CAPÍTULO 2

Factors affecting the nutritive value and fermentation of high-moisture corn

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1. Introduction

High-moisture corn (HMC) is an important feedstuff to help meet the nutrient demands of a lactating cow. Even though HMC has greater ruminal and total-tract starch digestibility compared to dry corn (FERRARETTO *et al*., 2013), some limitations to maximize its fermentation and starch availability exist. From the perspective of the corn plant, starch present in the kernel endosperm exists primarily to nourish the germ. Therefore, the corn kernel possesses key structural features that aid in its ability to protect this valuable resource. From the perspective of a ruminant nutritionist, however, these same structural features are the primary limitations to starch digestion in the animal. First, the pericarp surrounding the endosperm of corn kernels is highly resistant to microbial and enzymatic degradation (MCALLISTER *et al*., 1994). Therefore, breaking the pericarp is essential for digestion of starch. Even when the pericarp is broken successfully, however, starch degradation is inhibited by the hydrophobic zein protein matrix surrounding starch granules (KOTARSKI *et al*., 1992). Zein protein degradation in the rumen is very slow (PHILIPPEAU *et al*., 2000; LOPES *et al*., 2009). Ensiled feeds such as HMC have the complex starch-protein matrix that surrounds starch granules broken down during silage fermentation, making starch more accessible for microbial and enzymatic degradation in the rumen and small intestine, respectively. However, the low moisture content of HMC can make achieving a thorough fermentation challenging (KUNG *et al*., 2007) as organic acid production may be reduced due to limited water availability (MUCK, 1988). High-moisture corn is also prone to aerobic deterioration because of its reduced fermentation capacity and high starch content, which can be metabolized by spoilage yeasts (KUNG *et al*., 2007).

Fortunately, there are numerous strategies available to optimize fermentation and overall quality of HMC. Likewise, some existing strategies may improve the accessibility of microbial and host enzymes to starch in HMC and are essential for the efficient utilization of these feedstuffs by dairy cattle. The objective of this review is to discuss factors that affect the nutritive value and fermentation of HMC, as well as strategies available to producers to optimize its nutritive value and fermentation.

2. Storage

It is well-understood that, as plant sugars are fermented, organic acids accumulate in the silo during storage reducing pH (KUNG *et al*., 2018). The primary fermentation phase is thought to last from 7 to 45 d (PAHLOW *et al*., 2003). However, there is evidence to suggest that the fermentation may last much longer in HMC (KUNG *et al*., 2018). Studies with HMC have reported a gradual increase in concentrations of organic acids and other fermentation end-products, like alcohols, for 120 d or more (HOFFMAN *et al*., 2011; KUNG *et al*., 2014). Furthermore, prolonged storage allows for the continual degradation of the zein proteins that surround starch granules in corn kernels (HOFFMAN *et al*., 2011) and the accumulation of nitrogenous compounds, like ammonia-N and soluble CP, over time (BARON *et al*., 1986; HOFFMAN *et al*., 2011; KUNG *et al*., 2014). The continual degradation of zein proteins with prolonged ensiling of HMC makes starch more accessible for digestion (KUNG *et al*., 2018). In a study evaluating the effect of ensiling time on HMC, Hoffman *et al*. (2011) observed that the α, γ, δ, and β prolamin-zein subunits of the starch-protein matrix were reduced from 10 to 40% when comparing HMC stored for 0 d with 240 d. Ferraretto *et al*. (2014) used month of sample submittal as a proxy for storage length when analyzing a data set comprised of more than 6,000 HMC samples obtained from a commercial laboratory. It was observed that in vitro starch digestibility of HMC increased 9%-units when month of sample submittal increased from October to August of the following year. Concentrations of soluble CP and ammonia-N followed a similar trend (FERRARETTO *et al*., 2014). Junges *et al*. (2017) reported that bacterial proteolysis was the primary contributor to the disruption of the starch-protein matrix in corn silages (60%), followed by plant enzymes (30%), fungi (5%), and fermentation end-products (5%) underscoring the importance of good ensiling practices not only to achieve preservation but also to enhance starch availability. Benefits of fermentation on starch digestibility, however, is not solely related to the chemical effects on zein proteins. There is some evidence suggesting prolonged storage may further improve digestibility of starch by means of kernel particle size reduction. Studies evaluating the influence of storage length on kernel particle size are scarce. Saylor *et al*. (2020) found that mean particle size decreased from d 0 to d 14 of storage when HMC was coarsely ground, but not when it was finely ground. In that study, mean particle size was unchanged for both coarsely and finely ground HMC as storage increased from 14 to 28 d. Therefore, space- and feed inventory-permitting, increasing storage length of HMC is a valuable strategy for increasing starch digestibility. Although prolonged storage can make starch of various types of silage more available, it is crucial to understand that fermentation may not overcome and sometimes not even attenuate lower starch digestibility due to other factors.

3. Hybrid selection

Optimizing starch digestibility of HMC starts with hybrid selection. Grain characteristics are altered primarily through modifications in nutrient concentration or starch composition (FERRARETTO; SHAVER, 2015). For example, modifications in nutrient concentration are often related to greater CP and fat, usually at the expense of starch. Modified starch composition can be observed in hybrids selected for starch high in amylopectin over amylose, or hybrids with a greater proportion of floury than vitreous endosperm (FERRARETTO; SHAVER, 2015). Increased kernel vitreousness has been shown to reduce ruminal in situ degradation of corn starch (CORREA *et al*., 2002; LOPES *et al*., 2009). Therefore, selection of hybrids with a more floury, less vitreous endosperm has potential to enhance digestibility of starch in HMC. In an assessment of U.S. dent corn hybrids and Brazilian flint unfermented corn hybrids, Correa *et al*. (2002) found a strong negative correlation (r^2 = 0.87) between kernel vitreousness and ruminal in situ starch availability. Kernel vitreousness, determined by manual dissection, of the Brazilian hybrids averaged 73.1%, while that of the U.S. hybrids averaged 48.2%. In this experiment, corn grain samples were dried and ground through a 4 mm screen prior to ruminal incubation. Lopes *et al*. (2009) conducted an experiment to evaluate the effect of corn endosperm type on nutrient digestibility in lactating dairy cows. Cannulated Holstein cows were fed diets containing dry rolled vitreous-, floury, or opaque-endosperm corn. The percentage of vitreous endosperm was 0 for the floury and opaque endosperm hybrids, and 64% for the vitreous corn. Additionally, concentrations of zein protein in the floury and opaque hybrids was 30% of that in the vitreous endosperm hybrid. Lopes *et al*. (2009) observed that in vitro and in situ starch digestibility were 42 and 32% greater on average, respectively, for floury and opaque endosperm corn than vitreous endosperm corn. It is important to note that these two trials assessed effects of corn hybrid on starch digestibility using dry corn, not HMC. Although the magnitude of corn endosperm effects on starch digestibility of HMC could be lesser due to benefits of fermentation on starch digestibility (KUNG *et al*., 2018), the hybrid effect should still be considered. Allen and Ying (2021a, b) evaluated the effects of endosperm type (floury or vitreous) in dry ground corn and HMC. Vitreousness of floury and vitreous corn was 8.6 and 81.0% of endosperm, respectively, in dry ground corn and 0 and 40.5% in high-moisture corn (before ensiling). Ruminal in vivo starch digestibility was 18.9%- and 13.1%-units greater for floury than vitreous endosperm in dry ground corn and HMC, respectively. However, compensatory intestinal digestion was observed in HMC (similar between endosperm types) and dry ground corn (2.0%-units greater for floury endosperm), and minor effects on production.

4. Maturity

Delayed maturity at harvest is known to impair the utilization of the kernel fraction of HMC. Ferraretto *et al*. (2014) observed that the 7-h ruminal in vitro starch digestibility of HMC was negatively correlated to HMC DM concentration. The reduction in starch digestibility with advanced maturity is partially associated with the increase in the proportion of vitreous endosperm at greater maturities (CORREA *et al*., 2002; FERRARETTO *et al*., 2018b). The increase in kernel vitreousness, which also increases kernel hardness, may also increase the resistance of kernels to mechanical processing (FERRARETTO *et al*., 2018b). Additionally, metabolic water available for the growth of bacteria in the silo becomes limiting as corn plants mature and DM concentrations (in the plant and within the kernel) increase (MUCK, 1988). Reduced concentrations of organic acids, suggesting a limited fermentation, are frequently observed in more mature corn silages (KUNG *et al*., 2018).

Baron *et al*. (1986) observed an increase in pH and a reduction in concentrations of acids as DM levels in ensiled corn grain increased from 78 to 64%. Goodrich *et al*. (1975) ensiled corn grain at harvest (67% DM) or after air drying to DM concentrations of 72.5 or 78.5%. In this study, pH increased as DM concentrations decreased. Concentrations of lactic acid were greater in corn grain ensiled at 67% and 72.5% DM compared to those in corn grain ensiled at 78.5% DM. Considering that up to 60% of the proteolysis that occurs in the silo can be attributed to bacterial activity (JUNGES *et al*., 2017), it is possible that a reduction in bacterial proteolysis may also be contributing to the reduction in starch digestibility frequently observed in more mature HMC. In an assessment of over 6,000 HMC samples, Ferraretto *et al*. (2014) observed that pH of HMC increased as DM concentrations increased from 65 to 80%. In this same study, a negative correlation between DM concentration and indicators of proteolytic activity was observed (FERRARETTO *et al*., 2014). Increasing DM concentrations decreased concentrations of ammonia-N and soluble CP. Since proteolytic activity is the primary mechanism by which starch digestibility of HMC is increased with ensiling, DM concentration was also found to be negatively associated with 7-h ruminal in vitro starch digestibility of HMC (FERRARETTO *et al*., 2014). Recently, our laboratory conducted a study to evaluate the effect of fermentation on starch digestibility without having different vitreousness (SAYLOR *et al*., unpublished). Briefly, this was accomplished by increasing DM concentrations in HMC through oven-drying at a low temperature as an alternative to a delayed harvest, like the method used by Goodrich *et al*. (1975). A more robust fermentation, proteolysis, and ruminal in situ starch disappearance were achieve with progressing storage when HMC was ensiled at 65% compared to 70% DM (SAYLOR *et al*., unpublished). These findings highlighted harvesting HMC at the appropriate maturity is essential for improving starch digestibility. It is recommended that HMC be harvested near 70% DM to optimize nutritive value and lactation performance.

Fernandes *et al*. (2021) conducted an experiment to evaluate the influence of hybrid, moisture, and length of storage on the fermentation profile and starch digestibility of corn grain silages. Two corn hybrids (flint and soft) were harvested at either 50% DM, 70% DM, or 80% DM. The 50 and 70% DM corn grain was ground and ensiled. The 80% DM corn was rehydrated with water to reach an approximate DM concentration of 70%, ground, and then ensiled. Silos were stored for 0, 7, 21, 60, and 120 d. Fernandes *et al*. (2021) found that concentrations of zein proteins were 24% higher for the flint hybrid compared to soft hybrid in the 70% DM and rehydrated corn grain at ensiling. However, after 7 d of storage, there were no differences in zein protein concentrations between the two hybrids. Furthermore, it was observed that ruminal in situ DM and starch digestibility at 24 h was similar for all silages after 60 d of storage, independent of the hybrid type or moisture content. Extended time in storage increased ammonia-N, soluble CP, and in vitro starch digestibility in whole-plant corn silage of various hybrids, maturities, and chop lengths (FERRARETTO *et al*., 2015a). However, extended fermentation did not attenuate the negative effects of kernel vitreousness and maturity at harvest on in vitro starch digestibility (FERRARETTO *et al*., 2015a). A similar approach was used to evaluate the effects of prolonged storage in earlage harvested at either ½ of the kernel milk line or black layer stage in earlage (FERRARETTO *et al*., 2016; FERRARETTO *et al*., 2018a). Both studies reported greater ruminal in vitro starch digestibility at 7 h for early maturity earlage from 0 to 240 d of storage.

Further research studying the effects of storage length on starch digestibility of whole-plant and fractionated corn silage of different maturities or DM concentrations is warranted. Because literature is conflicting about fermentation eliminating differences in starch digestibility, targeting for proper moisture is advised.

5. Particle size

Intact kernels reduce starch digestibility of whole-plant and fractionated corn silage (FERRARETTO *et al*., 2018b). Therefore, thorough kernel processing is essential for exposing starch in the endosperm to microbial degradation in the rumen and enzymatic digestion in the small intestine. Corn kernels that have been extensively processed have increased surface area available for microbial attachment and enzymatic degradation (HUNTINGTON, 1997). Ekinci and Broderick (1997) conducted an experiment to evaluate the effect of processing HMC on ruminal fermentation and milk yield. In this study, HMC was rolled prior to ensiling. Ground HMC was processed further by grinding through a 9.5-mm screen which reduced the geometric mean particle size from 4.33 to 1.66 mm. Intake and 4% FCM yield were greatest for cows fed a diet containing ground HMC and lowest for cows fed a containing the rolled HMC. Additionally, grinding HMC was found to increase digestibilities of DM, organic matter, and starch. In the same study, it was observed that grinding of HMC decreased pH and increased total volatile fatty acid concentration in ruminal in vitro incubations. Overall, authors concluded that grinding of HMC improved its utilization by lactating cows by stimulating ruminal fermentation (EKINCI; BRODERICK, 1997). In a meta-analysis evaluating the effect of corn grain harvesting and processing methods on dairy cow performance, Ferraretto *et al*. (2013) observed that total-tract starch digestibility of HMC was 95.2% when the geometric mean particle size (GMPS) was < 2,000 µm (average of 1,450 µm), and 89.5% when GMPS was > 2,000 µm (average of 3,630 µm). Interestingly, lactation performance was found to not be different between cows fed HMC with a GMPS > 2,000 µm and those consuming HMC with a GMPS < 2,000 µm (FERRARETTO *et al*., 2013).

Hoffman *et al*. (2012) observed a moderate negative relationship between mean particle size (MPS) of HMC and the peak absolute rate of ruminal in vitro gas production, supporting the hypothesis that a reduction in MPS of HMC provides more available substrate for ruminal fermentation. However, the extensive degradation of the starch-protein matrix during storage of HMC complicates this relationship, suggesting that the MPS of HMC “might not represent the true surface area available to rumen bacteria” when they degrade HMC starch (HOFFMAN *et al*., 2012). As a result, Hoffman *et al*. (2012) developed a measurement of effective MPS (eMPS) for HMC in which MPS is adjusted for concentrations of ammonia-N in the sample (an indicator of the extent of proteolytic activity in the silo) to predict HMC fermentation potential and subsequent animal responses (HOFFMAN *et al*., 2012). The use of eMPS, rather than MPS, was found to describe the relationship between processing and ruminal fermentability of HMC more-completely (HOFFMAN *et al*., 2012). Tools generated based on this model suggest reduced particle size partially compensate lower starch digestibility due to short-term fermentation.

Few studies have evaluated the effect of kernel processing on fermentation of HMC. Baron (1986) observed greater concentrations of soluble

N in ground compared to whole HMC ensiled for 30 d at 67% DM. Recently, Gomes *et al*. (2020) conducted a study to evaluate the effects of processing, moisture, and storage length on the fermentation, particle size, and ruminal DM disappearance of rehydrated corn grain silage. Dry corn was ground or rolled and then rehydrated to 30, 35, or 40% moisture prior to ensiling. Silos were stored for 0, 14, 30, 60, 90, 120, or 180 d. In this study, processing, moisture, and storage length were found to influence pH and concentrations of fermentation end-products. Compared to rolling, grinding slightly increased concentrations of acetic acid which the authors attributed to greater availability of substrate for fermentation (GOMES *et al*., 2020). Saylor *et al*. (2020) evaluated the effect of microbial inoculation and particle size on fermentation, aerobic stability, and starch digestibility of HMC ensiled for a short period. In this study, concentrations of lactic acid were found to be greater in finely ground (958 µm) compared to coarsely ground (4,448 µm) HMC after 28 d of storage. Authors attributed these differences to greater exposure of kernel sugars to microbial fermentation with finely compared to coarsely ground HMC. In a study by Saylor *et al*. (2021), pH was reduced, and concentrations of lactic, acetic, and total acids were greater in ensiled corn grain with broken kernels compared to that with intact kernels. Results from these two studies (SAYLOR *et al*., 2020, 2021) also suggested that proteolytic activity (indicated by greater concentrations of soluble CP and ammonia N) was increased when ensiled corn grain was more extensively processed.

Overall, research studies suggest finely grinding HMC improves fermentation and starch availability. Assessment of machinery, labor, time, and energy costs is advised when determining the best particle size for each operation.

6. Enzymes

In recent years, interest in additives capable of enhancing starch digestibility of HMC has grown. Most of the research in this area has studied the ability of proteases to increase starch digestibility by hydrolyzing the protein matrix that surrounds starch granules (KUNG *et al*., 2014; FERRARETTO *et al*., 2015b). Ferraretto *et al*. (2015b) observed a 6.5%- to 7.5%-unit increase in starch digestibility when HMC was treated with an exogenous protease. In a study by

Kung *et al*. (2014), starch digestibility increased with ensiling in both control- and protease-treated HMC, but the effect was greater in HMC treated with the protease. Although it is still uncertain if the cost of proteases can be offset by the economic value gained by improving starch digestibility, the data suggest that protease application in HMC is effective and may offer producers with a greater degree of flexibility regarding storage length. In addition to exogenous proteases, certain microbial inoculants may have potential to improve starch digestibility of HMC.

7. Microbial inoculation

When the epiphytic microbial population present on the plant at harvest is insufficient, silage may be treated with exogenous microorganisms, in the form of bacterial inoculants, to improve the rate, extent, and quality of fermentation that occurs during the storage period. Numerous benefits of microbial inoculation exist, making it one of the most effective strategies to optimize the nutritive value of HMC.

Silages treated with homofermentative bacteria have a lower pH, reduced concentrations of acetic acid, butyric acid, and ammonia N, as well as greater concentrations of lactic acid and improved DM recovery compared to untreated silages (MUCK; KUNG, 1997). Silages treated with heterofermentative bacteria, specifically *L. buchneri*, are known to have increased concentrations of acetic acid and a greater pH compared to untreated silages. Losses of dry matter can also be greater with *L. buchneri* due to the loss of carbon dioxide associated with the conversion of lactic to acetic acid (OUDE ELFERINK *et al*., 2001). A recent meta-analysis of 158 peer-reviewed manuscripts by Arriola *et al*. (2021) evaluated the effects of inoculation with *L. buchneri* on various silage quality parameters. Arriola *et al*. (2021) found that *L. buchneri* inoculation tended to increase pH in HMC. When *L. buchneri* converts moderate amounts of lactic to acetic acid, pH increases as acetic acid is a much weaker acid than lactic acid (KUNG *et al*., 2018).

Under laboratory conditions, aerobic stability, an indicator of the extent of aerobic deterioration, is defined as the time required (h) for the temperature of silage exposed to air to increase 2°C above the ambient or silage baseline temperature (MORAN *et al*., 1996). Effects of microbial inoculation on counts of spoilage organisms (like yeasts and molds) and aerobic stability of HMC are of great interests to producers. Muck and Kung (1997) reported that the use of homofermentative inoculants reduced aerobic stability in a third of the studies they summarized, and that this response occurred primarily in corn silage. In the meta-analysis by Oliveira *et al*. (2017), aerobic stability of various forages and crops was not affected by inoculation with homofermentative LAB, although yeast counts were greater in inoculated silage. As the initiators of aerobic deterioration, elevated yeast counts may contribute to reduced aerobic stability in these silages (Pahlow *et al*., 2003; Muck *et al*., 2018).

One of the primary benefits of inoculation with heterofermentative LAB is the potential improvement in aerobic stability, associated with elevated concentrations acetic or propionic acid. Acetic and propionic acid have strong antifungal activity which make them uniquely capable to inhibit the growth of yeasts and molds, preventing aerobic deterioration and maintaining aerobic stability. Arriola *et al*. (2021) reported that inoculation with *L. buchneri* reduced yeast and mold counts and improved aerobic stability in HMC. Across multiple crop types, these responses were found to be dose-dependent, however. Inoculation with 10^5, 10^6, and ≥ 10^7 cfu of *L. buchneri* per g of fresh forage reduced counts of yeasts and molds. No effects of inoculation on yeasts and molds were observed when a rate of ≤ 10^4 cfu/g was applied (Arriola *et al*., 2021). All inoculation rates were found to increase aerobic stability, but the magnitude of improvement was lower when *L. buchneri* was applied at ≤ 10^4 cfu/g. It should be noted that the risk of aerobic deterioration and potential benefits associated with heterofermentative microbial inoculation increase substantially during summer months, as elevated temperatures can increase the growth of spoilage organisms in silage (BERNARDES *et al*., 2018). Additionally, in warm weather, plastic films used to cover silos can become more permeable to air (BERNARDES *et al*., 2018).

Effects of microbial inoculation on dairy cow performance have been inconsistent. In a meta-analysis of 31 lactating dairy cattle studies, Oliveira *et al*. (2017) found that inoculation with homofermentative LAB increased milk production and tended to increase DMI. Inoculation also tended to increase concentrations of milk fat and milk protein (Oliveira *et al*., 2017). However, total-tract DM digestibility was unaffected by inoculation (Oliveira *et al*., 2017). An explanation for the improvement in animal performance associated with homofermentative microbial inoculation has been difficult to ascertain. In some studies, changes in silage characteristics cannot explain the observed production responses. In other studies, inoculation improved cow productivity without affecting silage fermentation (Muck *et al*., 2018). There is some evidence to suggest that improved performance associated with microbial inoculation may be a result of the inhibition of detrimental microorganisms in silage (ELLIS *et al*., 2016), an interaction between LAB and rumen microbes (MUCK *et al*., 2018), or altered rumen fermentation (WEINBERG *et al*., 2003). Contreras-Govea *et al*. (2011) found that corn silages treated with *L. plantarum* or *Lactococcus lactis* yielded more rumen microbial biomass than untreated silages. In the meta-analysis by Arriola *et al*. (2021), 12 studies evaluated the effect of inoculation with *L. buchneri* on dairy cow performance. It was observed that feeding silage inoculated with *L. buchneri* did not improve DMI, total-tract DM digestibility, or milk yield.

9. Concluding Remarks

Production of HMC is very important to the dairy industry. Understanding the factors that affect the nutritive value and fermentation of HMC is essential for optimizing its inclusion in dairy diets. Numerous management factors influence the nutritive value of HMC, including hybrid type, maturity at harvest and fineness of grind. Furthermore, the foundational principles of silage microbiology and biochemistry have paved the way for numerous strategies that have potential to enhance the fermentation, nutritive value, and digestibility of the HMC fed to dairy cattle.

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