in digestibility estimates. No studies were conducted to determine the magnitude of such a depression.

Starch Recovery in Freeze Dried and Oven Dried Fecal Samples

Comparisons were run utilizing enzyme hydrolysis to determine the effect on starch recovery of drying feces in an oven vs. freeze drying. Means for steam processed flaked treatments were not different ($P < 0.05$) for the two drying methods in either trial, although the oven dried samples tended to show a lower recovery in trial 1 (Table 8). With the dry rolled treatments, statistical analysis (Table 16, Appendix) indicated a lower ($P < 0.05$) recovery for the oven dried samples as compared to freeze drying. The differences found for the dry rolled treatments would not produce a large change in calculated digestibility for the rations fed in these trials (Table 7), although the effect on a low starch ration is not known. These results indicate that there are situations in which freeze drying would produce more precision in measuring starch content in feces. Comparisons between freeze drying and oven drying were not run on digesta samples, and possible interaction effects between drying method and hydrolyzing method were not tested.

Table 8. Mean starch recoveries from freeze dried or oven dried fecal samples.*

Trial	Treatment	Freeze dried	Oven dried
		<u>mg starch/g</u>	<u>mg starch/g</u>
1	Restricted steam flaked	17.3 ^a	13.8
	Full-fed steam flaked	17.3	14.2
2	Restricted steam flaked	40.7	39.7
	Full-fed steam flaked	60.2	62.7
	Restricted dry rolled	107.0 ^b	93.0 ^c
	Full-fed dry rolled	172.3 ^b	157.3 ^c

*. Means of two runs of three replications each (dry basis).

a. See appendix Table 16 for analysis of variance.

b,c. Means on same line with unlike superscripts are significant (P>0.05).

Digestibility Studies

The two digestion trials which were conducted provide an opportunity to examine the digestibility of starch in the total tract, whereas the collection of digesta samples permits the determination of digestion at different points along the tract. Together, these two methods enable one to obtain a more complete picture of digestion throughout the gastro-intestinal tract.

Trial 1

Steers receiving the 80% concentrate ration (Table 1) containing approximately 57% starch had intakes ($\text{g starch/W}_{\text{kg}}^{0.75}$) of 42.6 and 52.3 g for the restricted and full-fed treatments, respectively (Table 9). In spite of the difference in intake, starch and dry matter digestion and percentage fecal starch were essentially the same for both treatments. Differences in intake are reflected in the nearly 50% greater gain for the full-fed steers (Table 10), whereas feed efficiency (feed/gain) was not different ($P > 0.05$), though it tended to favor the full-fed treatment (8.14 vs. 8.89 for restricted intake).

Trial 2

The second trial was designed to consider a second factor, processing, in addition to level of intake. The milo content of the ration was increased from 63.3% for trial 1 to 79.5% (with approximately 66% starch) for trial 2 in an attempt to maximize starch intake, and the restricted intake steers were reduced from 80% of full-fed (trial 1) to approximately 75% of full-fed in trial 2 to accentuate the

Table 9. Daily intake and digestibility of dry matter and starch (trial 1).^{a,b}

	Treatment	
	Restricted	Full-fed
Dry matter intake, g	6420	8337
Starch in ration, %	57.6	57.2
Starch intake, g	3685	4786
Starch intake, g/ ^{0.75} kg	42.6	52.3
Fecal dry matter, g	1898	2442
Fecal starch, %	1.2	1.1
Fecal starch, g	23.2	27.0
Dry matter digestion, %	70.4	70.8
Starch digestion, %	99.4	99.4

a. Means of six animals per treatment (dry basis).

b. See appendix Table 17 for analysis of variance.

Table 10. Average daily gain and feed per gain (trials 1 and 2).*

	Treatment	Gain/day, kg	Feed/gain
Trial 1 ^a	Restricted	0.84 ^c	8.89
	Full-fed	1.18 ^d	8.14
Trial 2 ^b	Restricted steam flaked	0.88 ^{e,f}	6.29 ^e
	Full-fed steam flaked	1.09 ^e	6.86 ^{e,f}
	Restricted dry rolled	0.71 ^f	7.91 ^f
	Full-fed dry rolled	1.06 ^e	6.96 ^{e,f}

*. See appendix Table 18 for analysis of variance.

a. Six steers for restricted intake, five steers for full-fed intake.

b. Three steers per treatment.

c,d. Means within columns and trials with unlike superscripts are significant ($P < 0.01$).

e,f. Means within columns and trials with unlike superscripts are significant ($P < 0.05$).

differences in starch intakes between full-fed and restricted treatments. Resulting starch intakes ($\text{g starch/W}_{\text{kg}}^{0.75}$) reached 53.3 and 54.3 g for the full-fed dry rolled (FDR) and the full-fed steam processed flaked (FSF) treatments, respectively, and 40.6 and 38.6 g for the restricted dry rolled (RDR) and restricted steam processed flaked (RSF) treatments, respectively (Table 11).

Percentage fecal starch tended to be higher for the dry rolled treatments with full-fed higher than restricted. Total fecal starch content followed the same pattern as percentage fecal starch, and differences were significant ($P < 0.05$) between RSF and FDR. Starch digestion was highest (99.6%) for RSF and lowest (94.9%) for FDR. These differences were significant ($P < 0.05$). Dry matter digestibility tended not to follow the pattern of starch digestion. Whereas starch digestion favored the steam processed flaked treatments, dry matter digestion appeared to be affected more by intake level, the lower intakes tending to have higher digestibilities.

Average daily gains for the full-fed steers (1.09 kg for FSF and 1.06 for FDR) were similar and tended to be higher than the RSF (0.88 kg), though not significantly at the 5% level (Table 10). They were, however, higher ($P < 0.05$) than the 0.71 kg average gain per day for RDR. Feed efficiency (feed/gain) was superior for RSF (6.29) as compared to RDR (7.91) with similar intermediate values of 6.86 and 6.96 for FSF and FDR, respectively. Daily feed and fecal dry matter and percentage starch for individual steers in trials 1 and 2 are

Table 11. Daily intake and digestibility of dry matter and starch (trial 2).^{a,b}

	T r e a t m e n t ^c			
	RSF	FSF	RDR	FDR
Dry matter intake, g	4759	7089	5002	6701
Starch in ration, %	66.0	66.7	65.7	67.0
Starch intake, g	3142	4780	3288	4474
Starch intake, g/W ^{0.75} kg	38.6	54.3	40.6	53.3
Fecal dry matter, g	879	1549	1051	1521
Fecal starch, %	1.6	3.7	7.4	14.8
Fecal starch, g	14 ^d	60 ^{d,e}	88 ^{d,e}	239 ^e
Dry matter digestion, %	81.5	77.8	79.1	77.8
Starch digestion, %	99.6 ^d	98.7 ^{d,e}	97.4 ^{d,e}	94.9 ^e

a. Means of three animals per treatment.

b. See appendix Table 17 for analysis of variance.

c. RSF = restricted steam processed; FSF = full-fed steam processed flaked; RDR = restricted dry rolled; FDR = full-fed rolled.

d,e. Means on same line with unlike superscripts are significant ($P < 0.05$).

presented in Tables 19 and 20 (Appendix) and chromic oxide recovery for trial 2 in Table 20.

Digesta Collection

Starch intakes at the time of slaughter were somewhat higher than for the digestion trial, reaching a total of approximately 5200 and 5600 g (56.8 and 60.2 g/W_{kg}^{0.75}) for FDR and FSF, respectively (Table 12). Dietary starch for each of the restricted treatments amounted to approximately 4200 g daily. Percentage ruminal starch digestion was not different ($P > 0.05$) for any of the treatments, although there appeared to be a definite division between the steam processed flaked and dry rolled treatments in digestibility. It should be noted, however, that one steer in each of the dry rolled treatments had an extremely low apparent digestibility coefficient in the rumen of 21% for RDR and 17% for FDR. Had these values not been included in the treatment means, these means would have been 77 and 61% for the RDR and FDR treatments, respectively. The apparent abnormality of these two values appeared to be related to the use of chromic oxide powder as the indigestible marker. The ramifications of this possibility are discussed later.

Total starch digested in the small intestine was considerably higher for the dry rolled treatments, reflecting the greater quantities of starch escaping ruminal digestion. The 2500 g digested in the small intestine of the FDR is double the quantities for either of the steam flaked treatments. An examination of digestibility estimates through the different portions of the small intestine, the colon, and the

Table 12. Mean starch recovery and digestibility in different segments of the digestive tract.^{a, b}

	T r e a t m e n t ^c			
	RSF	FSF	RDR	FDR
Starch in feed, %	66.0	66.7	65.7	67.0
Intake				
DM, g	6385	8421	6300	7832
Starch, g	4236	5587	4180	5196
Starch, g/W _{kg} ^{0.75}	46.1	60.2	45.5	56.8
Starch presented, g				
Small intestine	1229	1341	1747	2866
Colon	85	206	184	332
Starch disappeared, g				
Rumen	3007	4246	2433	2331
Small intestine	1145	1136	1563	2534
Starch digestion, % (disappeared as % of presented)				
Rumen	71.1	76.0	58.5	45.3
Small intestine	94.1	81.3	87.2	88.6
Starch digestion, %				
Mouth to:				
Small intestine				
1st part	93.8	90.1	79.9	78.2
2nd part	95.2	92.0	91.9	91.2
3rd part	97.4	93.8	88.2	90.2
Colon	98.0	96.3	95.6	92.6
Rectum ^d	98.6	98.5	95.2	95.9
Total DM digestion, %	81.0	78.7	77.5	76.9

a. Three steers per treatment.

b. See appendix Table 21 for analysis of variance.

c. RSF = restricted steam processed flaked; FSF = full-fed steam processed flaked; RDR = restricted dry rolled; FDR = full-fed dry rolled.

d. Processing effects are significant ($P < 0.05$).

rectum reveals relatively high digestibility estimates throughout the post-abomasal tract (Table 12). With only three exceptions all values exceed 90% digestibility. The two lower values (79.9 and 78.2%) for the RDR and FDR in the first segment of the small intestine are reflected in the lower ruminal digestion coefficients for these two treatments. Total starch digestibilities were similar within processing method and tended to be higher for the steam processed flaked treatment. Analysis of variance (Table 21; see Appendix for Tables 15 through 24) indicated a significant ($P < 0.05$) processing effect.

Total dry matter digestibilities were not different for any of the treatments. These values, estimated from rectal contents and using marker techniques, gave similar values to those obtained from the total collection digestion trial (Table 11). Starch values, with the exception of RDR, were similar also. Individual steer data for dry matter content and percentage starch and chromic oxide are presented in Table 22.

Effects of Processing Grain on Starch Digestion

Grain processing is an area which is presently under active investigation as workers attempt to maximize feed utilization and efficiency and unravel the bioenergetic ramifications of starch digestion in different portions of the gastro-intestinal tract.

Starch Digestion in the Rumen. The digestibility values found in this study (Table 12) indicate an increase in starch digestibility in the rumen resulting from steam processing and flaking vs. dry

rolling (71 to 76% vs. 45 to 58%). This trend is in agreement with other studies involving the effects of processing sorghum grains on ruminal digestion. McNeill et al. (1971) found steam processed flaked sorghum grain to have the highest ruminal starch digestibility coefficient (83%) when compared to reconstituted ground (67%), dry ground (43%), or micronized (42%). Similarly, Holmes et al. (1970) found a processing effect on ruminal digestibility of milo starch when the grain was either steamed for 8 min at atmospheric pressure or steamed under a pressure of 3.5 kg/cm^2 for 1.5 min. Digestion in the rumen ranged from 89 to 90% for the 8 min treatment and 94 to 96% for the 1.5 min treatment.

Studies also indicate that corn starch digestion is affected by the method of processing. Orskov et al. (1969) found lower ruminal digestion of starch in cracked (86%) and ground (88%) corn than in steam flaked corn (95%). Beever et al. (1970) found an even greater difference between ground and flaked corn with the former having a ruminal starch digestibility of 78% and the latter of 96%.

The digestion of starch in barley appears to be less dependent on processing than either corn or sorghum grains. When barley was rolled (Orskov et al. 1969), 93% of the starch was digested in the rumen, whereas 96% was digested when the barley was ground. MacRae and Armstrong (1969b) found only small differences in the ruminal digestibility of starch in barley when fed either whole (95%) or rolled (97%).

The studies cited indicate that ruminal starch digestion can be altered by processing method in either corn or sorghum grain, but that

these effects are minimal in altering barley starch digestion. Of the methods studied, steam processing and flaking has given the greatest response as measured by an increase in ruminal digestion. The findings of this experiment tend to support this conclusion regarding sorghum grain, although the differences found were not significant ($P > 0.05$).

Total Starch Digestion. Total digestibility of starch seems to be less markedly affected by processing than is ruminal digestion, and the magnitude of any difference appears to relate to the source of starch. For this study, starch digestion in dry rolled milo was consistently lower (95.2 to 97.4%) than in steam processed flaked milo (98.5 to 99.6%) in both the digestion trial (Table 11) and at the time of slaughter (Table 12). Analysis of variance (Tables 17 and 21) indicated a significant processing effect ($P < 0.05$) in both instances. The lower digestibility of the dry rolled milo is also indicated by the total quantities of starch in the digestive tract three hours after feeding (Table 23). The total starch in the tract expressed as percentage of daily intake tended to be higher for the dry rolled grain (32 to 34%) than for steam processed flaked grain (27 to 29%).

Other studies appear to give conflicting results concerning the alteration of starch digestion by different methods of processing. These include not only starch studies, per se, but also studies involving factors which may relate to starch digestion such as dry matter or nitrogen free extract (NFE) digestibility.

In agreement with this study, McNeill et al. (1971) found higher ($P < 0.05$) starch digestibility coefficients for reconstituted

ground (99.5%) and steam flaked sorghum grain (99.7%) than for the dry ground (96.8%) or micronized (97.1%) treatments. A similar trend was found by Buchanan-Smith et al. (1968) who fed 78% grain sorghum rations to cattle and sheep. No differences ($P > 0.05$) were found when the grain was coarse ground, fine ground, steam processed rolled (flaked), or reconstituted rolled, although the two grinding treatments tended to have lower digestibilities than either the reconstituted or flaked, particularly in the cattle. Digestibilities in cattle were 91.3, 91.9, 94.3, and 94.6% for the four treatments, respectively. However, non-protein organic matter digestibility was higher ($P < 0.05$) for the moisture treated grain rations (85%) than for the dry ground rations (79%). Holmes et al. (1970) found no difference in total starch digestion (96 to 99%) in cattle or sheep when fed milo which had either been steamed for 8 min at atmospheric pressure and rolled or steamed under pressure (3.5 kg/cm^2) for 1.5 min and rolled.

Keating et al. (1965) found no difference in starch digestion in dry rolled or cooked milo (79 vs. 80%) but did have an increase ($P < 0.05$) in NFE digestibility for the latter (70 vs. 76%). Husted et al. (1968) found an increase ($P < 0.05$) in NFE digestibility when milo was steam flaked (84%) vs. dry rolled (71%), steam cut (73%), or water soaked cut (75%). In a second experiment NFE digestibility was higher for steam flaked (87%) and pressure cooked flaked (87%) than for either dry rolled (76%) or fine ground (78%). In agreement with this, Hale, Cuitun et al. (1966) found the NFE digestibility of steam flaked milo (78%) to be higher than for dry rolled milo (69%).

The observed increase in starch digestibility due to steaming and flaking is supported by the in vitro studies of Frederick (1968). Incubation of milo grain with lyophilized beef pancreas for 30 min did not show an increase in starch digestion for steam processed poorly rolled milo over dry rolled milo. In contrast, a flat flake in combination with steam processing increased digestion nearly threefold over dry rolling. These observations and those of Osman et al. (1970), which implicate the degree of steaming and flatness of flaking in starch digestibility, might explain the apparent discrepancy between work which shows an increase in starch digestibility due to steam processing and that which does not.

Studies involving the digestibility of starch in corn indicate that total digestibility is less affected by processing than is found in sorghum grains. Orskov et al. (1969) found starch digestibilities ranging from 99.2 to 99.5% when corn which had been cracked, ground, or steam flaked was fed to sheep. These findings of nearly complete digestion of corn starch is supported by the work of Beever et al. (1970) who found digestibilities exceeding 99.6% when sheep were fed ground or flaked corn. This work is in apparent conflict with the results obtained by Johnson et al. (1968) who fed steers corn which had been steam flaked, flaked-cracked (flaking followed by rolling the dried flake), cracked, or steam-cracked (steamed, dried, and then cracked). Dry matter digestibility was greater for the flaked and flaked-cracked treatments than for the cracked or steam-cracked corn. In contrast,

dry matter digestibility was not affected by cracking, grinding, or steam flaking in the work reported by Orskov et al. (1969).

Total digestion of barley starch, as in the rumen, is only minimally changed by processing. Orskov et al. (1969) found digestion to be very high (99.7 to 99.8%) when sheep were fed ground barley or rolled barley in combination with hay. This is in apparent agreement with Parrott et al. (1969) who found no difference in dry matter, organic matter, or NFE digestibility when steers were fed an 85.5% barley ration in which the barley had been either dry rolled or steam processed followed by regular or flat flaking.

An increase in rate of starch digestion was shown in in vitro trials by Osman et al. (1970) and Frederick (1968) due to steam processing and flaking vs. unflaked barley. However, rate of digestion would not necessarily affect total digestion unless the rate of passage were excessively high or starch intake levels exceeded the ability of the digestive tract to digest it.

Total starch digestion appears to be only minimally affected by processing method when corn or barley is fed. However, this and other studies indicate that digestion of starch in sorghum grains can be significantly altered by processing.

Effects of Intake Level on Starch Digestion

The results of studies involving level of starch intake have been inconclusive, although generally they suggest little effect of intake level on starch digestion. It should be noted that in previous

studies no intake level trials have been conducted using sorghum grains and that the studies utilizing other grains involve starch levels considerably lower than might be consumed in a finishing ration by a steer in the feedlot. Most starch digestion trials have had intakes falling within the range of from 1 or 2 g of starch/ $W_{kg}^{0.75}$ on some hay diets (MacRae and Armstrong 1969b; Topps, Kay, and Goodall 1968) to approximately 45 g/ $W_{kg}^{0.75}$ for some high concentrate diets (Topps, Kay et al. 1968). Although there are several trials reported which do not provide sufficient information to calculate intakes on a metabolic weight basis, it would not appear that the above levels have been exceeded. Thus, the maximum level reported in the literature is only slightly higher than the restricted intake level for trial 1 (42.6 g/ $W_{kg}^{0.75}$) and equals the restricted intakes for trial 2 at the time of slaughter (45.5 to 46.1 g/ $W_{kg}^{0.75}$). Therefore, the ad libitum intakes for both trials provide starch levels considerably higher than any other thus far reported for a starch digestion study.

Starch Digestion in the Rumen. The results of this study do not indicate any difference in ruminal starch digestibility attributable to level of intake (Table 12). Thus, the digestibility for FSF is slightly higher (76%) than the RSF (71%), whereas the FDR (45%) is lower than the RDR (58%). The failure to find any consistent pattern in digestibility due to level is in agreement with other workers.

Karr et al. (1966) worked with steers and Tucker et al. (1968) with sheep, feeding equal quantities of rations containing 20, 40, 60, or 80% ground corn. The former found the highest ruminal digestibility

(73%) with the 40% ration and the lowest (62%) with the 80% ration, whereas the latter found the 80% ration to be most fully digested (79%) and the 40% ration the least digested (67%). Waldo et al. (1971) conducted two experiments involving similar rations (i.e., 20, 40, 60, and 80% ground corn) using heifers or bulls. In the first trial a high starch digestibility value of 90% was obtained for the 20% ration, whereas the 60% ration was only 76% digested. In the second trial starch digestion ranged from 50% for the 60% ration to 74% for the 80% ration. Other studies have also failed to show an effect on ruminal digestibility by level of starch intake with barley (Orskov et al. 1969; Topps, Kay et al. 1968) or hay, dairy cubes, and flaked corn (Nicholson and Sutton 1969).

Total Digestion of Starch. The identical starch digestion coefficients (99.4%) for ad libitum and restricted intakes in trial 1 indicate no difference due to level of starch intake (Table 9). Although there is some suggestion of a level effect in trial 2 with the FDR (Table 11), statistical analysis (Table 17) indicates that variation due to processing is approximately 3 times that for level and accounts for most of the total variation. When digestibilities were determined from the rectal contents at the time of slaughter, digestibilities were very similar within processing method, again suggesting no level effect.

Most experiments in which corn or barley were used in level studies produced digestion coefficients equal to or exceeding 98% (Karr et al. 1966; Nicholson and Sutton 1969; Orskov et al. 1969; Topps, Kay

et al. 1968; Waldo et al. 1971). Tucker et al. (1968) found increasing digestion (86 to 96%) of the starch in ground corn with increasing levels of corn in the ration (20, 40, 60, and 80%). In another trial involving the same animals, digestibilities reached or exceeded 98% for all levels.

The consistently high overall digestibility of grain starch under a wide variety of conditions and the relatively low ruminal digestibilities exhibited in this study, as well as in other studies, suggest that considerable quantities of starch may be degraded in the small intestine. This quantity reached 2500 g (Table 12) for the FDR with a minimum of 1100 g for the steam flaked treatments.

Little et al. (1968) suggested that starch digestion in the small intestine may be limiting when higher levels escape ruminal digestion such as might occur on high concentrate rations. To test this hypothesis, quantities of up to 600 g of purified cornstarch were infused twice daily into the abomasum and samples collected from the posterior ileum and feces. Their results indicate that less than 300 g per treatment were digested in the small intestine at the 600 g infusion level. Whether these data can be considered to be truly indicative of the digestive capacity of the small intestine for grain starch is questionable. The site and interval of infusion, the basal diet fed (alfalfa hay), the medium in which the starch was introduced, and the purified nature of the starch itself would appear to limit any extrapolations one might apply to starch from processed grain which has passed through the rumen and enters the small intestine as a portion of the total digesta.

The levels reported by these workers are somewhat lower than the post-abomasal digestion reported by Drennan et al. (1970) with milo (753 g) and considerably lower than the 1100 to 2500 g found in this study.

The results of this study suggest that at levels as high as 60 g of starch/ $W_{kg}^{0.75}$ the ability of the digestive tract to digest starch is not exceeded when steam processed flaked milo is fed. There are indications that this may not be the case with dry rolled milo.

Feed Efficiency as Related to Starch Digestion

The effects of level of intake or processing method on feed efficiency suggested by this study are inconclusive. Feed efficiency (feed/gain) in trial 1 favored the full-fed steers as it did for the dry rolled treatment (full-fed vs. restricted) in trial 2 (Table 10). However, the RSF had a higher efficiency than FSF, giving apparently contradictory results. The tendency toward greater efficiency for the ad libitum intakes can be explained on the basis of a greater intake above maintenance available for production. However, other factors apparently were involved with the steam flaked comparisons in trial 2.

The effects of grain processing on efficiency appear to be inconsistent with the digestibility data, as well as with other trials measuring the effects of processing on feed efficiency. No differences were found in dry matter or starch digestibility, and intakes were similar for RSF and RDR treatments; yet the RSF was higher ($P < 0.05$) than the RDR in rate of gain (0.88 vs. 0.71 kg/day) and in feed

efficiency (6.29 vs. 7.91 units of feed/unit of gain). In contrast, the FDR, which had a lower total starch digestion than the FSF (95 vs. 99%), showed no difference from the FSF in gain per day or feed efficiency (1.06 vs. 1.09 kg gain/day and 6.96 vs. 6.86 units of feed/unit of gain for the FDR and FSF, respectively). One possible explanation for these seemingly inconsistent results would be the tremendous variation among the relatively few animals within treatments. Also, there were periods during which there were observed considerable quantities of whole grain in the steam processed flaked ration, a factor which would tend to minimize differences between processing treatments.

In feedlot trials Hale, Cuitun et al. (1966) found feed efficiency to favor steam processed flaked milo over dry rolled grain. Similarly, Hale, Theurer et al. (1966) found an approximate 10% decrease in feed required per unit of gain with steam processed flaked milo vs. steam rolled milo. Newson et al. (1968) found a 15 to 20% improvement in feed efficiency with steam flaked sorghum grain as compared to dry rolled. In contrast, Garrett (1965) found no difference between grinding or steam rolling sorghum grain on efficiency. The apparent disagreement between the different trials may be related to the degree of steaming or flatness of flake or both.

Although the data from this study are inconsistent and apparently contradictory with respect to feed efficiency, other work suggests that the feed required to produce a given amount of gain can be altered by processing method.

Estimation of Starch as Affected
by the Indigestible Marker

Armstrong and Beever (1969) reviewed several papers dealing with the site of starch digestion in the ruminant. It was suggested that rations containing ground corn were less fully digested in the rumen than rations containing either barley or flaked corn. Other possible sources of variation mentioned were starch analysis techniques, species of animal used (sheep vs. cattle), and origin of starch (feed starch vs. protozoal starch). Drennan et al. (1970) discounted these factors as probable major sources of difference between the digestion of ground corn vs. barley or flaked corn in the rumen. These workers pointed out that where ground corn had been used, chromic oxide was administered as a powder or in a gelatin capsule to serve as an indigestible marker. On the other hand, other workers using flaked corn or barley had used chromic oxide impregnated on paper or polyethylene glycol (PEG) as a marker. It was suggested that chromic oxide given as a powder or in a gelatin capsule left the rumen faster than the digesta, thus lowering estimated digestion values. An examination of these papers, in addition to work published since then and observations stemming from this study, appear to support the claim of Drennan et al. (1970).

Five studies (Drennan et al. 1970, Karr et al. 1966, McNeill et al. 1971, Tucker et al. 1968, Waldo et al. 1971) in addition to the present study in which chromic oxide was administered as a powder or in capsule form show a general pattern of lower ruminal digestion of starch and greater variation within and between experiments (as compared to

studies in which some other form or type of marker was used). Forty ruminal starch digestion values reported in these studies (only treatments in which grain was the major starch source were included in this comparison) had a mean of 70% and a range of 42 to 91%. When the marker used was PEG, lignin, or chromic oxide impregnated on paper, ruminal starch digestion was generally higher and with less variation within and between experiments. Seven studies (Beever et al. 1970; Holmes et al. 1970; MacRae and Armstrong 1969b; Nicholson and Sutton 1969; Orskov et al. 1969; Topps, Kay, and Goodall 1968; Topps, Kay et al. 1968) reporting 32 ruminal starch digestion values had a mean of 93% and a range of 78 to 98%. Only one value fell below 86%. These figures, though not conclusive, indicate a greater uniformity for the second group, both within and between digestion studies.

Other studies have shown that chromic oxide powder leaves the rumen faster than the solid digesta. Johnson et al. (1964) found that chromic oxide passed out of the rumen faster than the bulk of the dry matter. However, when fed impregnated on paper, chromic oxide appeared to follow the dry matter more closely and gave digestibility values similar to those of lignin. Chromic oxide powder fed to sheep at a single feeding peaked more rapidly in the feces and then fell off faster than when given impregnated on paper. This is in agreement with the findings of Corbett et al. (1958) who measured the passage of chromic oxide through the duodenum at hourly intervals after administering a single dose of chromic oxide in the form of a gelatin capsule. It was found that the concentration in the digesta passing the duodenum

rose rapidly and reached a maximum about two hours later. After four hours a large part of the dose had already passed the duodenum. A similar quantity of chromic oxide administered in a plaster of paris mixture or impregnated on paper gave a slower, more sustained release.

To further test the validity of the ruminal starch digestibility values reported, an examination was made of dry matter (DM) or organic matter (OM) digestibilities since they must be equal to or greater than corresponding starch digestibilities in order to be theoretically possible. A summary of starch digestion, DM or OM digestion, and expected minimal DM digestion in the rumen is given in Table 13 for the above cited studies when this information was available and in Table 14 for the present study. For the studies involving chromic oxide impregnated paper, PEG, or lignin, only one DM value was lower than the minimal digestion expected. Where powder was used, Waldo et al. (1971) reported only one inconsistent value, although Drennan et al. (1970) and the present study showed no DM or OM digestibility values which would meet the expected minimum. The latter did, however, have one organic matter (RDR) value which approached this minimum.

Starch digestibility estimates were lower in three treatments (Table 14) when abomasal contents were used than when ruminal contents were used to estimate digestibility, giving a negative digestibility as the starch moved from the rumen to the abomasum. The negative digestion estimated for the RSF, however, was much less marked than for either of the dry rolled treatments. This fact and the positive digestibility for the FSF are possible indications that the chromic oxide

Table 13. Starch, dry matter (DM) and theoretical minimal dry matter (TDM) digestibilities in the rumen for previously published experiments.

	Percentage ruminal digestion		
	Starch	DM	TDM
Topps, Kay et al. (1968) ^a	95	53	44
	95	54	44
	96	52	47 ^f
	96	44	47 ^f
Topps, Kay, and Goodall (1968) ^a	98	59	47
	94	57	42
Nicholson and Sutton (1969) ^a	96	65	40
	95	60	47
	94	60	49
Orskov et al. (1969) ^b	94	47	45
	95	57	53
	88	59	48
	86	50	46
Drennan et al. (1970) ^c	89	71 ^e	56
	94	69 ^e	59
	90	61	56
	96	66	60
Drennan et al. (1970) ^d	56	3 ^e	35 ^f
	82	22 ^e	51 ^f
	78	16	49 ^f
	91	36	57 ^f
Waldo et al. (1971) ^d	90	33	17
	85	35	27
	76	38	35 ^f
	84	47	52 ^f
	59	36	9
	59	35	17
	50	30	20
	74	51	40

a. Chromic oxide paper used as the marker.

b. Polyethylene glycol used as the marker.

c. Lignin used as the marker.

d. Chromic oxide powder used as the marker.

e. Based on organic matter rather than dry matter.

f. Dry matter digestibility lower than theoretical minimum.

Table 14. Starch, dry matter (DM), organic matter (OM), and theoretical minimal dry matter (TDM) and organic matter (TOM) digestibilities in the rumen.

Treatment	Percentage ruminal digestion					
	Starch ^a	Starch ^b	DM ^b	TDM ^b	OM ^b	TOM ^b
Restricted steam flaked	75.8	71.1	10.1	47	24.6	49
Full-fed steam flaked	50.3	76.0	12.3	49	24.8	53
Restricted dry rolled	78.0	58.5	24.3	38	35.4	40
Full-fed dry rolled	65.5	46.3	-22.1	31	7.0	33

a. Estimated from ruminal contents.

b. Estimated from abomasal contents.

either adheres more closely to the steam processed flaked grain than to the dry rolled grain, or that the rate of digestion is faster for the steam processed flaked grain, or that the rate of passage of the steam processed flaked grain is faster, or some combination of the above.

The data on dry matter and starch content of each segment of the tract expressed as percentage of total tract contents or daily intake (Tables 23 and 24) suggest a rate of passage or rate of digestion difference between steam processed flaked and dry rolled grain in the rumen and abomasum. However, it is difficult to determine from these data what is actually occurring. Nevertheless, the negative digestibilities mentioned above and the inconsistent starch and DM digestibility estimates indicate that the chromic oxide passed through both the rumen and abomasum ahead of the digesta. This is in agreement with the findings of Corbett et al. (1958).

Dry matter digestibility did not appear to correlate with starch digestibility and was a negative 22.1% for the FDR (Table 14). Neither was there any apparent correlation between starch and organic matter digestibilities, although all of the latter values were positive. Taking samples repeatedly over a period of time through a fistula might reduce errors, although Drennan et al. (1970) found impossible results whether collecting samples through a duodenal fistula or collecting a single sample after slaughter.

Table 12 shows a much more uniform pattern of starch digestion in the intestines. There were some inconsistent values for the dry rolled treatments, although these were fewer and less marked than those

found in the rumen or abomasum. In all treatments there was a definite trend toward an increase in total starch digestibility as the digesta moved through the post-abomasal tract. Johnson et al. (1964) suggested that the chromic oxide powder leaving the anterior portion of the digestive tract builds up in the posterior portion. If so, this would tend toward the over-estimation of starch digestion in the lower gut. Although this finding could not be tested by these results, total digestion coefficients estimated from chromic oxide at the time of slaughter were similar to those for total collection (Tables 11 and 12). These results suggest that more credible results might be obtained by using chromic oxide powder for estimating digestion posterior to the abomasum than anterior to it.

Conclusions

An examination of the effects of intake level on starch digestibility revealed little or no digestibility differences due to level in either the rumen or the total digestive tract. At levels of up to 60 g of starch/ $W_{kg}^{0.75}$ the capacity of the tract of a 400 kg steer did not appear to be exceeded (when fed steam flaked milo) with approximately 99% of the starch being digested. There was some evidence suggesting that the digestion of starch in milo processed by dry rolling might be reduced by high levels of ingested starch.

The digestibility of starch in milo can be significantly altered by different processing methods. Steam processing and flaking milo increases digestibility ($P < 0.05$) over dry rolling in the total

tract. A similar trend was found in the rumen but was not significant ($P > 0.05$).

Differences between total and ruminal digestion coefficients suggest that considerable quantities of starch (up to 2500 g daily) are digested in the small intestine. Data regarding starch digestibility and feed efficiency are apparently contradictory. However, this study and other work indicates that those processing methods which give the highest ruminal digestion coefficients are also the methods associated with producing the greatest gain per unit of feed.

The use of chromic oxide powder for estimating ruminal digestion gave impossible results and appears to be unsuitable for estimating absolute starch digestion, although digestibility values may be relative. The data suggest that estimation of post-abomasal digestibility would be feasible with chromic oxide powder.

SUMMARY

Laboratory studies were conducted to compare glucose or starch recovery from a variety of substrates which had been hydrolyzed in dilute HCl (0.761 N) or with an amyloglucosidase (Agidex). A modification of the Agidex enzyme hydrolysis procedure, based on a volumetric rather than a gravimetric determination, was developed, and an apparatus for refluxing samples was designed. Purified starch samples, milo grain, and feces (containing approximately 40% starch) showed no difference between the two hydrolysis methods. Cotton, alfalfa hay, and feces (containing approximately 3% starch) had higher ($P < 0.01$) recoveries from acid than from enzyme hydrolysis. Differences were also found in alfalfa-milo mixtures which contained over 67% hay and in samples taken from the small intestine, colon, or feces of two steers fed an 80% milo ration, but no differences were found in alfalfa-milo mixtures containing less than 50% alfalfa or in abomasal or feed samples corresponding to the above two steers. Coefficients of variation ranged from 0.0 to 19.2%, generally being lowest for substrates with a high starch content and highest for low starch substrates.

It was concluded that starch content would be over-estimated when using acid hydrolysis if the substrate had a high cellulose content as compared to starch. For substrates high in starch, acid hydrolysis could be utilized with an accuracy comparable to enzyme hydrolysis, and acid hydrolysis could be used to analyze the samples

from a digestion trial involving a high concentrate diet with only minimal errors arising from possible cellulose hydrolysis.

Analysis of duplicate fecal samples which had been either freeze dried or oven dried showed no differences in starch recovery when steam flaked milo had been fed, but feces from steers fed dry rolled milo had lower ($P < 0.05$) recoveries when oven dried than when freeze dried.

Two digestion trials were conducted to study the effects of starch intake level (full-fed vs. restricted) on starch digestion. The second of the two trials was also designed to study the effects of dry rolling or steam processing and flaking milo on starch digestion, and the steers in this trial were slaughtered upon reaching 410 kg. The steers were sacrificed at three hours post-prandial and digesta collected to study the site and degree of starch digestion in the gut.

There were no apparent effects due to intake level in either trial. Steam processing and flaking increased ($P < 0.05$) starch digestion in the total tract (98.5 to 99.6% vs. 94.9 to 97.4% for dry rolled). Steam processing and flaking also tended to increase ruminal digestion. Digestion was higher ($P < 0.05$) for the restricted steam flaked than for the full-fed dry rolled treatment in the total tract. Dry matter digestibility was similar for all treatments in both trials.

Feed efficiency favored the full-fed treatment in trial 1 and was approximately equal for the full-fed treatments in trial 2. Effects of steam processing and flaking varied with intake, with the restricted steam flaked treatment being most efficient and greater ($P < 0.05$) than for the restricted dry rolled treatment.

Starch and dry matter digestibilities in the rumen were inconsistent, with dry matter digestibility estimates being too low to account for the starch digestion. There was evidence that the chromic oxide powder used for the indigestible marker passed through the rumen and abomasum ahead of the digesta

APPENDIX

ANALYSES OF VARIANCE AND SUPPLEMENTARY DATA

Table 15. Analysis of variance of the effects of hydrolysis method on the estimation of feed and fecal starch content and starch digestibility.

Source of variation	df ^a	M e a n s q u a r e ^b		
		Feed	Feces	Digestibility
Total	47			-
	23	-	-	
Method	1	13.0	80.6 ^c	5.0 ^c
Steer	11	4.7	180.9 ^c	13.9 ^c
M x S	11	3.3	1.1	0.1
Replication	24	0.7	0.3	-

a. df = degrees of freedom; also in Tables 16, 17, 18, and 21.

b. Interaction effects were tested against replication; method and steer effects against interaction.

c. (P < 0.01).

Table 16. Analysis of variance for starch recovery from freeze dried and oven dried fecal samples from trials 1 and 2.

Source of variation	df	M e a n s q u a r e ^a					
		Trial 1		Trial 2			
		Restricted	Full-fed	RSF ^b	FSF	RDR	FDR
Total	35			-	-	-	-
	17	-	-				
Method	1	111	85	4	30	875 ^c	1006 ^c
Steer	2	330	279 ^c	586 ^c	6272 ^c	33036 ^d	18499 ^d
S x M	2	101 ^d	12	10	179 ^d	20	32
Replication	30	4	7	-	-	-	-
	12	-	-	5	17	43	37

a. Main effects tested against interaction.

b. RSF = restricted steam processed flaked; FSF = full-fed steam processed flaked; RDR = restricted dry rolled; FDR = full-fed dry rolled.

c. (P < 0.05).

d. (P < 0.01).

Table 17. Analysis of variance for fecal dry matter (DM) and starch content and digestibility for trials 1 and 2.

Trial	Source of variation	df	M e a n s q u a r e ^a			
			Fecal starch g	Fecal starch %	DM digestion %	Starch digestion %
1	Total	11				
	Treatment	1	53.7	4.4	0.5	0.01
	Error	10	49.5	9.4	2.4	0.03
2	Total	11				
	Level	1	29,126	67.4	18.2	8.58
	Processing	1	48,336 ^b	215.5 ^b	4.6	26.31 ^b
	L x P	1	8,332	20.7	4.6	1.98
	Error	8	8,974	23.3	18.2	4.18

a. Main and interaction effects tested against experimental error.

b. ($P < 0.05$).

Table 18. Analysis of variance for average daily gain and feed efficiency for trials 1 and 2.

Trial	Source of variation	df	Mean square ^a	
			Gain/day	Feed/gain
1	Total	10		
	Treatment	1	1.47 ^b	12.95
	Error	9	0.10	7.40
2	Total	11		
	Level	1	0.23 ^c	0.11
	Processing	1	0.03	2.21 ^d
	L x P	1	0.01	1.74
	Error	8	0.03	0.60

a. Treatments in trial 1 and main and interaction effects in trial 2 tested against experimental error.

b. ($P < 0.01$).

c. ($P < 0.05$).

d. ($P < 0.10$).

Table 19. Daily feed and fecal dry matter and percentage starch for individual steers in trial 1.

Treatment	Steer no.	Feed dry matter	Fecal dry matter	Feed starch	Fecal starch
		g	g	%	%
Restricted	030	6866	1950	53.4	0.89
	835	6617	2038	57.0	1.38
	039	6706	1938	61.3	1.18
	726	6657	1984	54.2	0.99
	144	6009	1821	62.1	1.38
	746	5664	1660	57.4	0.88
Full-fed	058	8411	2523	62.6	1.48
	087	7400	2250	59.6	1.38
	043	7189	1812	54.7	1.08
	141	8544	2486	58.4	1.78
	136	9568	2851	51.8	0.99
	056	8912	2727	56.4	0.79

Table 20. Chromic oxide recovery and daily feed and fecal dry matter and percentage starch for individual steers in trial 2.

Treatment	Steer no.	Feed dry matter	Fecal dry matter	Feed starch	Fecal starch	Cr ₂ O ₃ Recovery
		g	g	%	%	%
Restricted steam processed flaked	133	4988	831	66.6	1.10	86.6
	137	4644	1014	67.4	1.93	97.0
	198	4644	792	64.2	1.67	86.6
Full-fed steam processed flaked	148	6067	1608	67.4	6.45	92.0
	176	7580	1248	66.1	1.19	84.7
	181	7620	1791	66.5	3.40	90.1
Restricted dry rolled	140	5274	1044	64.9	5.48	102.1
	151	4692	827	64.5	1.39	94.7
	164	5040	1282	67.7	15.28	93.3
Full-fed dry rolled	171	5878	1105	67.4	7.91	83.4
	194	7935	2186	65.5	18.35	103.3
	169	6290	1271	68.1	18.08	88.1

Table 21. Analysis of variance for starch digestion and recovery in different segments of the digestive tract and total dry matter (DM) digestion.

	Mean squares for sources of variation ^a				
	Total	Level	Processing	L x P	Error
df:	11	1	1	1	8
Starch presented					
SI ^b		1,136,521	3,126,302	760,537	1,374,807
Colon		54,136	38,081	533	17,831
Starch disappeared					
Rumen		967,872	4,645,096 ^c	1,349,381	961,202
SI		694,564	2,474,300	720,790	1,128,605
Starch digestion (% of presented)					
Rumen		36.0	1373.9	228.8	524.6
SI		96.1	0.1	152.7	57.8
Starch digestion (% of total)					
Rumen		36.0	1373.9	228.8	524.6
SI		9.0	1076.2	226.6	437.0
Total tract		0.5	29.6 ^d	0.8	5.0
Total DM digestion		0.7	2.4	0.2	9.2

a. Main and interaction effects tested against experimental error.

b. SI = small intestine.

c. (P < 0.01).

d. (P < 0.05).

Table 22. Dry matter (DM) contents and percentage starch (S) and chromic oxide (CO) in the feed (daily intake) and in the digesta three hours after feeding for individual steers (dry basis).

Steer no.	Item	Feed	Rumen	Abomasum	Small intestine			Colon	Rectum
					1	2	3		
133 ^a	DM, g	6454	4994	250	150	73	100	331	54
	S, %	66.6	21.0	23.8	1.0	1.5	4.8	4.4	3.4
	CO, %	0.200	0.200	0.225	0.250	0.380	0.525	0.985	1.070
137 ^a	DM, g	6500	5039	213	109	150	114	377	50
	S, %	67.4	26.7	26.9	7.6	8.2	12.8	13.0	9.3
	CO, %	0.215	0.330	0.210	0.280	0.425	0.880	0.965	1.130
198 ^a	DM, g	6200	3904	77	82	141	100	322	104
	S, %	64.2	26.2	18.3	6.5	7.4	3.6	2.8	2.6
	CO, %	0.200	0.350	0.255	0.190	0.270	0.960	1.350	1.045
148 ^b	DM, g	7941	4222	50	154	91	254	381	59
	S, %	67.4	33.5	15.7	5.5	10.9	14.6	10.9	6.8
	CO, %	0.170	0.200	0.375	0.165	0.305	0.525	0.790	0.800
176 ^b	DM, g	8636	5266	309	91	118	64	182	- ^c
	S, %	66.1	23.6	18.5	6.4	6.4	7.7	5.8	1.2
	CO, %	0.210	0.175	0.215	0.390	0.540	0.805	0.940	1.080
181 ^b	DM, g	8685	5280	272	177	232	204	518	23
	S, %	66.5	27.4	23.4	10.6	13.8	15.1	10.8	7.0
	CO, %	0.180	0.090	0.150	0.145	0.265	0.380	0.430	0.780

Table 22.--Continued

Steer no.	Item	Feed	Rumen	Abomasum	Small intestine			Colon	Rectum
					1	2	3		
140 ^d	DM, g	6300	8808	263	109	132	73	227	27
	S, %	64.9	17.7	26.2	7.8	6.6	11.8	10.2	8.6
	CO, %	0.190	0.545	0.495	0.275	0.575	0.835	1.010	0.945
151 ^d	DM, g	6200	5039	345	109	150	127	458	77
	S, %	64.5	22.6	34.8	8.3	14.0	14.3	9.8	9.7
	CO, %	0.200	0.320	0.340	0.205	0.550	0.790	0.930	1.075
164 ^d	DM, g	6400	2860	68	177	250	200	282	41
	S, %	67.7	34.2	44.0	26.0	22.6	41.9	23.9	24.4
	CO, %	0.195	0.275	0.150	0.175	0.480	0.435	0.915	0.675
171 ^e	DM, g	6888	6038	390	136	145	114	282	54
	S, %	67.4	25.8	21.8	8.9	11.2	11.5	10.3	11.6
	CO, %	0.195	0.225	0.165	0.195	0.380	0.825	0.970	0.900
194 ^e	DM, g	8362	4630	291	263	154	282	291	- ^c
	S, %	65.5	22.0	35.6	8.6	12.2	22.9	22.8	9.9
	CO, %	0.200	0.200	0.135	0.230	0.305	0.460	0.570	0.810
169 ^e	DM, g	8246	5312	245	45	136	86	468	- ^c
	S, %	68.1	30.2	35.8	26.3	10.7	24.3	19.1	17.7
	CO, %	0.195	0.185	0.195	0.150	0.400	0.610	0.670	0.850

a. Restricted steam processed flaked.

b. Full-fed steam processed flaked.

c. Estimated from feces.

d. Restricted dry rolled.

e. Full-fed dry rolled.

Table 23. Starch in different segments of the digestive tract expressed as percentage of total tract starch content or percentage of daily starch intake (dry basis).

Segment	% of total content				% of intake			
	RSF ^a	FSF	RDR	FDR	RSF	FSF	RDR	FDR
Rumen	92.9	91.3	84.5	85.7	26.8	24.5	29.3	27.3
Abomasum	3.4	2.9	5.1	5.9	1.0	0.8	1.7	1.8
Small intestine								
1st part	0.4	0.7	1.6	1.0	0.1	0.2	0.5	0.3
2nd part	0.6	1.0	2.1	1.1	0.2	0.3	0.7	0.3
3rd part	0.6	1.6	2.8	2.3	0.2	0.4	0.9	0.6
Colon	1.8	2.3	2.4	3.9	0.6	0.7	1.1	1.1
Rectum	0.3	0.2	0.3	0.3	0.1	0.1	0.2	0.1
Total	-	-	-	-	28.9	26.9	34.4	31.5

a. RSF = restricted steam flaked; FSF = full-fed steam flaked; RDR = restricted dry rolled; FDR = full-fed dry rolled.

Table 24. Dry matter in different segments of the digestive tract expressed as percentage of total tract dry matter content or percentage of daily dry matter intake.

Segment	% of total content				% of intake			
	RSF ^a	FSF	RDR	FDR	RSF	FSF	RDR	FDR
Rumen	83.2	82.3	81.7	82.3	72.6	58.3	88.6	69.1
Abomasum	3.1	3.4	3.3	4.7	2.8	2.4	3.6	4.1
Small intestine								
1st part	2.0	2.3	2.5	2.3	1.8	1.6	2.1	1.9
2nd part	2.2	2.4	3.4	2.3	1.9	1.7	2.8	1.8
3rd part	1.9	3.0	2.7	2.6	1.8	2.1	2.1	2.0
Colon	6.2	6.0	5.7	5.4	5.4	4.3	5.1	4.4
Rectum	1.3	0.7	0.8	0.8	1.1	0.5	0.7	0.8

a. RSF = restricted steam flaked; FSF = full-fed steam flaked; RDR = restricted dry rolled; FDR = full-fed dry rolled.

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