The rate and extent of ruminal digestion of cereal starch depends on a number of complex interactions among rumen microorganisms, grain kernel structure, and the method of grain processing. The pericarp of cereal grains is the foremost barrier to microbial digestion and its destruction via processing (i.e., grinding, rolling) or mastication is essential for efficient starch utilization in ruminants. Upon exposure of the endosperm, rumen microbes readily digest endosperm cell walls, but their ability to digest the protein matrix surrounding starch granules depends on the type of cereal grain. Corn and sorghum contain dense protein matrices within the vitreous endosperm that surround starch granules and limit the access of amylolytic microbes to starch granules. In contrast, the protein matrices of wheat and barley are more diffuse and do not impede the access of rumen microbes to granules. More severe processing methods such as steam-flaking disrupt the protein matrix of corn and sorghum and increase the rumen availability of starch within the vitreous endosperm. In contrast, starch from barley and wheat is readily digested with less extensive processing. Once free of the protein matrix, starch granules from all grains are digested readily by rumen microbes, which reflects the myriad of amylases produced by these diverse strains of microbes that can digest starch. However, the “inside-out” method of microbial digestion is more prevalent for starch granules from corn and sorghum than for starch granules from wheat or barley. This strategic difference may reflect differences in surface lipids and or proteins among starch granule types. Steam-flaking effectively disrupts barriers to microbial starch digestion; the degree of gelatinisation is highly correlated with the destruction of the protein matrix. Post flaking reductions in ruminal starch degradation likely reflect reformation of starch-protein complexes rather than starch retrogradation. An increase in extent of ruminal digestion of starch often results in improved growth and feed efficiency because by-pass starch often results in a decline in total tract starch digestibility. Future strategies aimed at enhancing starch digestion in ruminants must include a deeper appreciation for the microbial processes involved in cereal grain digestion.

CEREAL GRAIN STRUCTURE

The outer surface of cereal grains consists of a thick, multilayered pericarp that protects the inner germ and endosperm from microbial onslaught (Figure 1.1). High concentrations of lignin are deposited during secondary thickening of the pericarp and wax esters often are associated with the surface as a further deterrent to microbial invasion and water uptake. In addition to the pericarp that accounts for 3 to 8% of the total kernel weight, barley and oats have a fibrous hull or husk that may amount to up to 25% of total kernel weight (Evers et al., 1999). Chemically, the pericarp and husk are composed of about 90% fiber and, due to their highly lignified nature, their digestion is likely limited to less than 40% (Van Barneveld, 1999). Ruminal digestibility of the hull and pericarp likely is impaired further by the low ruminal pH (i.e., < 6.2) commonly associated with high grain diets. The endosperm consists of two distinct tissues, starchy endosperm (60 to 90% of kernel weight) and aleurone (2 to 7% of kernel weight); the aleurone consists of 1 to 3 layers depending on the type and genetics of the cereal grain (Kent, 1983). Endosperm cell walls of wheat and corn are composed primarily of arabinoxylans, whereas those in oats and barley are predominately composed of (1→3, 1→4) - β-glucans. Endosperm cell walls are largely devoid of lignin and, given the high arabinoxylanase and β-glucanase activity of rumen microbes (McAllister et al., 2001), are unlikely to be a significant barrier to starch digestion. Endosperm cell walls surround starch granules embedded within a protein matrix (Figure 1.2). The endosperm has two distinctly different regions in both corn and sorghum grain. In the vitreous endosperm region, starch granules are densely compacted within a protein matrix, whereas in the floury endosperm region, starch granules are only loosely associated with the protein matrix. In corn, starch granules are so tightly associated with the protein that the granules frequently fracture upon grinding; this exposes the concentric rings that are formed during the deposition of starch in the
granule during kernel development (Figure 1.3). In barley and wheat, the protein matrix is loosely associated with starch granules throughout the entire endosperm (Figure 1.4).

**Figure 1.** Scanning electron microscopy of (1) the pericarp (P) of corn (2) endosperm cells in wheat (3) horny endosperm of corn with starch granules (s) and (4) the endosperm of wheat with starch granules (s) Bars = 10 µm. (From McAllister and Cheng, 1996).
The principal carbohydrate in the endosperm is starch. Starch is composed of linear and branched glucose polymers called amylose and amylopectin (French, 1973). The glucose units in amylose are linked by $\alpha$-(1-4) bonds; in amylopectin, additional $\alpha$-(1-6) linkages are present which result in branch points. Starch is deposited in granules within the endosperm. Depending on the grain type, granules vary widely in their shape (round, lenticular, polygonal), size, size distribution (uni- or bi-modal), and association either as individual (simple) or granule clusters (compound) (Table 1, Tester et al., 2004). Starch granules are formed by the deposition of growth rings that consist of alternating semi-crystalline and amorphous layers. These rings extend from the centre of the granule (hilum) towards the surface of the granules in a manner analogous to the layers of an onion (Figure 1.4). The amorphous regions in starch granules are thought to represent the amylopectin branch points whereas the crystalline area represents the more compact double-helical structure of amylopectin. Starches are defined as waxy when the ratio of amylose to amylopectin is low $< 15\%$, normal when the amylose makes up 16 to 35% of the granule, and high-amylose when amylose content exceeds 36% of the granule. Although several studies have shown that the amylose/amylopectin ratio is negatively correlated with starch digestion in non-ruminants (Svihus et al., 2005), the impact of the ratio on starch degradation by ruminal microorganisms is less certain.

**Table 1.** Characteristics of starch granules from different cereals

<table>
<thead>
<tr>
<th>Cereal source</th>
<th>Distribution</th>
<th>Shape</th>
<th>Size, $\mu$m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>Bimodal</td>
<td>Lenticular (A-type)</td>
<td>15 – 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spherical (B-type)</td>
<td>2 – 5</td>
</tr>
<tr>
<td>Corn</td>
<td>Unimodal</td>
<td>Spherical/polyhedral</td>
<td>2 – 30</td>
</tr>
<tr>
<td>High amylose corn</td>
<td>Unimodal</td>
<td>Irregular</td>
<td>2 – 30</td>
</tr>
<tr>
<td>Millet</td>
<td>Unimodal</td>
<td>Polyhedral</td>
<td>4 – 12</td>
</tr>
<tr>
<td>Oat</td>
<td>Unimodal</td>
<td>Polyhedral</td>
<td>3 – 10 (single)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>80 (compound)</td>
</tr>
<tr>
<td>Rye</td>
<td>Bimodal</td>
<td>Lenticular (A-type)</td>
<td>10 – 40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spherical (B-type)</td>
<td>5 – 10</td>
</tr>
<tr>
<td>Sorghum</td>
<td>Unimodal</td>
<td>Spherical</td>
<td>5 – 20</td>
</tr>
<tr>
<td>Triticale</td>
<td>Unimodal</td>
<td>Spherical</td>
<td>1 – 30</td>
</tr>
<tr>
<td>Wheat</td>
<td>Bimodal</td>
<td>Lenticular (A-type)</td>
<td>15 – 35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spherical (B-type)</td>
<td>2 – 10</td>
</tr>
</tbody>
</table>

1 Adapted from Tester et al. (2004).

**Starch Granule Digestion by Rumen Microorganisms**

As a result of their numerical predominance and metabolic diversity, ruminal bacteria are likely responsible for the majority of the starch digestion in the rumen. *Streptococcus bovis, Ruminobacter amylophilus, Prevotella ruminicola, Butyribrio fibrisolvens, Succinimonas amylolytica* and *Selenomonas ruminantium* are the principal starch-digesting bacteria in the rumen (Cotta, 1988). Recent work using molecular techniques has suggested that less than 10% of the bacteria in the rumen lend themselves to culture under anaerobic conditions in the laboratory (McAllister et al., 2006). This raises the possibility that many amylolytic bacterial species within the rumen may remain to be identified and characterized. Although starch granule type can have considerable influence on the ability of isolated amylolytic enzymes to digest starch (Zhang et al., 2006), this variation in digestion is less marked when isolated starch granules are subject to digestion by a mixed rumen microbial population (McAllister et al., 1993a; Fondevila and Dehority, 2001).

This likely reflects the wide diversity of amylases produced by rumen microorganisms as well as the formation of complex microbial consortia that frequently are observed on the surface of starch granules (Figure 2A). This microbial consortium can more readily produce the array of enzymes required to overcome additional digestive barriers that exist on the surface of starch granules such as lipids and proteins.
(Tester et al., 2004). Microbial digestion of starch granules from wheat and barley radiates from a central point of microbial attachment on the surface of the granule (Figure 2B). In contrast, with corn starch granules amylolytic bacteria tunnel into the interior of corn starch granules (Figure 2C) such that corn starch granules are digested from the inside out. As a consequence, as digestion nears completion, the granule interior often is hollow with only the outer surface layer remaining (Figure 2D). Although differences in the microbial approach to digestion of different types of starch granules do exist, their overall impact on rate and extent of starch digestion pales in comparison to the influence of more recalcitrant barriers to starch digestion such as the protein matrix in some grains and the pericarp and husk.

Both Holotrich and Entodiniomorphid protozoa are capable of degrading starch; protozoa may be responsible for as much as 50% of the starch digestion in the rumen (Jouany and Ushida, 1999). Protozoa readily engulf starch granules at a rate inversely related to the size of the starch granule (Figure 3A). Consequently, the engulfment of starch granules by Entodinium exiguum is more rapid for the small rice starch granules (diameter 3-8 \( \mu \text{m} \)) than for the larger corn starch granules (with diameter 9-30 \( \mu \text{m} \); Fondevila and Dehority, 2001). To date, no studies have determined if differences in granule composition alter the ability of protozoa to utilize starch.

Perhaps the most significant impact of protozoa on cereal grain digestion is their ability to modulate pH (Ushida et al., 1991) as a result of their capacity to sequester starch granules intracellularly and their ability to be predatory toward amylolytic bacteria (Nagaraja et al., 1992). Engulfed starch granules may require up to 36 h to be completely metabolized by protozoa (Coleman, 1986). Protozoal numbers typical increase when grain is included in forage-based diets (Hristov et al., 2001) and their number also may be sensitive to the type of grain fed or if mixed grains are included in the diet (Mendoza et al., 1999). Inclusion of very high concentrations of grain in the diet (i.e., >90%) may cause the diversity and number of protozoa to decline, a factor that may exacerbate a low ruminal pH and increase the risk of acidosis in cattle fed these types of diets. A decline in protozoa during the first eating bout of a high grain diet may make cattle more susceptible to acidosis during the second eating bout.

Although ruminal fungi are often considered only in relation to the digestion of recalcitrant plant cell walls, their contribution to rumen biomass increases when grains are included in the diet (Faichney et al., 1997). Our laboratory conducted studies that showed three species of ruminal fungi, Orpinomyces joyonii, Neocallimastix patriciarum and Piromyces communis digested starch in corn more than in wheat and barley (McAllister et al., 1993b). The rhizoids in ruminal fungi are capable of penetrating directly through the protein matrix in corn, enabling complete digestion of encased starch granules (Figure 3B). Although ruminal fungi are not a major contributor to ruminal starch digestion, many fungal species exhibit amylase activity and logically lead one to conclude they will digest starch under some circumstances. Their characteristic ability to penetrate through recalcitrant barriers may make their role more prevalent for digestion of more vitreous rather than flourier endosperm grains.
Figure 2. Scanning electron microscopy of (A) formation of a microbial biofilm on the surface of a wheat starch granule (Bar = 3 µm); (B) formation of concentric digestive rings on the surface of a wheat starch granule (Bar = 10 µm); (C) microbial biofilm on the surface of a corn starch granule, notice absence of rings observed in (B) (Bar = 1 µm) and (D) hollow corn starch granules after microbial digestion (Bar = 5 µm); (Adapted from McAllister et al., 1990a and 1990b).
Enzymology of Starch Digestion

Several enzymes are involved in the digestion of starch (Table 2). Although there is a plethora of information on the ruminal enzymes involved in the digestion of plant cell walls, only a handful of studies have examined the nature of ruminal amylases. The majority of these have focused on alpha-amylases from \textit{S. bovis} (Clark et al., 1992; Cotta and Whitehead, 1993; Satoh et al., 1993) with only a single report of an alpha-amylase from \textit{B. fibrisolvens} (Rumbak et al., 1991). Studies to isolate and identify amylases capable of hydrolyzing the \(\alpha-(1-6)\) linkages in amylopectin have not been conducted, but given that free starch granules are rapidly hydrolyzed in rumen fluid (Cone, 1991), such amylases presumably do not represent a rate limiting step to microbial starch digestion in the rumen.

Table 2. Enzymes involved in the hydrolysis of starch\(^1\)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Bond specificity</th>
<th>End product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylase</td>
<td>(\alpha-(1-4))-glucosyl</td>
<td>Glucose-1-phosphate</td>
</tr>
<tr>
<td>Alpha-amylase</td>
<td>(\alpha-(1-4))-glucosyl</td>
<td>Linear and branched oligosaccharides</td>
</tr>
<tr>
<td>Beta-amylase</td>
<td>(\alpha-(1-4))-glucosyl</td>
<td>Maltose and limit dextrins</td>
</tr>
<tr>
<td>Amyloglucosidase</td>
<td>(\alpha-(1-4))-glucosyl and (\alpha-(1-6))-glucosyl</td>
<td>Glucose</td>
</tr>
<tr>
<td>Isoamylase</td>
<td>(\alpha-(1-6))-glucosyl</td>
<td>Linear (\alpha-(1-4))-glucan chains</td>
</tr>
<tr>
<td>Pullulanase</td>
<td>(\alpha-(1-6))-glucosyl</td>
<td>Linear (\alpha-(1-4))-glucan chains</td>
</tr>
</tbody>
</table>

\(^1\)Adapted from Tester et al., 2004.
Figure 4. Scanning electron microscopy of (A) Starch granules embedded in the protein matrix of corn. Note that bacteria are preferentially colonizing and forming digestive pits on the surface of starch granules (Bar = 7.5 µm); (B) Protein matrix showing the locations previously occupied by starch granules (Bar = 7.5 µm); (C) Vitreous endosperm cells from corn. Note that the majority of the starch granules have been digested from the protein matrix (Bar = 75 µm); (D) Corn endosperm cell in which all of the starch granules have been digested and only the protein matrix and the endosperm cell wall remains (Bar = 10 µm). (A), (B), (C) Wang and McAllister, unpublished data; (D) From McAllister et al., 1990a.

Role of the Protein Matrix in Starch Digestion

For those cereal grains that are commonly fed to cattle, the nature of the protein matrix that surrounds starch granules has a far greater impact on the rate and extent of starch digestion than the properties of the starch itself (McAllister and Cheng, 1996). The yellow dent corn typically fed to cattle in North America arose as a result of crossing flint and floury genotypes. Flint corn contains high concentrations of vitreous endosperm and is less rapidly digested in the rumen than corn that contains higher concentrations of floury endosperm, based on in situ measurements (Philippeau and Michalet-Doreau, 1997). Rumen bacteria preferentially colonize exposed starch granules that are embedded within the vitreous protein matrix (Figure 4A). As digestion proceeds, they hydrolyze the starch granules, tunnelling into the interior of the endosperm cells but leaving the protein matrix intact (Figure 4B) and the shape of the endosperm cell readily discernable (Figure 4C). With prolonged exposure to
rumen bacteria, all of the starch granules are digested and only the surrounding protein matrix and endosperm cell wall remain (Figure 4D). Properties of the protein matrix also may be related to the type or location of proteins, given that starch digestibility is negatively correlated with zein proteins but positively correlated with glutelins (Philippeau et al., 2000). Opaque 2, a corn genotype selected for its low zein concentration, exhibits a more rapid rate of ruminal starch digestion and a higher total tract starch digestibility than its isogenic counterpart when both genotypes are dry rolled (Ladely et al., 1995). Similar relationships between endosperm vitreousness and starch digestion also have been identified for sorghum (Kotarski et al., 1992). Many of the differences in digestion between more slowly fermented grains (e.g., corn, sorghum) and those that are more rapidly fermented (e.g., wheat, barley) can be attributed to differences in the properties of the protein matrix between these grains (McAllister et al., 1990b).

Impact of Grain Processing on Starch Digestion

Processing of cereal grains, whether by grinding, rolling, pelleting, tempering (i.e., addition of water prior to rolling), steam rolling (i.e., exposure to steam prior to rolling) or steam flaking (i.e., longer duration of exposure and higher grain temperature), breaks down recalcitrant barriers such as the hull, pericarp and protein matrix and allows microbes access to the starch harbored within endosperm cells. Furthermore, these processes reduce the particle size of the grain, increasing the surface area available for microbial attachment and colonization; combined, these actions increase the rate and extent of starch digestion (McAllister et al., 1994). Steam rolling and steam flaking expose grain to moisture and heat. At temperatures above 80ºC, a portion of the starch in grain is gelatinized. Differential scanning calorimetry can be used to measure the extent of starch gelatinization and often is used to assess the effectiveness of steam conditioning. Steam conditioning and flaking gelatinizes less than half the starch (i.e., < 20%); extent of gelatinization increases with exposure to a higher temperature for a longer period of time (Svihus et al., 2005). Exposure of grain to temperatures above 120ºC, such as those encountered during autoclaving, eliminates any differences in the rate and extent of microbial digestion of starch between corn and wheat (McAllister et al., 1991).

The performance of feedlot cattle fed barley, which has a readily digestible protein matrix, was not improved as a result of steam processing as opposed to dry-rolling (Engstrom et al., 1992). In contrast, in corn, steam flaking as opposed to dry-rolling eliminated the adverse affects of increasing endosperm vitreousness on total tract starch digestibility in steers (Corona et al., 2006). This observation indicates that the benefits of steam flaking on digestion of corn are related not only to gelatinization of the starch, but also to enhanced destruction of the protein matrix. Recent work with high-moisture corn hybrids that differed in degree of vitreous endosperm supports this hypothesis. Szasz et al. (2007) found that ruminal, intestinal and total tract digestibility of starch in high-moisture corn were at least equal or in some instances higher for a vitreous hybrid than for a floury hybrid. In that experiment, all hybrids were rolled without steam conditioning, thus gelatinization of the starch should not be a significant factor in determining the efficiency of starch digestion. Surprisingly, after rolling the high moisture corns, the particle size was smaller for the vitreous than the floury hybrid, exposing 15.8% more surface area for microbial colonization and enzymatic digestion (Szasz et al., 2007). We also have found that a large degree of the variation in ruminal starch digestion among barley varieties can be attributed to the degree to which the kernels shatter during processing and to the size of the processed particles (McAllister et al., unpublished). Consequently, a processing method × particle size interaction may be a major factor that determines the relative efficiency of starch utilization among varieties within a cereal grain species and between cereal grain species. With this point in mind, it seems logical that defining the factors within and among grain types that are responsible for the post-processing variation in particle size would be a prudent means of predicting the value of a processing method and of different batches of cereal grains for ruminants.

Post-processing Changes in Starch Digestion

Gelatinized starch can undergo a process known as retrogradation whereby starch molecules reassociate and form tightly packed structures stabilized by
hydrogen bonding. Retrograde starch resists digestion by amylases. This phenomenon is primarily associated with amylose, because retrogradation of amylopectin takes weeks or months to develop (Lii et al., 2004). Consequently, cereal grains that contain starch granules with a high amylose content (e.g., high amylose corn) are more subject to retrogradation than those that contain starch granules with a low amylose content. Storage of grain at higher temperatures can dramatically accelerate the rate of amylose retrogradation (Jouppila et al., 1998). However, under commercial production conditions in feedlots, the duration of storage is likely too short and the temperature too low for significant retrogradation of starch to occur.

Although retrogradation may be most prevalent in high amylase grains exposed to high temperatures, researchers have reported a “retrograde response” in steam-flaked corn held at temperature for much shorter periods of time (Ward and Galyean 1999; McMeniman and Galyean 2007). Thus, the amount of available starch in corn subjected to various processing procedures may be described as portrayed in Figure 5. Heat treatment at low moisture levels can decrease the digestibility of starch due to the formation of starch-protein complexes (Ljokjel et al., 2003), but it is not known to what extent similar complexes may be formed in grain subjected to steam processing. The formation of these complexes may also impede the precise measurement of starch in feed byproducts such as distillers grains. At this point it is not known if rumen microbes can hydrolyze these complexes and make the starch available for ruminal fermentation, but others have observed no difference in ruminal or total tract starch digestion between fresh and air-dried steam flaked corn (Zinn and Barrajas, 1997). Consequently, if such a phenomenon does occur, this type of “retrograde starch” is unlikely to limit starch digestion in ruminants. In light of the expanding use of corn and wheat distillers grains in feedlot diets, studies on the potential impact of these complexes on the performance of feedlot cattle are warranted.

![Figure 5](image)

**Figure 5.** Changes in total or available starch in four corn hybrids as a result of tempering, steaming and crushing of corn. Note that an apparent “retrograde response” was observed in flaked corn that was held at temperature for 1 h. Data adapted from Ward and Galyean (1999) by Owens (personal communication).

**SUMMARY AND IMPLICATIONS**

The utilization of starch in cereal grains by ruminants is limited primarily by kernel structures rather than the nature of the starch itself. Presence of the pericarp restricts bacterial and enzymatic access so that whole cereal grains are poorly digested. The pericarp
must be disrupted by processing or mastication for starch digestion to proceed. Once endosperm cells are exposed, starch digestion can still differ among cereal grains, limited by a dense protein matrix surrounding the starch granules. Processing techniques, such as steam flaking, that involve the application of heat and shear force are more effective than dry rolling for exposing the starch within the vitreous endosperm to digestion. Particle size also plays a key role in determining the efficiency of starch digestion because smaller particles have a larger surface area and consequently are more susceptible to digestion by both microbial and mammalian enzymes. Characterization of the properties of cereal grains that influence particle size distribution such as kernel uniformity, chemical composition, moisture content, and degree of shatter upon processing may provide a reliable index by which relative ruminal digestibility of starch from cereal grains can be predicted.

LITERATURE CITED


QUESTIONS AND ANSWERS
Q: Tim, based on your photomicrographs, the protein of corn seems quite indigestible in the rumen. Because corn byproducts and distillers’ products concentrate those fractions, the amount of protein degraded in the rumen must be quite low for such products.
A: Lower ruminal protein degradation matches with higher protein bypass for distillers’ grain. With wheat distillers’ products from Canada, the energy availability is greater than what we initially expected, so the level that we can substitute into the diet without having negative effects is much greater. Whether you have seen this with corn is not known.

Q: Tim, you commented that if protein barriers limit the accessibility of starch to enzymes in the rumen, then mammalian enzymes similarly are not likely to digest that starch either. But once you expose the product to the low pH and pepsin of the abomasum, doesn’t that change the structure of that protein so that you are looking at a different organizational structure than you have in the rumen?

A: Good point. Nobody has looked at that. Someone should collect samples from the small intestine of cannulated animals and examine the protein matrix with an electron microscope. When you are dealing with a product that has a high ruminal protein bypass because of its resistance to microbial enzymes, you may get some recovery of starch postruminally, but the starch will never become as available as when the grain is steam flaked that makes all the starch available.