

The Chemistry of High Moisture Corn

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Introduction

The first trial reported in the Journal of Dairy Science evaluating the nutritive value of high moisture corn (**HMC**) for lactating dairy cows was published by Zogg et al., (1961). The use of HMC on commercial dairy farms has grown, with approximately 65 % of Wisconsin dairy producers now utilizing HMC (Holmes, 2010). Despite wide spread use, HMC is an enigmatic feed because HMC per se is not a homogeneous feed. A specific HMC corn fed to a lactating dairy cow can be a highly variable feedstuff. Corns of varying endosperm type can be ensiled between 20%-40 % moisture, with or without inoculants, at ambient temperatures of 10-70°F, ensiled whole or ground, treated with or without organic acids, contain cob or husk, be fermented 1 to 365 days or more, stored in bags, bunkers and oxygen limiting silos and still be classified as HMC.

Variance associated with HMC production practices has been hypothesized or shown to create variance in fermentation characteristics, chemical composition, starch digestibility and milk yield in lactating dairy cows. The effects of chemical alteration of HMC on animal performance is challenging to quantify because most if not all HMC studies in the literature did not measure detailed chemical compositions of the HMC fed. As a result, general review articles (Firkins et al., 2001; Huntington, 1997, Owens et al., 1986) pertaining to starch digestibility in ruminants have focused on animal responses in trials where chemically undefined HMC was fed. Differences in ruminal, post-ruminal or total tract starch digestibilities between various grain sources and HMC can be generally categorized, but animal responses are challenging to directly link to HMC chemistry. Absent from the literature, are defining chemical mechanisms associated with fermentation, which explain altered starch digestion of HMC in ruminants. In short, altered starch digestion in lactating dairy cows fed HMC is presently binomially classified (i.e. dry vs HMC) but mechanisms explaining chemical alterations in HMC as compared to dry corn and variance of these alterations within a multitude of HMC production practices are poorly defined.

This paper will focus on the chemistry of HMC during fermentation and attempt to provide inference regarding why HMC starch digestibility in ruminants is altered as compared to other grain sources such as dry corn. A greater understanding of HMC chemistry may yield a better understanding of animal performance variance associated with feeding HMC of various origins.

High Moisture Corn-Starch Digestibility

Reviews (Firkins et al., 2001; Huntington, 1997, Owens et al., 1986) pertaining to factors and limitations of starch digestibility in ruminants have been previously published and will not be re-reviewed in this proceedings paper. From these reviews and trials on feeding HMC the following general concepts of HMC starch digestibility in lactating dairy cows can be defined (refer to Table 1).

- 1) The NE_L value of HMC is estimated to be 5-10 % greater than dry corn of similar origin and particle size (Tyrrell and Varga, 1987; Wilkerson et al., 1997). Greater NE_L values for HMC are primarily due to increased total tract starch digestion of HMC as compared to dry corn.
- 2) Ensiling of corn alters the site of starch digestion. Ruminal starch digestibility of HMC starch is commonly 20-30 percentage units higher than dry corn (Firkins et al., 2001; Owens, 2005)
- 3) The rate of ruminal starch digestion is faster for HMC as compared to dry corn (Sniffen et al., 1992).
- 4) Increased ruminal starch digestion and increased starch digestion rates may decrease ruminal NDF digestion (Firkins et al., 2001, Knowlton et al., 1998, Oba and Allen, 2003).
- 5) Feeding high levels of HMC with high rumen fermentability may depress DMI, rumen pH or milk fat test (Owens et al., 1986, Bradford and Allen, 2007, Firkins et al., 2001). Depressions in DMI are thought to be modulated by a glucose-insulin response effect (Bradford and Allen, 2007).

Starch Digestibility – From a Corn Seed Perspective

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 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, as compared to the germ which contains 17% NDF, and the pericarp with 33% NDF (Van Kempen et al., 2003). Corn endosperm contains storage proteins (albumins, globulins, glutelins and the abundant prolamins (zein protein) which are hydrophobic. 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).

The basic morphological parts of the corn seed are not equally digestible in ruminants. The pericarp is the primary morphological structure protecting both the embryo and endosperm. In native form, the pericarp of corn is poorly digested by rumen bacteria with minimal post ruminal digestion potential. Corn pericarp is relatively resistant to rumen bacteria attachment (Huntington, 1997) and whole corn seeds with the pericarp intact are largely indigestible in the small and large intestines of ruminants (Owens et al., 1986).

Digestion of starch, contained in the endosperm, by ruminants is enigmatic because starch isolated from cereal grains, regardless of source is rapidly attacked by rumen microbes and fermented rapidly (Hibberd et al., 1983). Starch in corn endosperm is, however, not in isolated form. Starch in a corn seed is protected by hydrophobic (repels water) proteins called prolamins (zein). The combination of starch, prolamins and other proteins (albumins, globulins, glutelins) in corn endosperm is often referred to as the starch-protein matrix. The starch-protein matrix binds starch granules together and the degree of binding alters the grinding efficiency of

corn (Fox and Manley, 2009) and the ability of, and surface area for, rumen bacterial attachment (Huntington, 1997).

In corn, prolamin proteins named zein are the primary protein in the starch protein matrix, 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 subclasses ($\alpha, \beta, \gamma, \delta$) (Buchanan, et al., 2000). Because prolamin-zein proteins are synthesized on the rough endoplasmic reticulum within the amyloplast without the presence of 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 α - 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).

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, shiny or glassy are classified as, horny, translucent or vitreous (Kempfen, 1921).

The Starch-Protein Matrix 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 is not well 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. At 0 and 240 d, the α , γ , δ and β zein regions of the starch-protein matrix were profiled using high performance liquid chromatography. The effect of fermentation (storage time) on the starch-protein matrix of HMC after 240 d of storage is presented in Figure 1. Fermentation (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 surface localized and 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 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. The observations of Benton et al. (2005) and Hoffman et al. (2010b) are presented in Figure 2. The data are similar and when combined suggest DM that the digestion potential of HMC increases chronically as storage time increases.

High Moisture Corn Fermentation is Poor and Slow

In our recent research (Hoffman et al., 2010a) we evaluated the nutrient composition (CP, prolamin, starch, ADF and NDF), fermentation (pH, lactate and acetate) and protein degradation markers (buffer-soluble CP and $\text{NH}_3\text{-N}$) of HMC at 0, 15, 30, 60, 120 and 240 d of storage. The data provided a holistic view of nutri-chemical transformations in HMC by storage time. The CP, origin prolamin, starch, ADF and NDF contents of HMC are relatively static, but pH, lactate, acetate, buffer-soluble CP and $\text{NH}_3\text{-N}$ contents chronically changed with advancing storage time. The chronic changes in HMC $\text{NH}_3\text{-N}$ content by storage time are presented in Figure 3. Ammonia ($\text{NH}_3\text{-N}$) in HMC did not stop accumulating even at 240 d. Ammonia is an important

marker in fermented feeds, because $\text{NH}_3\text{-N}$ is intrinsic to deamination of amino acids which is the terminal phase of proteolysis (protein breakdown). The data in Figure 3 suggest that proteolysis in HMC did not abate even after 240 d of storage. These data present an indication that fermentation of HMC is very slow and chemical alterations are occurring over an extended storage period.

It is logical that HMC ferments slower than legume or corn silage for a number of reasons. Normally HMC, prior to ensiling, does not contain high levels of sugars or water soluble carbohydrates for conversion to volatile fatty acids. The levels of mono-disaccharides and water soluble carbohydrates of legumes, whole-plant corn and corn grain prior to ensiling (Dairy One Laboratories, Ithica, NY) are presented in Figure 4. Fresh corn contains significantly less mono-disaccharides or water soluble carbohydrates than legumes or whole-plant corn. Because the level of fermentable substrate in fresh corn is lower than fresh legumes or whole-plant corn, the production of volatile fatty acids in HMC is reduced when compared to legume or corn silage (Figure 5).

High moisture corn is also ensiled at DM contents near 70.0%. High DM contents increase the osmotic potential in silage (or HMC), which decreases the growth rate of lactic acid producing silage bacteria (Pitt et al., 1985). As a result, silage or HMC with higher DM content ferment slower than silage or HMC with a lower DM content. Slower growth rates of silage bacteria induced by ensiling at high DM contents can be further exacerbated by ensiling at low temperatures (Pitt et al., 1985). The effect of silage mass temperature on growth of lactic acid producing bacteria is presented in Figure 6. High moisture corn is commonly ensiled when ambient temperatures are $< 50^\circ\text{F}$, which does not facilitate rapid bacterial growth. The combination of limited substrate for fermentation, high DM content, and ensiling at cooler temperatures suggest HMC fermentations are destined to be protracted. Protracted fermentations would mean a protracted proteolytic breakdown of the starch-protein matrix in the endosperm. In our recent work (Hoffman et al., 2010ab), we observed this effect; protracted fermentations and protein alterations in HMC which resulted in chronic increases over time in storage for 12 h in vitro gas production and rates of gas production.

Conclusions

- The starch protein matrix in HMC is significantly altered by the fermentation process, especially γ zein proteins which cross link starch granules together.
- Fermentation induced degradation of γ zein proteins in HMC appears to yield a general disassociation of starch granule clusters yielding more individual starch granules and (or) surface area for potential bacterial attack.
- The degradation of the starch protein matrix in HMC appears to be chronic and slow.
- Fermentation and storage time chronically increases the DM digestion potential of HMC.
- Traditional feed chemistry nutrients in HMC (ADF, NDF, CP and starch) are static across the storage period and do not appear well suited for determining biochemical factors that influence starch digestibility of HMC in ruminants.
- High moisture corn is not a static feedstuff with a fixed or book value nutrient composition. Nutrient availabilities in HMC chronically change and changes are likely dependent on physical processing, the strength of the starch-protein matrix at ensiling, fermentation conditions at ensiling (DM and temperature), and the length of the storage period.

TABLES AND FIGURES

Table 1. Site and extent of starch digestibility of various corn sources in lactating dairy cows.

Review	Corn Source	Trials/diets	Starch digestibility		
			Total tract % diet	Ruminal % diet	Post-ruminal % flow
Owens, 2005	Dry rolled	22	89.9	49.2	77.7
Firkins et al., 2001	Dry rolled	9	85.0	44.6	
Firkins et al., 2001	Dry ground	12	90.7	52.1	.
Firkins et al., 2001	Dry ground, fine	2	91.4	.	.
Owens, 2005	High moisture	4	96.0	76.3	82.9
Firkins et al., 2001	High moisture	12	.	86.8	.
Firkins et al., 2001	High moisture, rolled	3	94.2	.	.
Firkins et al., 2001	High moisture, ground	2	98.8	.	.
Owens, 2005	Steam flaked	5	93.9	51.8	88.4
Firkins et al., 2001	Steam flaked	10	94.2	56.9	.
Owens, 2005	Steam rolled	3	94.2	55.7	88.3
Firkins et al., 2001	Steam rolled	10	88.8	.	.

Combined review data of Firkins et al. (2001) and Owens, (2005).

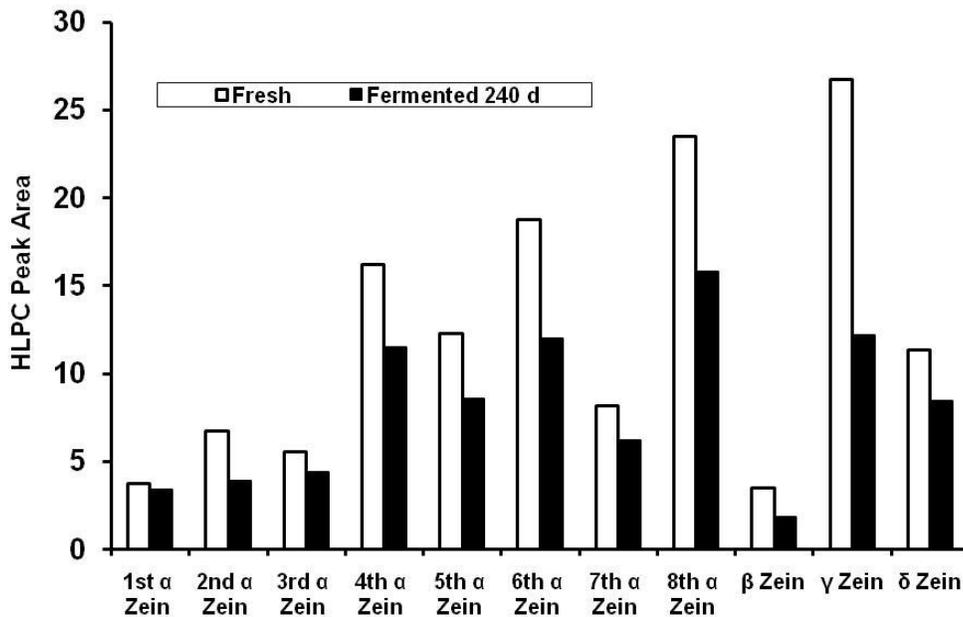


Figure 1. The effect of storage period (240 d) on hydrophobic prolamins-zein proteins in the endosperm of high moisture corn (Hoffman et al., 2010a).

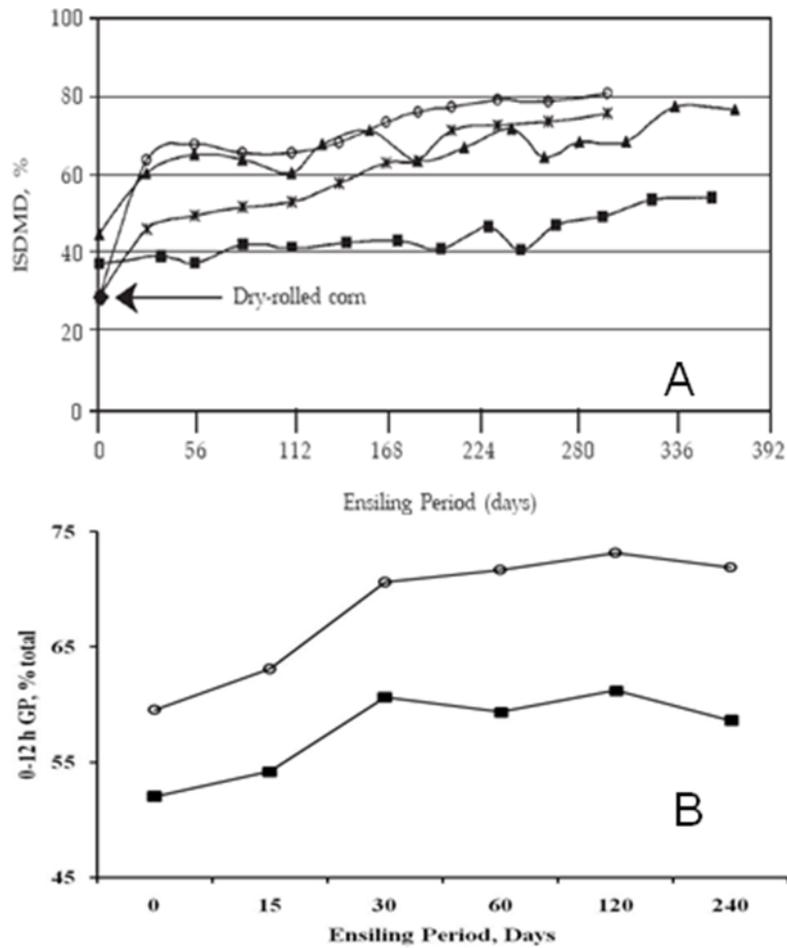


Figure 2. Changes in high moisture corn DM degradation across long ensiling periods. A= Benton et al., 2005 (■ = 24 % moisture HMC, x = 28 % moisture reconstituted corn, ▲ = 30 % moisture HMC, ○ = 35 % moisture reconstituted corn). B = Hoffman et al., 2010b (○ = 29.3 % moisture HMC, ■ = 25.7 % moisture HMC). In situ Dacron bags and in vitro gas production were used as evaluation techniques by Benton et al., (2005) and Hoffman et al., (2010b) respectively.

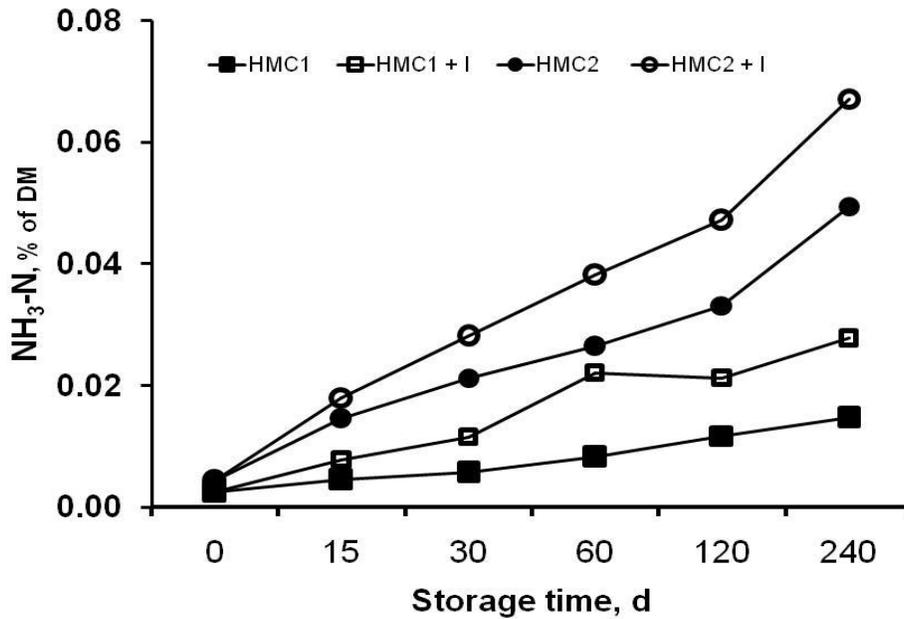


Figure 3. The effect of storage time of on NH₃-N of four high moisture corns (Hoffman et al., 2010a)

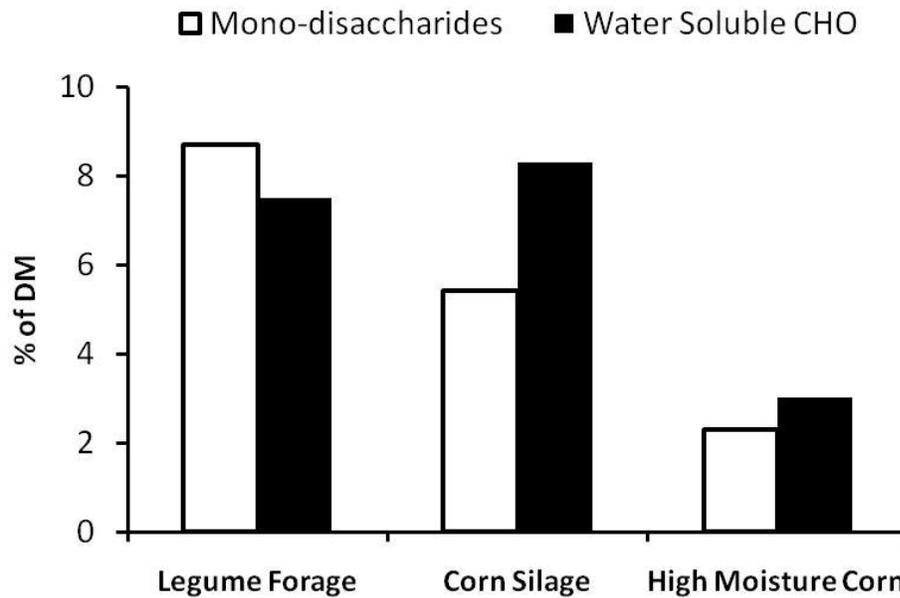


Figure 4. Typical contents of mono-disaccharides and water soluble carbohydrates (CHO) in legumes, corn silage and high moisture corn prior to ensiling (Dairy One Laboratories, Ithaca NY).

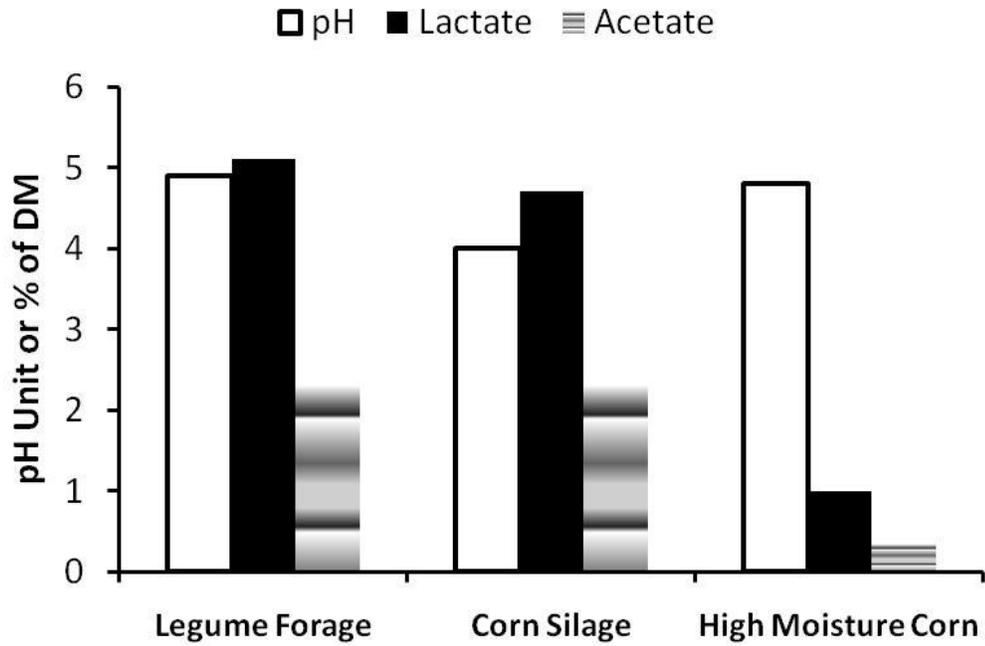


Figure 5. Typical pH, lactate and acetate contents of legume silage, corn silage and high moisture corn (Dairy One Laboratories, Ithaca, NY).

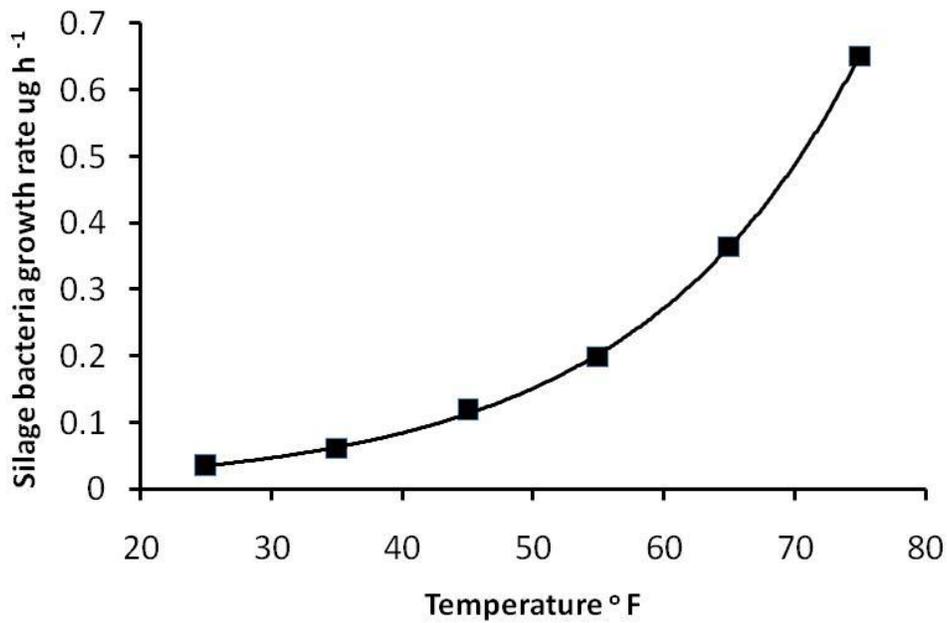


Figure 6. Effect of ensiling temperature on silage bacteria growth rate (Calculated from Pitt et al., 2005)

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