Innovations in Feed Grain Evaluation for Lactating Dairy Cows

P. Hoffman and R. Shaver
Department of Dairy Science
University of Wisconsin, Madison, WI

Take Home Message

- 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 in dry corn is negatively related to starch digestibility in dairy cows.
- The strength of starch protein matrix bonding in the endosperm at harvest can be related to hybrid, N fertility or maturity.
- The strength of starch protein matrix bonding in the endosperm of dry corn is related to crude protein, prolamin protein or vitreousness.
- Greater ruminal starch digestibility potential of high moisture corn is the result of degradation of starch encapsulating proteins by proteolysis during fermentation.
- The weakness of starch protein matrix bonding in high moisture corn is related to soluble crude protein or ammonia nitrogen.
- Determination of mean particle size from un-dried, un-ground HMC will result in an overestimation of HMC mean particle size.
- Integrated evaluation systems that utilize multiple nutrient and physical components of feed grains and or corn silage to estimate nutritive value for lactating dairy cows offer promise.

Introduction

Digestibility of starch in lactating dairy cows can be altered or improved by numerous mechanisms. Management practices, such as grinding corn, (Remond et al., 2004), steam flaking corn (Callison et al., 2001), fermenting corn (Oba and Allen, 2003), or feeding corns with weak starch protein matrixes (Lopes et al., 2009), 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 routinely used to improve the feeding characteristic of corn grain. The net merit or degree to which a management practice improves starch digestibility however has been difficult to quantify. New information and technologies are however emerging which allow dairy nutrition consultants and producers to better understand and evaluate factors associated with starch digestibility in lactating dairy cows.

Some Basics

The corn seed is comprised of three basic morphological parts, pericarp, germ, and endosperm. The endosperm represents 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, 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 starch and abundant storage proteins (albumins, globulins, prolamins, and glutelins). The combination of starch, prolamins and other proteins (albumins, globulins, glutelins) in corn endosperm is often referred to as the starch-protein matrix. 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).

Prolamins are primary endosperm storage proteins in grains and have specific and historical names: wheat (gliadin), barley (hordein), rye (secalin), corn (zein), sorghum (kafirin) and oats (avenin). The small grains (wheat, oats, and barley) have lower prolamin contents as compared to corn although modified endosperm types exist 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 not soluble in water or rumen fluid (Momany, et al., 2006; Lasztity, 1984).

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.

Improving Starch Digestibility

Processing/Grinding

Processing corn silage (Bal et al., 2000) and dry or high moisture corn (**HMC**; Owens et al., 1986) are well established management practices to improve starch digestibility. Dry and high moisture corns are typically processed prior to feeding to enhance their nutritional value for lactating dairy cows. The degree of processing invoked on dry or HMC is typically measured by assessment of mean particle size (**MPS**) and effects of MPS on extent and site of digestion of grains by ruminants has been extensively reviewed (Huntington, 1997, Firkins et al., 2001). Across trials, a 1000 *u*m reduction in MPS increased total tract starch digestibility approximately 5.14 and 2.82 percentage units for dry and HMC respectively (Hoffman and Shaver, 2009).

In vivo, ruminal digestion of corn grains should be a simple inverse relationship with MPS, because as mean MPS decreases, surface area for bacterial digestion or enzymatic degradation of starch increases (Huntington, 1997). This simple inverse relationship however, between MPS and *in vivo* starch digestibility is not holistically observed (Firkins et al., 2001). The classic breach between MPS of corn grains and *in vivo* starch digestibility is when ruminants are fed HMC or corn silage grain particles. At a similar MPS, digestibility of HMC or grain particles in corn silage, are recognized to be more digestible than dry corn (Knowlton et al., 1998, Firkins et al., 2001). These observations suggest there is no universal upholding relationship of physical MPS and starch digestibility between corn silage grain particles, dry or HMC. As a result, relationships between MPS and starch digestibility are typically employed within grain types and not between grain types.

Ensiling

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).

In a recent study, (Hoffman et al., 2011a) the fate of the starch-protein matrix in HMC across a long storage period (240 days) was monitored. 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. Ensiling time greatly affected the starch-protein matrix of HMC and data are presented in Figure 1. 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. Upon fermentation and storage for 240 d, the disassociation of starch-granule clusters in HMC could be readily seen using electron microscopy (Figure 2). Fermentation resulted in a greater number of individual starch granules (and surface area) for potential attack by rumen bacteria.

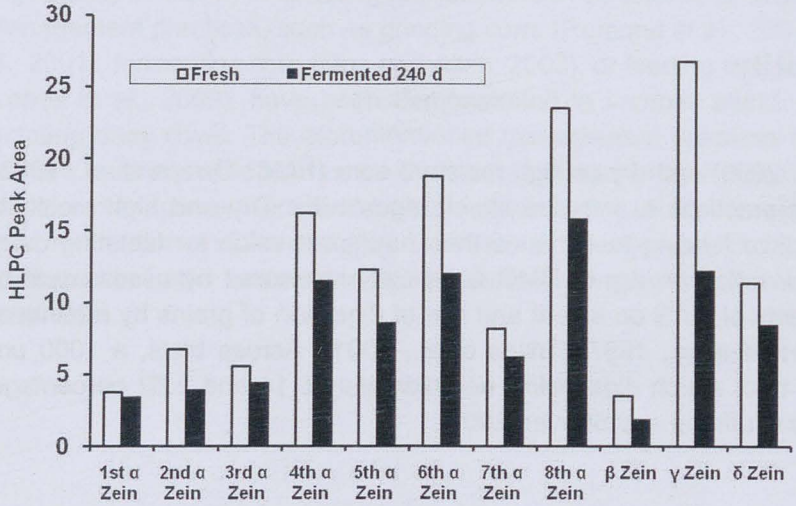

Figure 1. The effect of storage period (240 d) onhydrophobic prolamin-zein proteins in the endosperm of high moisture corn (Hoffman et al., 2011a).

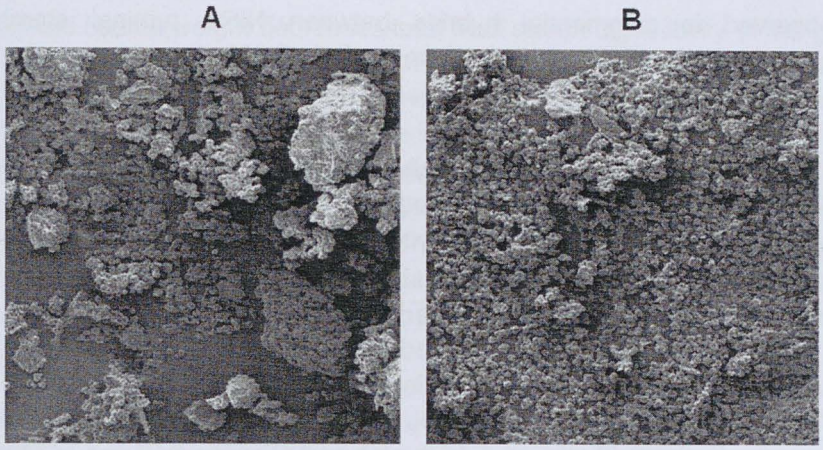

Figure 2. Electron micrographs of high moisture corn starch granule clusters prior to ensiling (A), and after 240 d of ensiling (B).

In second study (Hoffman et al., 2010), the digestibility of HMC fermented and stored for 0, 15, 30, 60, 120 and 240 d 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.

Hybrid Selection

It is well defined that floury and opaque corn endosperm types are less vitreous and 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). Increased kernel vitreousness has been demonstrated to reduce ruminal in situ corn starch degradation (Correa et al., 2002; Ngonyamo-Majee et al., 2008). Taylor and Allen (2005) reported greater ruminal and total tract starch digestibility in ruminally and duodenally cannulated lactating dairy cows for floury (3% vitreousness) vs. normal dent (67% vitreousness) endosperm dry corn. Lopes et al. (2009) conducted an experiment to evaluate the effect of type of corn endosperm on nutrient digestibility in lactating dairy cows using near-isogenic variants of a normal dent endosperm hybrid carrying floury-2 or opaque-2 alleles. The percentage vitreous endosperm was zero for floury and opaque endosperm corns and 64% for the vitreous corn. Prolamin protein content of floury and opaque endosperm corns was 30% of the content found in vitreous corn. Starch disappearance after 8-hr ruminal in situ incubation was 32%-units on average greater, respectively, for floury and opaque endosperm corns than vitreous corn. Total-tract starch digestibility was 6.3%-units, on average, greater for cows fed diets containing floury and opaque endosperm corns than vitreous corn.

At present, there are seed industry claims of altered endosperm types specifically designed for livestock applications; however, there is no industry method or index employed to define the endosperm characteristics (vitreousness, zein etc.) of commercial corn hybrids. Ngonyamo-Majee et al. (2010) conducted two studies to determine the variability of Monsanto corn germplasm on kernel physicochemical properties. Results from these studies reported a wide genetic variability of NIRS estimates of ruminal starch digestibility and other physico-chemical traits in commercial corn hybrids.

Negative correlations were observed in commercial hybrids between NIRS ruminal starch digestibility predictions and kernel protein (r = −0.79) and vitreousness (r = −0.42).

Corn Nitrogen Fertility

Application of N to corn is well known to improve corn yield however environmental conditions can alter corn N status. Excessive rainfall can result in N losses from leaching and denitrification. Likewise, lack of moisture during the pollination can result in poor N status. When corn is deficient in N, yield is reduced because the nitrogen sink, which facilitates starch accumulation and increased kernel weight, is altered (Tsai et al., 1980). Correspondingly, the amount and type of protein in the endosperm is altered. The data of Tsai et al. (1978) whom applied 0, 67, 134 and 201 kg/ha of N to a Pioneer hybrid (3369A) is shown in Figure 3. The CP of grain increased (6.68, 7.23, 8.10, 8.88 % CP) with increasing N fertility. The primary proteins affected by N fertility status were prolamins (zein). Zein (mg) in N deficient corn endosperm (0 N) was 1.86 mg as compared to 6.21 mg in corn receiving 201 kg/ha of N fertilizer. Further, the N status of Pioneer 3369A altered translucence (vitreousness) of the kernels. Corn kernels from corn fertilized with 0 N were primarily opaque and kernels from corn receiving 201 kg/ha of N were highly translucent. Recently, Masoero et al., (2011) demonstrated alterations kernel CP and zein content induced by N fertility, maturity or hybrid altered *in vitro* gas production of corn. Zein content of corn increased and *in vitro* gas production (2 h) decreased with greater N fertility and advancing maturity in corn.

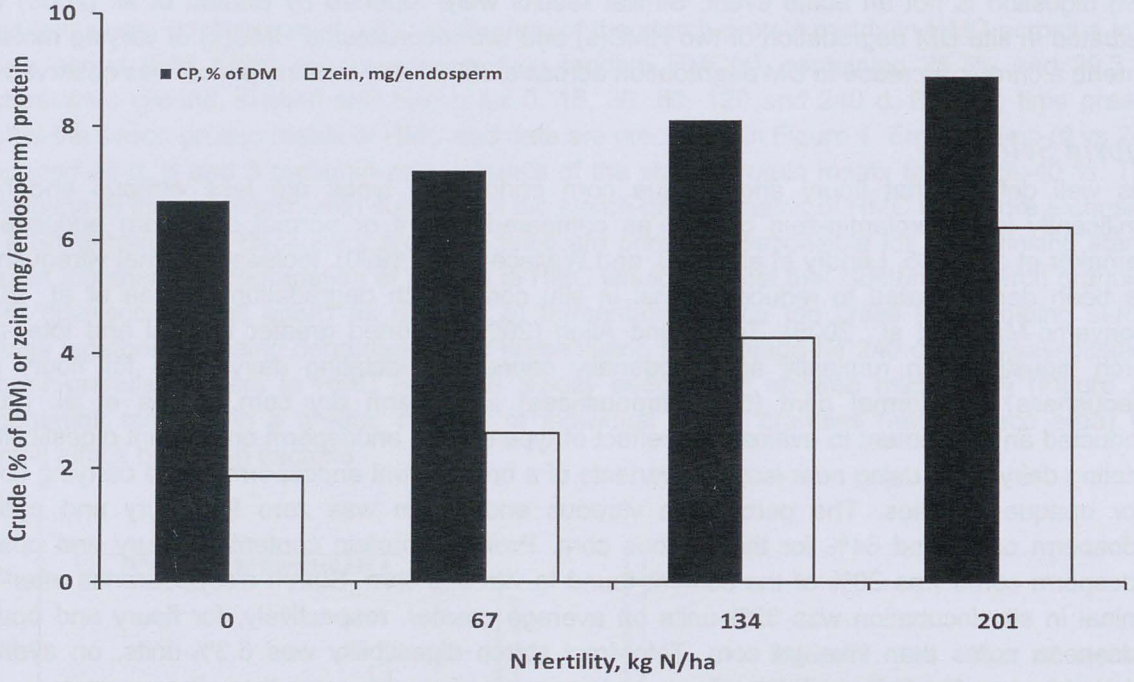

Figure 3. The effect of corn N fertility on total and zein protein in grain (Tsai et al., 1978).

Steam Flaking

Steam flaking is a process which imparts heat, moisture and rolling to increase starch digestibility of dry corn. The effects of steam flaking corn have been extensively reviewed (Zinn, 2002). The degree of damage of to the pericarp and extent of denaturing of protein in endosperm varies with processing conditions. Typically, flake bushel weigh is used as quality control index at the processing facility. As

flaked bushel weight decreases total tract starch digestibility of the grain increases. In lactating dairy cows ruminal digestibility of starch is marginally altered (50-60 %) by steam flaking corn. The effect of steam flaking corn is more pronounced on post-ruminal starch disappearance. Steam flaking corn can alter the digestibility of the post-ruminal flow of starch in lactating dairy cows up to 40 percentage units. Corns steam flaked to a lower density (<30 lb/bu) have greater post-ruminal disappearance than grain processed to a higher flake density (>30 lb/bu). For lactating dairy cows, a flake bushel weight, less than 25 pounds/bu, is generally preferred.

Evaluating Starch Digestibility

Mean Particle Size

Procedures to determine MPS of dry ground corn are well defined (ASAE, 2008, Baker and Herman, 2002). Approximately 150 g of dried, un-ground corn is placed in a series of 14 screens with nominal apertures of 4750, 3350, 2360, 1700, 1180, 850, 600, 425, 300, 212, 150, 106, 75, 53 *u*m and a pan. The series of screens and pan are placed on an oscillating sieve shaker for 15 minutes. Procedures to determine MPS of HMC are nebulous because nether ASAE (2008) or Baker and Herman, (2002) define whether HMC is to be dried prior to sieving. The MPS of dry or HMC is often determined on farm by dairy producers or nutrition consultants by sieving un-dried samples through a short stack of sieves (Baker and Herman, 2002). Typically corns are sieved through 6 screens (4000, 2000, 1000, 500 and 250 um) and pan. Recent data (Hoffman et al., 2011b) from our laboratory suggest on-farm determination of MPS for dry corn is very effective but on-farm determination of MPS on un-dried, un-ground HMC causes a large bias, overestimating the MPS of HMC (Figure 4).

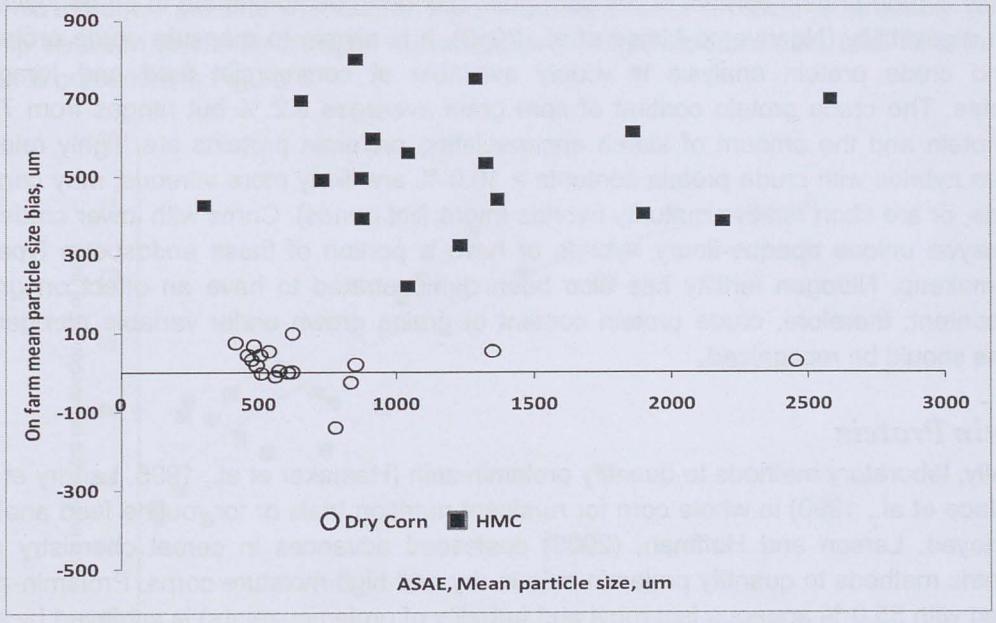

Figure 4. Mean particle size (MPS) bias, between ASAE (2008) and on farm determination of MPS on un-dried, un-ground samples of dry and high moisture corn (HMC).

Kernel Processing Score

Quantifying the mean particle size (**MPS**) of starch in corn silage with or without kernel processing in commercial forage testing systems has been challenging. As a commercially viable surrogate,

Ferriera and Mertens (2005) recommended determining the starch content of the total sample and the starch content of the DM remaining on screens > 4.75 mm for corn silage. This yields a kernel processing score (**KPS**), that serves as a starch particle size index for corn silage. Corn silage is defined as optimally processed if > 70 % of starch passes through the 4.75 mm screen. Corn silage is can also be defined as adequately (50-70%) or inadequately (<50 %) processed by the KPS scoring system. The MPS of corn particles in corn silage can be estimated (Zwald et al., 2008) from KPS using the equation (MPS, um = 7,491+ (−93.81KPS + 0.37KPS^2).

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. Vitreousness of corn will primarily be influence by hybrid, maturity or corn N status.

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 digestibility (Ngonyamo-Majee et al., 2010). It is simple to measure crude protein in corn grain and crude protein analysis is widely available at 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 variable nitrogen growing conditions should be recognized.

Prolamin Protein

Historically, 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 were not employed. 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) are solubilized with 55.0 % aqueous isopropyl and turbidity of prolamin-zein(s) is achieved by addition to a turbidity solvent. Degree of turbidity is measured on a spectrophotometer and prolamin-zein was quantified using a standard absorbance curve developed form purified zein. Dry flint and dent corns contain significantly more prolamin-zein/100 g of starch as compared to floury- opaque corns or corns with poor N status at pollination. 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, are short relative maturity hybrids (more flint genes) and had adequate N fertility. Corns with lower prolamin protein < 3.0 % maybe unique opaque-floury hybrids or be grain from N deficient corn. The prolamin assay of Larson and Hoffman, (2008) does not measure the degradation of prolamin proteins in HMC as induced by fermentation (Hoffman et al., 2011a).

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 but in extensively fermented high-moisture corns, up to 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 > 6 % 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. A relationship between ammonia nitrogen concentration in high moisture corn and 12 h *in vitro* gas production is presented in Figure 5.

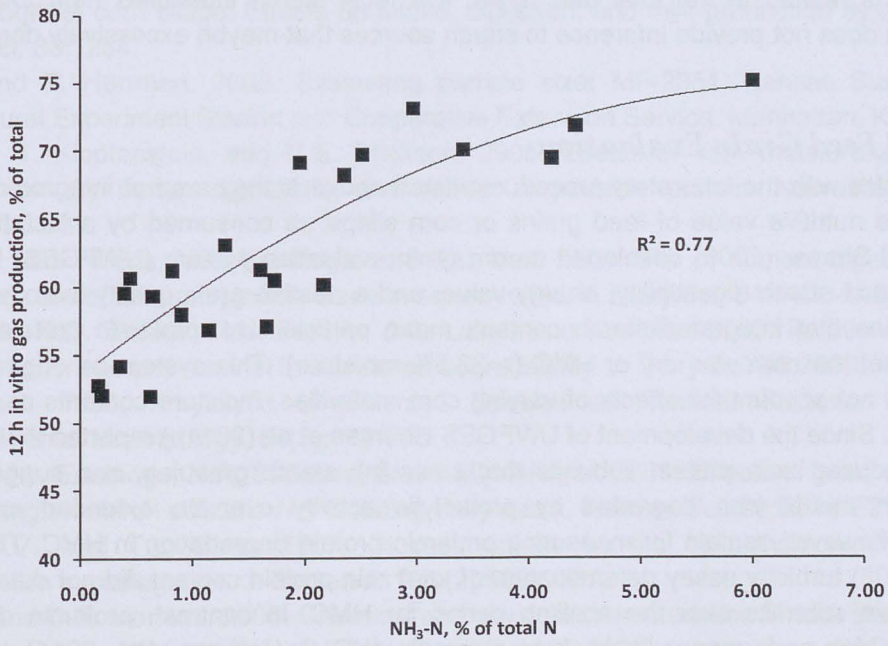

Figure 5. Relationship between NH_3-N concentration of high moisture corn and 12 h *in vitro* gas production (% of total gas; Hoffman et al., 2010).

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 and are useful in ranking or indexing relative starch digestibility potential by dairy cows. The challenge with *in vitro* starch digestibility measurements is integrating the values with MPS and other corn nutrients.

Fecal Starch

Commercial feed and forage testing laboratories are currently offering fecal starch analysis as an on-farm diagnostic tool to assess starch digestibility in lactating cow diets. Fecal starch testing has merits because on farm fecal starch is representative of the actual diet fed on a given farm. Fecal starch contents < 3.0 % indicate total tract starch digestibility is high. When fecal starch is > 5.0 % issues with dietary starch digestibility are likely. There are challenges to fecal starch evaluation. First, there is a high degree of variance of fecal starch concentration between cows, meaning fecal composites from multiple cows are required but fecal starch sampling protocols are not well defined. Second, fecal starch evaluation does not identify which starch containing feed maybe problematic as fecal starch is related to the total diet. Third, low fecal starch indentifies high total tract starch digestion but does not provide inference to starch sources that maybe excessively degradable in the rumen.

Integrated Feed Grain Evaluation

Major obstacles with the laboratory procedures listed above is they are not integrated into systems to predict the nutritive value of feed grains or corn silage as consumed by a lactating dairy cow. Hoffman and Shaver (2009) developed a corn grain evaluation system (**UWFGES**) for dairy cows where total-tract starch digestibility, energy value, and a relative grain quality index were predicted from equations that integrated starch content, mean particle size, prolamin protein content, and whether or not the corn was dry or HMC (> 22.5% moisture). This system as originally proposed, however, did not account for effects of varying corn maturities, moisture contents or extents of silo fermentation. Since the development of UWFGES Hoffman et al. (2011b) reported that ensiling HMC for 240 d reduced zein protein subunits that cross-link starch granules, and suggested that the starch-protein matrix was degraded by proteolytic activity over an extended ensiling period. Challenges, however, remain for measuring prolamin protein degradation in HMC. The Larson and Hoffman (2008) turbidity assay determination of total zein protein content did not detect a reduction in zein protein subunits over the ensiling period for HMC. In contrast, prolamin degradation as measured by high-performance liquid chromatography (HPLC; Hoffman et al., 2011) demonstrated a clear degradation of prolamin proteins in HMC over the ensiling period. However, Hoffman et al.,

(2011) did observe as HPLC prolamin (zein) protein subunits in HMC decreased, ammonia nitrogen and soluble CP contents increased in HMC (Hoffman et al., 2011). These results suggest that ammonia nitrogen or soluble CP analyses could be used potential markers of prolamin protein degradation in HMC. Presently, a second proposed version of UWFGESV2.0 which integrates the effects of moisture content, mean particle size, prolamin concentration (dry corn) and soluble protein (HMC) on total tract and ruminal starch digestibility is in progress and schedule for release in 2012.

Integrated Corn Silage Evaluation

Laboratory analyses typically used in an integrated system, to predict the nutritive value of corn silage, typically include dry matter (**DM**), crude protein (**CP**), neutral detergent fiber (**NDF**) and starch concentrations and ruminal in-vitro NDF digestibility (IVNDFD). Quality indexes, such as milk per ton or milk per acre (Schwab et al., 2003), are calculated using energy values estimated using summative energy equations (NRC, 2001) combined with dry matter intake (**DMI**) and DM yield respectively. Integrated corn silage evaluation systems such as (Milk2006) serves as the basis for evaluating corn hybrids for corn silage production at the University of Wisconsin (Lauer et al., 2009), other land-grant universities, and many commercial seed corn companies. Lacking in these integrated systems are direct determinations of starch digestibility for corn silage. This is an on-going area of research that remains challenging with regard to incorporation into MILK2006, because MPS of grain within corn silage is challenging to measure and starch digestibility has been found to increase with time in storage in silos (Hoffman et al., 2011).

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Form-A-Feed, Inc.
Gary Asche
740 Bowman Street
Stewart, MN 55385
800-422-3649 (toll free)
320-562-2413 (local)
garyasche@formafeed.com

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