Corn Biochemistry: Factors Related to Starch Digestibility in Ruminants

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Introduction

In general, review articles (Firkins et al., 2001; Theurer, 1986) pertaining to corn starch digestibility in ruminants, focus on factors or management practices that increase ruminal or total tract starch digestibility in ruminants. Management practices, such as grinding corn, (Remond et al., 2004), steam flaking corn (Callison et al., 2001), feeding high moisture corn (Oba and Allen, 2002), or feeding floury corn (Allen et al., 2008), have been demonstrated to improve ruminal or total tract digestion of corn starch by lactating dairy cows. Inference from these studies however, brings to light a broader question—why is corn starch within the corn kernel only partially digestible by ruminants? This paper will review the biochemical properties of corn which are potentially related to starch digestibility in ruminants.

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. Presented in Figure 1 is the general morphology of a corn seed. 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 which 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 imbibition (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, 1997; 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 2.

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 were 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 3).

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 4 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 soluents 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 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) coalesced the potential 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. An example of prolamin-zein contents expressed as prolamin-zein/100g of starch, of dry flint, dent, opaque, and high moisture corns (**HMC**) are present in Figure 5. The procedure of Larson and Hoffman, (2008) delineated prolamin-zein encapsulation of starch across corn endosperm type and conservation method. Dry flint and dent corns contained significantly more prolamin-zein/100 g of starch as compared to floury or opaque corns. Prolamin-zein contents of HMC were similar or lower than prolamin-zein contents of floury or opaque corns.

Prolamins and High Moisture Corn

The research of Larson and Hoffman, (2008) offers a view of the prolamin-zein contents of HMC. In their work, prolamin-zein content of HMC expressed on a starch basis was 2.5 times lower than prolamin-zein contents of normal dent corns. Prolamin-zein contents of HMC were comparable or in many cases less than prolamin-zein contents of floury or opaque corns. Two possible explanations for these observations exist. First, HMC is assumed to be harvested at an earlier physiological stage than dry corn and because prolamin-zein increases with advancing maturity (Murphy and Dalby, 1971) lower prolamin-zein contents in HMC could be expected. This argument is 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(s). In addition, HMC and dry corn are often field harvested (combined) at very similar moisture-maturities with only post harvest handling and storage of the corn being different thereafter. Specifically, corn is commonly combined at 30-20 % moisture and mechanically dried thereafter yielding dry corn.

The more plausible explanation for lower prolamin-zein contents in HMC is fermentation acids and proteolysis degrades prolamin-zein via the ensiling process. The most recognized solvents of prolamin-zein are aqueous alcohol solutions, but fermentation acids (lactic and acetic) are also primary solvents of prolamin-zein (Lawton, 2002). Because fermentation of results in lactic and acetic acid formation some chemical solubilization of prolamin-zein in HMC may occur. Second, bacterial proteolysis is an intrinsic mechanism in corn-grain fermentation inducing 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 encapsulating starch granules were partially degraded by proteolysis. Likewise, Jurjanz and Monteils, (2005) observed the effective ruminal degradability of starch to be lower in kernel grains before (70.2%) as compared to 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 % versus 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 why higher ruminal and total tract starch digestibility is observed in HMC as compared to dry corn (Firkins, et al., 2001). Prolamin-zein values for HMC, as determined by Larson and Hoffman, (2008) anecdotally support this hypothesis.

Prolamins-Forage Chemistry vs. Cereal Chemistry

In 1970, Goering and VanSoest, (1970) published the methods defining the detergent system (NDF, ADF, acid detergent lignin) of forage-fiber analysis. Ironically, in 1970 Landry and Moureaux (1970) published laboratory methods to isolate and separate corn endosperm storage protein fractions. Presently, the methods of Goering and VanSoest, (1970) are the foundation of feed analysis for ruminants worldwide. Presently, the methods of Landry and Moureaux, (1970) are a foundation of corn endosperm protein differentiation for human nutrition but not for ruminant nutrition.

The objectives of Goering and VanSoest (1970) and Landry and Moureaux, (1970) are uniquely different. The objective of Goering and VanSoest, (1970) was to better quantify fiber in forages. The NDF and ADF reagents contain sodium lauryl sulfate or

cetyltrimethylammonium bromide, which are surfactants and are commonly used to prepare proteins for electrophoresis in SDS or CTAB-PAGE. The surfactants work by disrupting non-covalent bonds in proteins, denaturing them, and causing the molecules to lose their native shape (conformation). The net effect on ADF or NDF determination is the majority of proteins are denatured and purposely not retained because the prime objective of the Goering and VanSoest, (1970) technique is to determine fiber (ADF or NDF) not protein. Because hydrophobic proteins, not fiber, encapsulate starch in the endosperm of corn and because the ADF and NDF reagents are chemically designed to denature proteins, ADF, NDF, lignin, ADF-CP, and NDF-CP may not be strongly related to measures of starch digestibility in ruminants.

The relationship between forage-fiber chemistry and in vitro gas production of cereal grains was reported by Lanzas et al. (2007). Lanzas et al., (2007) compared in-vitro gas production rates of barley, wheat, corn and sorghum samples with their ADF, NDF, NDF-CP and ADF-CP contents in an evaluation of the Cornell Net Carbohydrate and Protein System. Fractional rates of gas production were significantly different between grains with mean fractional gas production rates of 0.26, 0.24, 0.15, 0.06 h^{-1} for wheat, barley, corn and sorghum respectively. Fractional gas production rates were weakly related to ADF, NDF, NDF-CP, ADF-CP and buffer soluble CP content of individual grains. A weak relationship between forage-fiber chemistry and in vitro gas production in cereal grains is understandable for two reasons. First, ADF, NDF, ADF-CP and NDF-CP would primarily define the fiber or the CP in the fiber of the smallest morphological structure (pericarp) of cereal grains not the endosperm because the endosperm is virtually devoid of fiber. Second, because starch encapsulating prolamins (soluble in aqueous alcohol, Landry and Moureaux, 1970) in the endosperm would likely be denatured by ADF and NDF regents and not be denatured by buffer solutions (buffer soluble CP) their net effect on in vitro gas production in cereal grains would be relatively unattainable.

The prolamin content barley, wheat, oats, corn and sorghum endosperms are well defined by their solubility in aqueous alcohol solutions (Lasztity, 1984; Hamaker et al., 1995, Landry et al., 2000) with barley and wheat containing low prolamins, corn containing medium-high levels of prolamins and sorghum containing high levels of prolamins. Speculatively, a negative relationship should exist between starch encapsulation by prolamin proteins and in-vitro gas production rates in cereal grains because the endosperm constitutes 70-80 % of cereal grain DM. This relationship is theoretically synonymous to the negative relationship between prolamin-zein and situ starch degradability observed by Philippeau et.al. (2000), who determined the prolamin-zein content of corn by the modified procedures (Landry et al., (2000) of Landry and Moureaux, (1970). The nuances of forage-fiber chemistry and cereal chemistry should be considered in future studies investigating the effect of corn chemical composition on starch digestion in ruminants. *(Authors note: J. Landry is co-author of Philippeau et al. (2000).*

Starch Type and Starch Granules

Starch is a polysaccharide and can exist in two basic forms, amylose and amylopectin. Amylose is essentially a straight chain of glucose units bound together with ά-(1-4) linkages with very few ά-(1-6) linkages. Amylopectin is a larger form of starch and is highly branched with numerous branches at the ά-(1-6) linkages sights (Stevnebo et al., 2006). Native starch sources with significant amylopectin are commonly referred to as waxy endosperms and genotypes commonly exist in corn and barley (Tester et al., 2006). Studies have demonstrated increased starch hydrolysis potential of amylopectin as compared to amylose starches (Stevnebo et al., 2008). Native starch granules also differ in size and volume (Tester et al., 2006). Ring et al.

(1988) demonstrated that starch hydrolysis potential (wheat>corn>pea>potato).was inversely correlated to starch granule size.

The effect of starch type and starch granule size on starch degradability, digestion and performance of dairy cows is not well defined. Offner et al. (2003) reported in situ starch degradabilities for oats, wheat, barley, pea, potato and corn to be 0.927, 0.942, 0.891, 0.790, 0.785 and 0.599 % of total starch which is not in order of starch granule size (Tester et al., 2006). Akay and Jackson, (2001) however observed increase starch digestibility and lactation performance when waxy (amylopectin) starch was fed to cows as compared to control (amylose starch) corns. In contrast, Foley et al. (2006) observed lower starch digestibility in lactating dairy cows fed high-amylopectin barley as compared to control barley. To date, effects of starch type and starch granule size on starch digestibility in ruminants is somewhat inconclusive.

Conclusions

- Corn is a seed and is comprised of three basic morphologic parts, pericarp, germ and endosperm. Starch is contained in the endosperm and thus the biochemistry of the endosperm would be most logical in influencing starch digestibility in ruminants.

- Vitreous endosperm is negatively related to starch degradability and in vivo starch digestibility in ruminants.

- Vitreous endosperm is visually determined and represents a starch-protein matrix where hydrophobic prolamin proteins are commissural with starch.

- Dry flint and dent corns contain more hydrophobic prolamin-zein per g of starch as compared to floury or opaque corns. Prolamin-zein contents of high moisture corn are similar or lower than dry opaque or floury corn.

- Lower prolamin-zein contents and correspondingly higher starch digestibility of high moisture corn is hypothesized to be the result of degradation starch encapsulating proteins by fermentation acids and proteolysis during fermentation and not solely due to moisture or harvest maturity per se.

- Traditional forage-fiber chemistry techniques may not be well suited for cereal grains in determining biochemical factors that influence starch digestibility in ruminants.

- The influence of starch type and starch granule size on starch digestibility in ruminants is not well defined.

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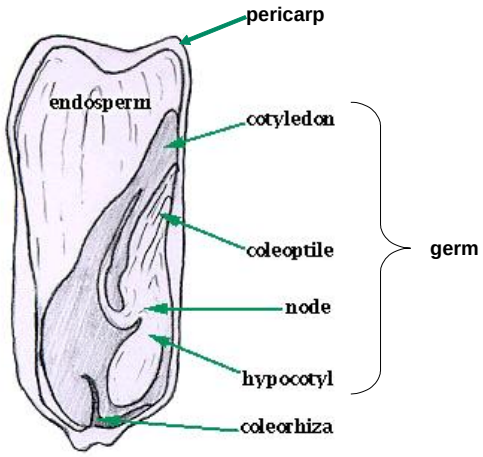

Figure 1. General morphology of corn.

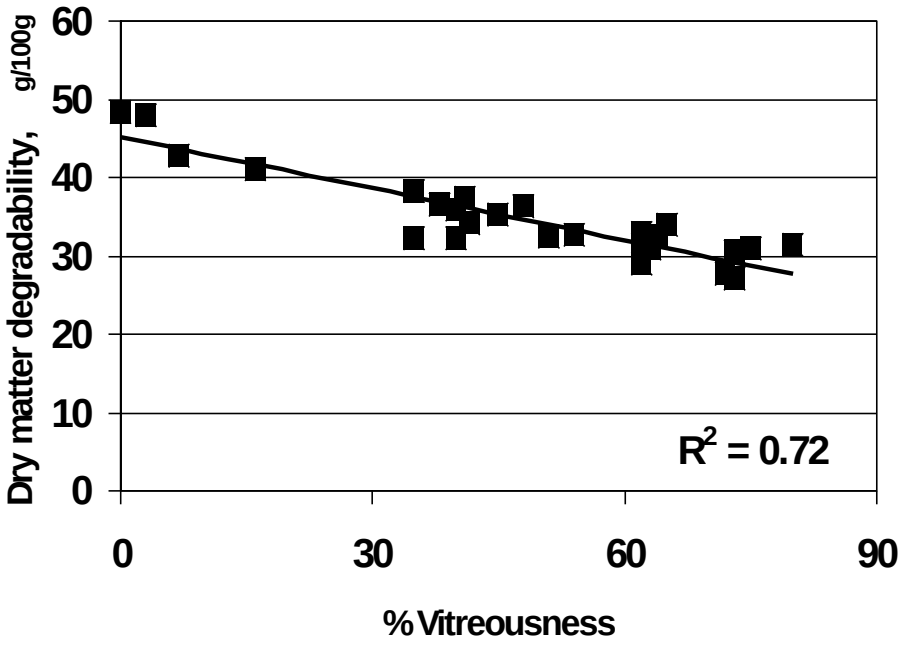

Figure 2. The relationship betwwen kernel vitreousness and in situ DM degradability of corn (Ngonyamo-Majee, et al., 2008).

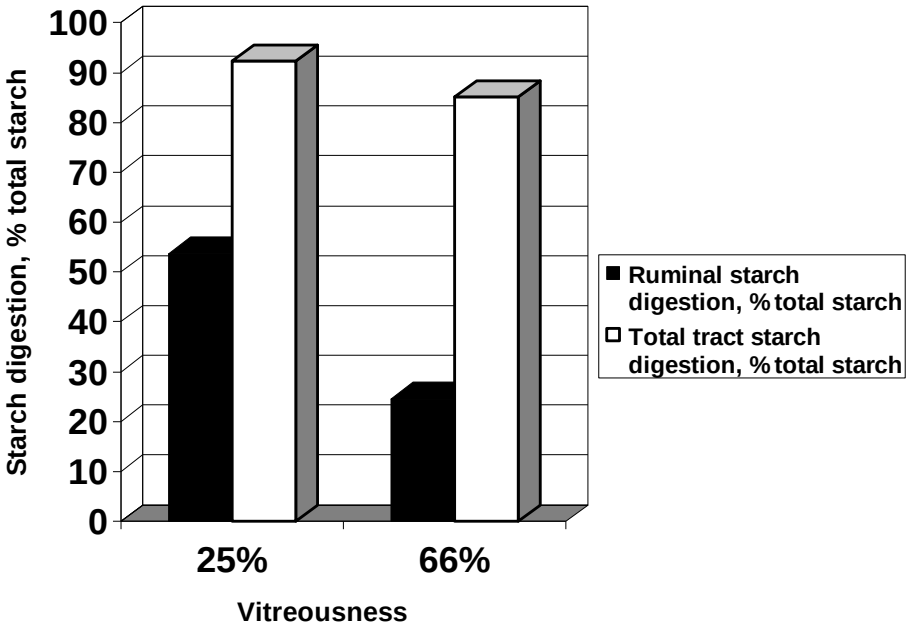

Figure 3. Effect of kernel vitreousness on ruminal and total tract starch digestibility in lactating dairy cows (Allen et al., 2008).

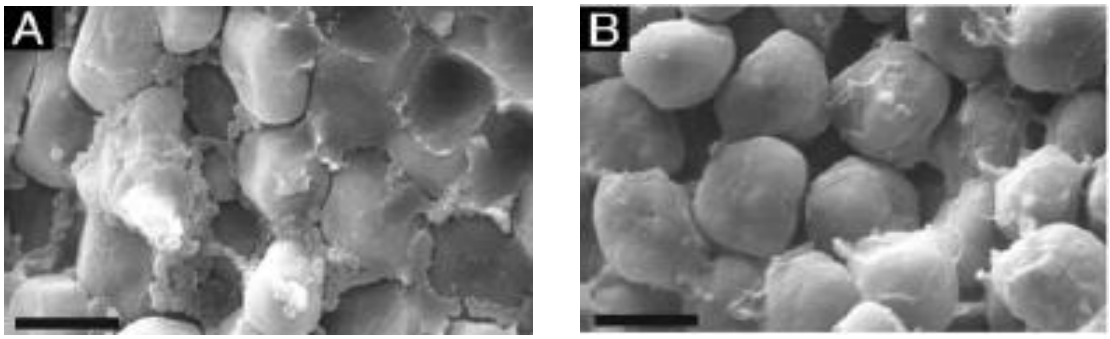

Figure 4. Scanning electron microscopy of starch granules in corn: A) starch granules heavily imbedded in prolamin-protein matrix, B) starch granules in opaque corn endosperm with less extensive encapsulation by prolamin-proteins (Gibbon et. al., 2003). Published with permission: *Copyright (2003) National Academy of Sciences, U.S.A*.

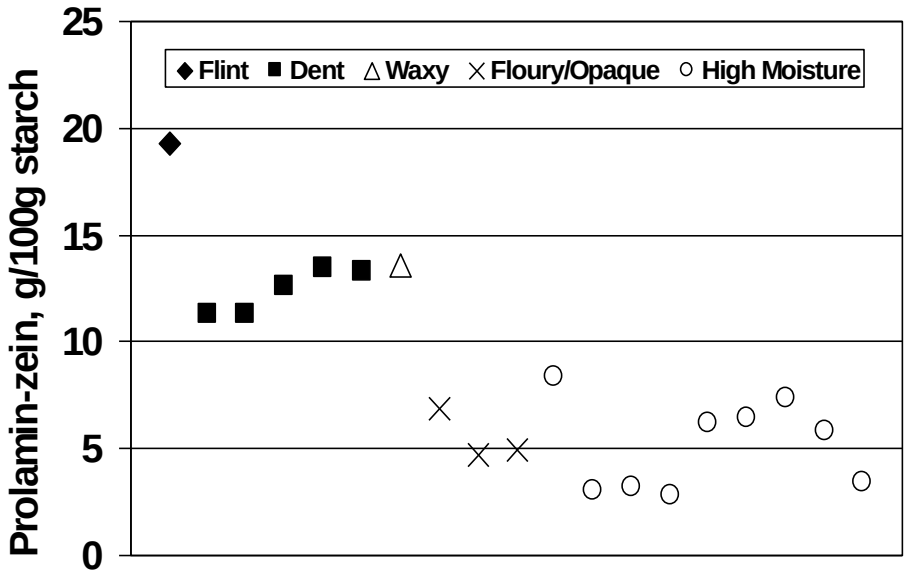

Figure 5. Starch encapsulation by prolamin-zein expressed as prolamin-zein,g/100g of starch in various corn endosperm types and high moisture corn (Larson and Hoffman, 2008).