The Nutritional Chemistry of Dry and High Moisture Corn

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Introduction

When fed to lactating dairy cows, management practices such as grinding corn, (Remond et al., 2004), steam flaking corn (Callison et al., 2001), feeding high moisture corn (Oba and Allen, 2003), or feeding floury corn (Allen et al., 2008), have been demonstrated to improve starch digestion and milk production of lactating dairy cows. The aforementioned management practices are common in the dairy industry and are deemed necessary to improve the feeding characteristic of corn grain. The use of these management practices brings to light a broader question—why is corn starch within the native corn kernel only partially digestible by dairy cows? This paper will review the nutritional chemistries of corn which are potentially related to starch digestibility in dairy cows.

Corn is a Seed

Corn per se is not a feed, it is a seed, and some understanding of corn seed anatomy and physiology are required to better understand chemical factors that potentially influence starch digestibility in ruminants. The corn seed is comprised of three basic morphological parts: pericarp, germ, and endosperm. The endosperm represents approximately 75-80 percent of the corn kernel by weight and is the morphological structure that contains starch. The endosperm contains primarily starch and protein but does contain small amounts of fat as phospholipids and ash. The endosperm of corn is virtually devoid of fiber (ADF or NDF). Specifically, corn endosperm contains < 4% NDF and 0.09% P (phosphorus), as compared to the germ which contains 17% NDF and 0.97% P, and pericarp with 33% NDF and 0.29% P (Van Kempen et.al., 2003). Corn endosperm contains abundant storage proteins (albumins, globulins, prolamins, and glutelins) which will be discussed in detail later in this paper.

The endosperm in cereal grains surrounds the germ and serves as the primary nutrient source for the germ which contains living tissue (roots, leaves, etc). Seed germination is initiated by water absorption and the seed undergoes renewal of enzymatic activity that results in cell division and ultimately embryo emergence through the pericarp. The endosperm's biological function is to serve as the primary nutrient source for the embryo until photosynthesis is initiated upon seedling emergence (Buchanan, et al., 2000; Mohr and Schopfer, 1995).

Corn Endosperm

Corn is an annual plant, reproducing only by seed, facilitated by the seed falling onto the ground where germination is reinitiated. Plant reproduction by seed requires protection of the embryo from improper environmental conditions until proper environmental conditions (moisture, temperature, seed coverage, dark) exist for germination. The fibrous pericarp is the primary morphological structure protecting the embryo but the starch in corn endosperm is also protected by hydrophobic (repels water) proteins called prolamins. Pure starch cannot be efficiently stored in corn endosperm because pure starch is highly hydrophilic (attracts water) and premature hydration of the endosperm would not properly facilitate germination. The combination of starch, prolamins and other proteins (albumins, globulins, glutelins) in corn endosperm is often referred to as the starch-protein matrix. Differences in the starch protein matrix can be visibly seen in dissected kernels of yellow dent corn. The visual appearance of all or portions of the starch-protein matrix in corn endosperm have historically been given visually descriptive classifications. Starch-protein matrices appearing white are commonly given the names floury, opaque or soft endosperm. Starch-protein matrices appearing yellow, shinny or glassy are classified as horny, translucent or vitreous (Kempten, 1921). The word vitreous means to exist in an amorphous, glassy-like state. A common example of something existing in a vitreous state would be a ceramic vase. The term vitreous is presently important because over the past decade animal and dairy scientist have adopted the word to semi-quantitatively define corn endosperm types in ruminant nutrition trials.

Vitreous Endosperm and Negative Effects on Starch Digestibility

Vitreousness of corn can be quantified in whole corn kernels by manual dissection (Correa et al., 2002). Corn kernels are soaked in water, the pericarp and germ are removed with a scalpel and the remaining endosperm is weighed. Using visual judgment, the floury (white, opaque) endosperm is separated from vitreous (yellow, glassy) endosperm with a scalpel and the weight of the vitreous endosperm is weighed and expressed as a percentage of the total endosperm.

Recent research has evaluated the relationship between in situ starch or DM degradability of corn (Philippeau and Michalet-Doreau, 1998; Correa et al., 2002; Ngonyamo-Majee, et al., 2008) and endosperm vitreousness. All studies have observed a strong negative relationship between endosperm vitreousness and in situ starch or DM degradability, meaning as endosperm vitreousness increases in situ starch or DM degradability decreases. Ngonyamo-Majee, et al. (2008) evaluated in situ DM degradability of 31 corns differing in vitreousness. Corn kernels were ground through a 6-mm screen, placed in dacron bags and incubated for 14 h in cannulated steers. The negative relationship ($R^2 = 0.72$) between endosperm vitreousness and in situ DM degradability of corn observed by Ngonyamo-Majee, et al. (2008) is presented in Figure 1.

Lebaka et al., (2007) reported the opaque (o2) gene alters vitreousness and endosperm storage protein composition of corn. The less-vitreous kernel texture of *o2* grain directly improved in situ starch degradability, but adversely affected agronomic performance. Lebaka et al. (2007) evaluated 140 recombinant inbred lines of corn for in situ starch degradability in combination with quantitative trait loci markers (QTL) to assess regions of the corn genome negatively or positively related to corn in situ DM degradability. Ruminal starch degradability of corns was negatively related QTLs on 2 primary chromosomes which have been previously associated with endosperm storage proteins (prolamin-zein) in corn. Similar results were observed in vivo by Allen et al. (2008). Allen et al., (2008) fed eight ruminally and duodenally cannulated lactating dairy cows, corns with 25 or 66 % vitreous endosperm. Feeding cows 66 % vitreous endosperm corn reduced ruminal and total tract starch digestion by 19.1 and 7.1 percentage units respectively (Figure 2).

Prolamins Make Corn Vitreous

Prolamins are endosperm storage proteins high in proline (amino acid) found in the seed of all cereal grains. Prolamins for each cereal grain have specific and historical names: wheat (gliadin), barley (hordein), rye (secalin), corn (zein), sorghum (kafirin) and oats (avenin). The small grains (wheat, oats, barley) have lower prolamin contents as compared to corn although modified endosperm types exist in corn which are low in prolamins. Prolamins are characterized by a high glutamine and proline content. Proline is a highly hydrophobic amino acid capable of complex folding and thus proteins with high proline contents develop tertiary structures that are intensely hydrophobic and are soluble in aqueous alcohol solutions (Momany, et al., 2006; Lasztity, 1984).

In corn, prolamin proteins are named zein and comprise 50-60 % of the total protein in whole corn (Hamaker et al., 1995). Prolamin-zein, defines a class of hydrophobic proteins synthesized on the rough endoplasmic reticulum of the amyloplast (starch producing organelle) envelope consisting of four zein sub-classess (ά,β,γ,δ), (Buchanan, et al., 2000). Because prolamin-zein proteins are synthesized on the rough endoplasmic reticulum within the amyloplast without the presence transit genes (Buchanan et al., 2000) prolamin-zein proteins are not intrinsic within the starch granule but are primarily surface localized on the exterior of starch granules (Mu-Forster and Wasserman, 1998). As prolamin-zein proteins enlarge and distend with advancing maturity β- and γ- zeins cross-link and ά- δ-zeins penetrate their network and occupy a more central position encapsulating starch into a starch-hydrophobic protein matrix (Buchanan et al., 2000, Mu-Forster and Wasserman, 1998).

The degree, amount, mechanisms and genetics associated with starch encapsulation by prolamin-zein in corn have been extensively investigated by plant physiologist and cereal chemist (Buchanan et al., 2000; Landry et al., 2000; Mu-Forster and Wasserman, 1998; Lasztity, 1984).

It is well defined that floury and opaque corn endosperm types have significantly lower prolamin-zein content as compared to flint or normal dent corn endosperms (Hamaker et al., 1995, Landry et al., 2000, and Wallace et al., 1990). The lower prolamin-zein content of floury, opaque or modified opaque corn is regulated by ά, and γ, prolamin-zein gene expression (Wallace et al., 1990). Philippeau et al. (2000) quantified the relationship between vitreousness and prolamin-zein content with vitreous flint corns containing more prolamin-zein than less vitreous dent corns. These data define differences in the chemical composition between vitreous endosperm (glassy, translucent) and floury or opaque endosperm. The starch in vitreous corn endosperm is more extensively encapsulated by prolamin-zein as compared to floury or opaque corn endosperm. Differences in corn starch encapsulation by prolamin-zein can be seen using scanning electron microscopy. Presented in Figure 3 are scanning electron micrographs of corn starch granules, (A) heavily encapsulated in a prolamin-protein matrix and (B) starch granules in opaque corn endosperm with less extensive encapsulation by prolamin-proteins (Gibbon et al., 2003).

The significance of prolamin-zein protein and it's chemistry in corn to ruminant nutrition implies sequential logic. Prolamin-zein is not soluble in water (hydrophobic) nor soluble in solvents normal to the innate rumen environment (Lawton, 2002). Potentially, starch digestion requires rumen bacteria to first degrade prolamin-zein via proteolysis before amylolytic activity in the rumen (Cotta, 1988) can actively hydrolyze starch to glucose. Because glucose uptake by rumen bacteria is momentary (Franklund and Glass, 1987) and the rumen has extensive amylotyic capacity (Cotta, 1988) to hydrolyze starch to glucose, proteolysis of hydrophobic prolamin-zein proteins in the rumen should therefore be a rate limiting step associated with starch digestion. The synergism between prolamin-zein and starch digestion in ruminants is compounded by poor attachment and slow degradation potential of prolamin-zein proteins by rumen bacteria. Romagnolo et al., (1994) observed the ruminal degradation rate of zein to be 0.026 %/h as compared to corn globulin-albumin proteins at 0.06 %/h.

McAllister et al., (1993) defined the influence of starch protein matrix on starch digestion in a classical study. McAllister et al., (1993) observed that when corn was treated with a protease (pronase E, Sigma Chemical) in vitro starch digestion increased approximately two fold and concluded the protein matrix in corn was a major factor in ruminal starch digestion. Lichtenwalner et al., (1978) executed as similar study treating sorghum (prolamin = kafirin) with a protease followed by incubation with glucoamylase and observed a marked increase in starch hydrolysis.

Measuring Prolamins in Corn

Prolamin-zein was first quantified by it's solubility in aqueous ethanol by Osborne, (1897). Presently, the methods of Landry and Moureaux (1970) are a recognized, but not the sole method

to quantify prolamin-zein in corn endosperm. Modifications of Landry and Moureaux (1970) have been evaluated (Hamaker et al., 1995, Landry et al., 2000, and Wallace et al., 1990) resulting in permutations. The basis of Landry and Moureax (1970) and other aforementioned methods consist of sequentially solubilizing corn endosperm proteins with saline, H_2O, aqueous alcohol and an alkali. The methods of Landry and Moureaux (1970) are arduous and designed to divide corn endosperm proteins into multiple fractions (albumins, globulins, prolamins, and glutelins), which may be over extensive for ruminant nutrition because only prolamins have been recognized to be negatively associated with starch degradability (Philippeau et al., 2000) in ruminants.

Due to labor, expense, procedural metamorphosis, and prolamin-zein analysis of isolated corn endosperm, laboratory methods to quantify prolamin-zein (Hamaker et al., 1995, Landry et al., 2000, and Wallace et al., 1990) in whole corn for ruminant nutrition trials or for routine feed analysis are not employed. Turbidimetric methods (Paulis et al., 1974, Aboubacar et al., 2003; Olakojo et al., 2007) to quantify prolamin-zein periodically occur in the literature and have been successfully used to singularly quantify prolamins zein or kafirin in ground whole corn or sorghum. Larson and Hoffman, (2008) coalesced advances in cereal chemistry and rapid turbidimetric methods to quantify prolamin-zein in dry and high moisture corns. Prolamin-zein(s) were solubilized with 55.0 % aqueous isopropyl and turbidity of prolamin-zein(s) was achieved by addition to a turbidity solvent. Degree of turbidity was measured on a spectrophotometer and prolamin-zein was quantified using a standard absorbance curve developed form purified zein. The procedure of Larson and Hoffman, (2008) delineated prolamin-zein encapsulation of starch across corn endosperm type. Dry flint and dent corns contained significantly more prolamin-zein/100 g of starch as compared to floury or opaque corns.

Prolamins and High Moisture Corn

The starch-protein matrix in corn has been previously defined as a physio-chemical impediment to starch digestion in ruminants (Owens et al., 1986), but the role of the starch- protein matrix in the digestion of HMC starch in ruminants has only recently been defined. Because prolamin-zein increases with advancing maturity (Murphy and Dalby, 1971), lower prolamin-zein contents in HMC at ensiling could be expected. This argument is somewhat illogical because Murphy and Dalby (1971) observed that maximum prolamin-zein accretion occurred near black layer formation (± 30 % moisture), which is similar to typical ensiling moisture contents of HMC. In addition, HMC and dry corn are often harvested (combined) at very similar moisture contents with only post-harvest handling and storage of the corn being different thereafter. Specifically, corn is commonly combined at 25%-30 % moisture and mechanically dried thereafter yielding dry corn.

A more plausible explanation for greater and more rapid starch digestion of HMC starch is that fermentation acids or proteolysis degrade prolamin-zein proteins during the ensiling process. Bacterial proteolysis is an intrinsic mechanism in corn-grain fermentation which induces degradation of corn proteins (Baron et al., 1986). Philippeau and Michalet-Doreau (1998) observed that ensiling grains increased ruminal starch degradability and hypothesized that ensiling increases accessibility of starch granules to rumen microorganisms, because hydrophobic prolamin-zein proteins encapsulating starch granules were partially degraded by proteolysis. Likewise, Jurjanz and Monteils (2005) observed the effective ruminal degradability of starch to be lower in corn kernels before (70.2%) than after (92.3%) ensiling. The ensiling process improved starch degradation by significantly altering the rapidly-degradable starch fraction (80.7% versus 65.6 %) and the starch degradation rate (12.4 vs 8.0 %/h). Combined, these data (Baron et al., 1986; Philippeau and Michalet-Doreau, 1998; Jurjanz and Monteils, 2005) result in a very plausible hypothesis as to why higher ruminal and total tract starch digestibility is observed for HMC as compared to dry corn (Firkins, et al., 2001).

In a recent study, (Hoffman et al., 2010a) we monitored the fate of the starch-protein matrix in HMC across a long storage period (240 days). Two random HMC(s), containing 25.7% and 29.3 % moisture were ground, ensiled and stored for 0, 15, 30, 60, 120 and 240 d. The HMC(s) were also inoculated with or without 600,000 cfu/g of *Lactobacillus buchneri* 40788 (Lallemand Inc., Milwaukee, WI). Inoculation improved fermentation of the HMC with inoculated HMC having lower pH and greater acetate contents but changes in fermentation acids did not affect proteins in the starch protein matrix. However, ensiling time greatly affected the starch-protein matrix of HMC and data are presented in Figure 4. Ensiling time (0 vs 240 d) reduced all α, β and δ prolamin-zein subunits of the starch-protein matrix from 10%-40 %. The degradation of the γ prolamin-zein subunits of the starch-protein matrix of HMC was more extensive with a 60 % reduction. Because γ prolamin-zeins are primarily responsible for cross-linking starch granules together, the degradation of γ zeins in HMC would suggest that clusters of starch granules should disassociate (fall apart) as a result of fermentation since the cross links holding starch granules together are being degraded. This was confirmed by electron microscopy (photos not shown) of HMC starch granules at 0 and 240 d. Upon fermentation and storage for 240 d, the disassociation of starch-granule clusters in HMC could be readily seen using electron microscopy. Fermentation resulted in a greater number of individual starch granules (and surface area) for potential attack by rumen bacteria. Electron micrographs also revealed no alteration in individual starch granules in HMC prior to fermentation or after 240 d of storage. Inferences from this investigation (Hoffman et al., 2010a) also suggested the proteins in the starch-protein matrix were more likely altered by bacterial proteolysis and may not have been simply solubilized by fermentation acids.

In second study (Hoffman et al., 2010b), the digestibility of HMC fermented and stored for 0, 15, 30, 60, 120 and 240 d inoculated with or without 600,000 cfu/g of *Lactobacillus buchneri* 40788

(Lallemand Inc., Milwaukee, WI) was evaluated using an in vitro gas production system. Gas production and rate (kd) of gas production by rumen bacteria during the first 12 h of incubation increased with increasing storage time, which indirectly validates the observations of greater ruminal starch digestion of HMC as compared to unfermented corn. Increases in 12 h gas production and rate (kd) of gas production increased chronically over the entire HMC storage periods suggesting that the increase in HMC (DM) digestion is not an acute event. Similar results were reported by Benton et al. (2005) who evaluated in situ DM degradation of two HMC(s) and two reconstituted HMC(s) of varying moisture content; a chronic increase in DM degradation across a 300(+) day ensiling period was observed.

Grain Quality: Simple Test

Vitreousness. Vitreousness of corn can be quantified in whole corn kernels by manual dissection but the task is tedious. A semi-quantitative method is to determine vitreousness of whole corn kernels (i.e. from a specific corn hybrid) is use of a light box scoring system. Because vitreous endosperm is translucent, light shines through it as opposed to opaque endosperm which is not translucent. A complete guide to light box scoring of corn grain for vitreousness is available in *Breeding Quality Protein Maize (QPM): Protocols for Developing QPM Cultivars.* **http://ideas.repec.org/p/ags/cimmma/56179.html**

Crude Protein: Crude protein in corn grain is of benefit and detriment to dairy cows. Greater crude protein content in corn grain reduces the need for supplemental protein but proteins in corn grain also serve a lignin-like function because hydrophobic (prolamin-zein) proteins encapsulate starch reducing starch digestibility. Crude protein content of dry corn grain has been demonstrated to be negatively related to starch degradability. It is very simple to measure crude protein in corn grain and crude protein analysis is widely available at numerous commercial feed and forage testing laboratories. The crude protein content of corn grain averages 9.2 % but ranges from 7.5-11.5%. Crude protein and the amount of starch encapsulating prolamin proteins are highly related. Corn grain from hybrids with crude protein contents > 10.0 % are likely more vitreous, may contain more flint genes, or are short relative maturity hybrids (more flint genes). Corns with lower crude protein < 8.0 % maybe unique opaque-floury hybrids or have a portion of these endosperm types in their genetic makeup. Nitrogen fertility has also been demonstrated to have an effect on grain crude protein content therefore crude protein content of grains grown under nitrogen deficient growing conditions should be interpreted with caution.

Prolamin Protein: A commercial test is available to determine the concentration of hydrophobic prolamin proteins that encapsulate corn starch. The prolamin protein assay is available at a number of commercial feed and forage testing laboratories. The prolamin content of dry corns ranges from 2.5-5.5 % of dry matter. Corns > 4.5 % prolamin as a % of DM are likely more vitreous, may contain more flint genes, or are short relative maturity hybrids (more

flint genes). Corns with lower prolamin protein < 3.0 % maybe unique opaque-floury hybrids or have a portion of these endosperm types in their genetic makeup.

Soluble Protein: The relationships between crude or prolamin protein and starch digestibility in lactating dairy cows applies primarily to dry corn. In ensiled corn (HMC) it is important to ascertain whether the hydrophobic proteins in the starch protein matrix have been degraded in the ensiling process. Prior to ensiling about 20 % of the protein in corn is soluble in a buffer solution. In extensively fermented high-moisture corns, more than 70% of the protein maybe soluble. The change in soluble protein is a marker of the degradation process of starch matrix proteins. As silage bacteria degrade hydrophobic proteins they become more soluble in buffer solutions.

Ammonia Nitrogen: Ammonia nitrogen may also be a marker of the status of starch-matrix proteins in high-moisture corn. At ensiling, corn has virtually no ammonia nitrogen and the appearance of ammonia in high moisture corn means that amino acids associated with the starch protein matrix are being degraded by silage bacteria. In extensively fermented high-moisture corn, ammonia nitrogen may represent >7% of the total nitrogen (or protein). High-moisture corns with <2% of the total nitrogen (or protein) as ammonia nitrogen indicate the degradation of starch-matrix proteins is probably minimal.

Starch: As compared to test weight or density, determination of starch content of corn grain prior to feeding is important. Corns harvested at immature stages due to late planting, early frost or lack of growing degree days will likely be lower in total starch content. High moisture corns harvested with cob or husk as a part of the feed will also be lower in starch content. Starch contents of corn fed to dairy cows ranges from 60-74 % of DM. Diets can easily be adjusted for starch content if the starch content of the grain is known.

In Vitro Starch Digestibility: Numerous feed and forage testing laboratories offer in vitro starch digestibility. There is no standardized method. Typical whole grains are ground through a mill fit with a 4-8 mm screen and incubated in rumen fluid from 6-12 h. More specialized in vitro gas production procedures are also available. These procedures result in lab specific numbers but are useful in ranking or indexing relative starch digestibility potential by dairy cows.

Conclusions

- Corn is a seed and is comprised of three basic morphologic parts, pericarp, germ and endosperm. Starch is contained in the endosperm and the biochemistry of the endosperm influences starch digestibility in dairy cows.
- Vitreous endosperm is negatively related to starch degradability in dairy cows.

- Vitreous endosperm is translucent and can be indexed using a light box scoring system.
- Dry flint and dent corns contain more hydrophobic prolamin-zein as compared to floury or opaque corns.
- The higher ruminal starch digestibility potential of high moisture corn is the result of degradation of starch encapsulating proteins by proteolysis during fermentation and not solely due to moisture or harvest maturity per se.
- The crude protein, prolamin protein or vitreousness of dry corn grain is negatively related to starch degradability in dairy cows.
- In high moisture corn the extent of degradation of the starch protein matrix can be evaluated using soluble crude protein or ammonia nitrogen as a marker.

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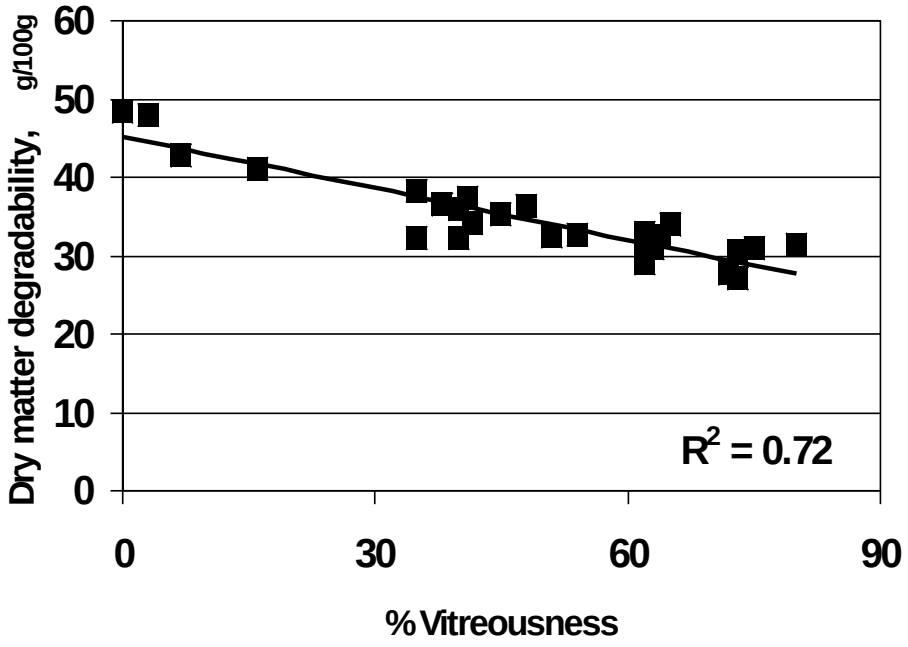

Figure 1. The relationship between kernel vitreousness and in situ DM degradability of corn (Ngonyamo-Majee, et al., 2008).

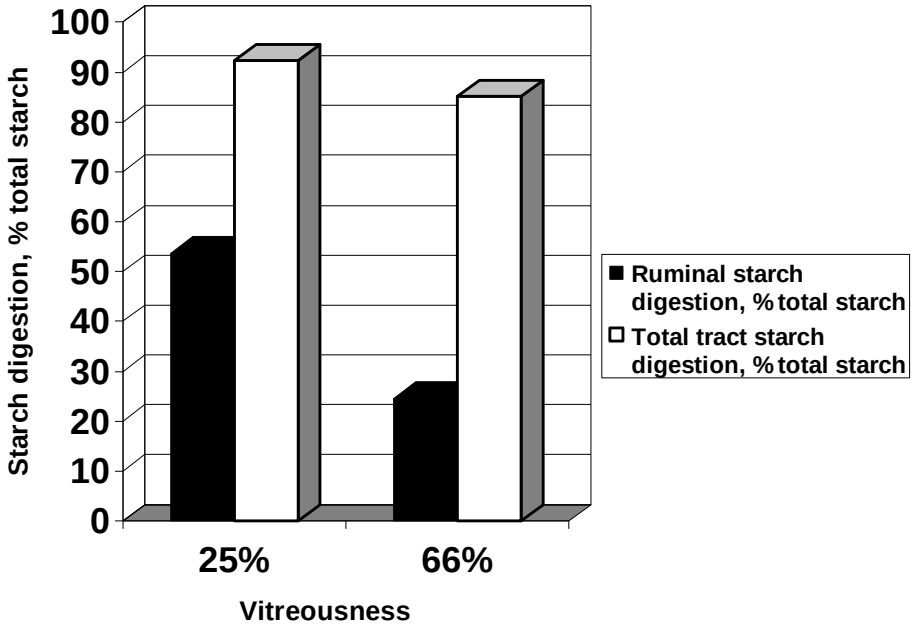

Figure 2. Effect of kernel vitreousness on ruminal and total tract starch digestibility in lactating dairy cows (Allen et al., 2008).

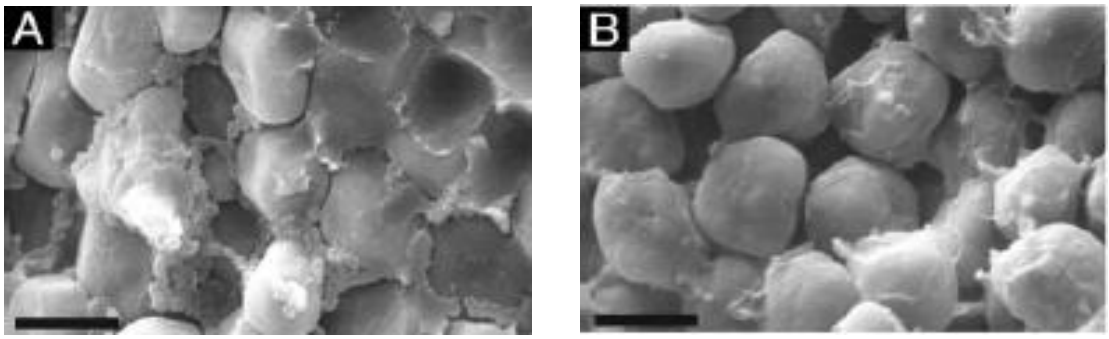

Figure 3. Scanning electron microscopy of starch granules in corn: A) starch granules heavily imbedded in prolamin-protein matrix, B) starch granules in opaque corn endosperm with less extensive encapsulation by prolamin-proteins (Gibbon et. al., 2003). Published with permission: *Copyright (2003) National Academy of Sciences, U.S.A.*

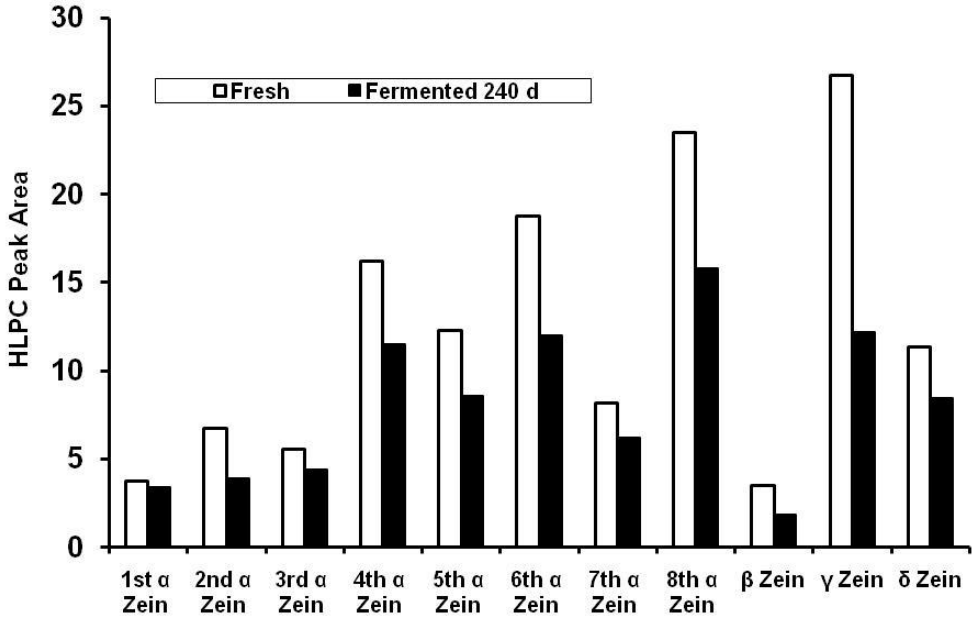

Figure 4. The effect of storage period (240 d) on hydrophobic prolamin-zein proteins in the endosperm of high moisture corn (Hoffman et al., 2010a).