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Silage Fermentation End Products and Microbial Populations: Their Relationships to Silage Quality and Animal Productivity

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Abstract

Understanding the roles that various microbes play in silage fermentation and the factors that affect their growth can help us understand why silages ferment in various ways. In many, but not all cases, the fermentation that a crop undergoes can be explained by how microbes interact with factors such as moisture content, buffering capacity, and sugar content. However, management factors such as silo packing speed, silage pack density, type of additive used, chop length, silo management during storage, and silo management during feed-out can also affect the silage fermentation. In some cases fermentation analyses can qualitatively explain poor silage nutritive value or low intakes, but they cannot be used to balance diets for cattle. Thus, they should always be used in conjunction with other standard chemical analyses (i.e. ADF, NDF, CP, RDP/RUP, NE_L , NDF digestibility, etc.). A large portion of this review was patterned after a paper by Kung and Shaver (2001) and an invited presentation by Muck and Kung (2002).

Lactic Acid Bacteria and Their End Products

Lactic acid bacteria (LAB) are responsible for converting water soluble sugars to various end products. The two types of LAB include heterolactic acid bacteria (HAL) and homolactic acid bacteria (HOL). The former make lactic and acetic acids, ethanol and CO_2 (representing a loss of energy and dry matter) whereas the later make only lactic acid when fermenting 6-carbon sugars like glucose and fructose. Fermentation from HOL is more efficient than from HAL but under practical conditions sometimes fermentation from the latter may be desirable (specifically when aerobic stability of silage is a problem). Standing crops average about 10^4 to 10^6 colony forming units (CFU) of LAB per gram of wet forage. Most of these organisms are “wild” HAL. Frost, drought conditions, and exposure to high heat and UV rays from the sun may lower their numbers. The population on hay crops usually increases while the crop is drying in the field. During the early stages of ensiling, populations of total LAB may reach 10^9 to 10^{10} CFU/g of forage but after months of storage, their numbers usually decrease to 10^4 to 10^6 CFU/g. HOL have traditionally been the microbes of choice for silage inoculants because they can rapidly produce lactic acid and minimize DM losses. Lactic acid is primarily responsible for lowering the pH of the forage crop during ensiling, but it has weak antifungal activity.

Some strains of lactic acid bacteria from silage inoculants may provide a “probiotic” effect in the rumen and have the ability to improve fiber digestion by possibly altering ruminal fermentations (Weinberg et al., 2007). In addition, several strains of lactic acid bacteria have been identified with the ability to express ferulic acid esterase activity and also have the potential to increase fiber digestibility (Nsereko et al., 2007). When utilizing microbial inoculants, even distribution of the microbes throughout the silo mass is important. In drier silages ($\geq 40\%$ DM), application

of the inoculant in water (rather than from a dry form) results in faster activation and better growth of the added microbes. When using water-based inoculants, keep the temperature of the water below 100°F. Enumerating for LAB in forages and silages does not provide helpful information to the end user.

Fungi and Their End Products

Yeasts and molds (fungi) are also naturally found on the standing crop. In general, corn silage, and high moisture corn usually contain 10^5 to 10^6 CFU of yeasts and molds per gram of wet forage, but their numbers are usually lower on alfalfa and grasses (10^3 to 10^5 CFU/g). Numbers of yeasts and molds may increase when the plant has been physically damaged (insects, birds, hail, lodging, etc.). Molds are undesirable because under certain conditions some molds on the plant may produce mycotoxins. Specifically, the mold *Aspergillus fumigatus* has been implicated in causing hemorrhagic bowel syndrome (Puntenney et al., 2004). Most molds generally do not survive the ensiling process, but ensiling has minimal effects on mycotoxins already formed in the field.

Yeasts are also undesirable microbes in the silage process because they compete with LAB for sugars and they can be relatively acid tolerant and survive the ensiling process. During anaerobic conditions yeasts can ferment sugars and produce substantial amounts of CO₂, ethanol (representing a loss in DM), some volatile acids and other minor alcohols. Most yeasts make ethanol when small amounts of air are present, but their growth can be inhibited when there is no oxygen present. Their growth is also probably limited by negative feedback from end product inhibition (high levels of alcohol) and lack of fermentable substrates. Thus, fast and tight packing of silos to remove as much oxygen as possible is one key to limit their growth. If the silage mass remains strictly anaerobic, non-lactate assimilating yeasts are the dominant yeasts at silo opening. However, if air enters the silage mass during storage, lactate assimilating yeasts (e.g. species of *Candida* and *Hansenula*) predominate, and at feed out when they are further exposed to air, they can destabilize the silage system leading to spoilage. After yeasts initiate the spoilage, growth of molds and aerobic bacteria follow causing more deterioration. The number and types of yeasts and molds on the standing plant are generally not well correlated with the numbers of yeasts and molds at silo opening. However enumerating yeasts and molds in silages may sometimes be useful as high numbers (usually $\geq 10^6$ CFU/g of silage) may be an indication for a high propensity of that silage to spoil when exposed to air. Many nutritionists feel that there is a correlation between high yeasts in silages and a variety of animal issues (e.g., low milk fat tests, low milk production and/or poor intakes). We speculate that spoilage yeasts may compete in the rumen for nutrients, alter fatty acid metabolism in the rumen, or may produce toxins that may negatively affect ruminal fermentations. However, to date, there is no published research that definitively links high numbers of spoilage yeasts in silages and poor animal performance.

If silages are high in yeasts and/or molds and the silage or total mixed ration is heating and spoiling, steps should be made to minimize further spoilage. These would include feeding more from the silage face each day, using a face shaver to minimize the infiltration of air into the silage mass, and/or adding a buffered propionic acid-based preservative to the silage or TMR. Use of buffered propionic acid-based preservatives added to the TMR should not be considered a preferred solution to spoiled feeds as significant amounts of spoilage could be occurring in the

silo. During hot weather yeasts may enter the TMR from sources other than silages. For example, dirty feed bunks may harbor large quantities of spoilage organisms and wet distillers grains that have been exposed to air for several days may bring contribute to their numbers.

Enterobacteria and Their End Products

Enterobacteria are usually quite high on standing forage crops (10^5 to 10^6 CFU/g of wet forage). Their numbers can increase quickly immediately after silo filling, and they are undesirable for a number of reasons. First, they compete for fermentable substrates with LAB. Although they produce primarily acetic and lactic acids, they also produce ethanol and butyric acid. They can also produce biogenic amines, but the results of research studies have varied on how severely these compounds may affect animal performance as some destruction of these compounds occurs in the rumen. Some enterobacteria are pathogenic (e.g. *E. coli* and *Listeria monocytogenes*), and some also have the ability to reduce nitrates that can result in large quantities of potentially toxic gasses (commonly called silo gas but containing various nitrogen oxides) that can affect humans and animals. Enterobacteria are extremely intolerant to low pH (< 5.0), and thus in fast fermenting silages their numbers decline rapidly as pH declines and they usually cannot be found in well fermented silages. However, pockets of air within a silo may provide suitable environments such that high numbers of these organisms can be found in some silage (Fenlon and Wilson, 2001). Enumeration of enterobacteria in silages has been not used in the field as a diagnostic tool.

Clostridia and Their End Products

Clostridial organisms can sometimes dominate silage fermentation especially if there is high moisture, a slow drop in pH and/or if there is a lack of fermentable substrates. The two general types of clostridia are saccharolytic (sugar degraders) and proteolytic (protein degraders) in nature. The undesirable fermentation of clostridial silages are dominated by putrid smells, high pH and high levels of butyric acid, acetic acid, propionic acid, ammonia-N, and amines. Clostridia have also been implicated in hemorrhagic bowel syndrome. Clostridia are unable to thrive when the osmotic pressure is high. Thus, wilting forage crops above 30-35% DM greatly reduces their chance of dominating silage fermentation. Excessive wilting times and rain damage often results in losses of fermentable substrates and thus should be avoided to prevent clostridial fermentations. The practice of chopping forages and leaving them in forage wagons overnight should be avoided as this leads to excessive respiration and a reduction in sugars.

Although not totally substantiated, Kung and Muck (2002) reported finding a general trend that suggested clostridial fermentations in alfalfa where more saccharolytic from the Midwest while they tended to be more proteolytic from the East. Relative to Midwestern alfalfa with high moisture contents, Eastern alfalfa silage with high moisture contents had a higher pH and ammonia-N but lower lactic and butyric acids. Enumeration of clostridia in silages has been not used in the field as a diagnostic tool because butyric acid is a good marker for their growth.

The Significance and Fate of Silage Fermentation End Products

A table with typical silage fermentation profiles is shown in Table 1. There have been many attempts to correlate the end products of silage fermentation with animal performance. The results of these studies have not always been in total agreement (Rook and Gill, 1990; Steen et al., 1998) relative to the effects that silage acids may have on affecting DM intake. Eisner et al.

(2006) reported that the concentration of acetic acid in silage was negatively correlated with intake when silage and concentrates were fed separately. However, total acid concentration was the best factor that increased the fit of a model for the prediction of DM intake prediction when animals were fed a total mixed ration. In a more recent summary, Huhtanen et al. (2007) reported that only total acid concentration and propionic acid concentration were negatively correlated with intakes in lactating cows. In steers Krizsan et al. (2007) reported that 71% of the variance of intake of 24 low DM grass silages was best explained by total acids, total volatile fatty acids, lactic acid/total acid ratio and propionic acid. Total acid concentration in silages is negatively correlated to dry matter content of the crop. In drier silages, fermentation becomes restricted because water activity limits the growth of microbes responsible for fermentation. Overall, these studies tend to support the anecdotal reports from the field that intakes are depressed when lactating cows are fed wet silages that tend to have high concentrations of total acids.

Ingested lactic acid from silage is rapidly converted to propionic acid in the rumen by *Selenomonas ruminantium*, *Megasphaera elsdenii* or Propionibacteria. This is especially true if the rumen microbes are well adapted to metabolize lactic acid. Propionic acid is then absorbed from the rumen and is converted to glucose by the liver of the cow. Under certain conditions, extremely high levels of lactic acid (and total acids) in silages may contribute to sub acute acidosis and may affect intakes.

Acetic acid ingested from silages is absorbed from the rumen and ultimately contributes to milk fat production and energy metabolism in the cow. Feedback from the field suggesting that silages high in acetic acid depress intake must be interpreted with caution because this acid can be produced from a variety of organisms (e.g., enterobacteria, lactic and acetic acid bacteria, clostridia and bacilli). Other negative end products from these organisms may also play a role in reduced intake from silages and thus acetic acid may only be a marker for poor fermentations. For example, Figueiredo et al. (2007) reported finding at least 168 volatile compounds in red clover silage and the effect(s) of most of these compounds on DM intake in ruminants has not been studied. Silages treated with the heterolactic acid bacterium *L. buchneri* have moderately higher concentrations of acetic acid than untreated silages, but because this is a “controlled” acetic acid fermentation, depressions in intake when feeding these treated silages have not been observed (Dreihuis et al., 1999; Kendall et al, 2002; Kung et al., 2003; Ranjit et al., 2002; Taylor et al., 2002).

Propionic acid is seldom found in well fermented silages. Although Propionibacteria are able to produce this acid from glucose and lactic acid it is doubtful if this occurs in good silage because these organisms are very intolerant of a low pH. It is more common to observe high levels of propionic acid (> 0.3 to 0.5%) in poorly fermented silages, especially because it can be an end product from some strains of Clostridia. Added amounts of propionic acid would be extremely difficult to detect. For example, if one added 2 lb (about 60% propionic acid) of a propionic acid-based additive to 35% DM corn silage, this would increase the concentration of propionic acid in that silage by less than 0.2% (DM basis).

Ethanol is a high energy compound and much of it is converted to acetic acid in the rumen. Moderate levels of ethanol in the diet do not adversely affect ruminal fermentation. However,

high levels of ethanol (>4-5%) may be a problem because of some direct absorption which can cause off flavors in milk in addition to wobbly cows.

Butyric acid from silages adds to the ketogenic load of ruminants as it is converted to beta-hydroxybutyrate and acetoacetate (ketone bodies) in the rumen wall before entering the general circulation. Oetzel (University of Wisconsin, personal communication) recommends limiting the intake of butyric acid from silages to 50 g/d to prevent the possibility of ketosis. If possible, clostridial silages should not be fed to high producing cows in early lactation or to cows in the transition period. Clostridial silages also tend to become worse the longer they remain in the silo. Thus, feeding these silages out as fast as possible is recommended. If you have a silage that could be susceptible to clostridial fermentation such as hay crop silage ensiled on the wet side or after significant rain damage during wilting, feed that silage out early before it has a chance to go clostridial.

High concentrations of ammonia (>12 to 15% of CP) are a result of excessive protein breakdown in the silo caused by a slow drop in pH or excessive growth by clostridia or enterobacteria. In general, wetter silages have higher concentrations of ammonia. Extremely wet silages (< 30% DM) have even higher ammonia concentrations because of the potential for clostridial fermentation. Theoretically, high amounts of ammonia (by itself) in silage should not have negative effects on animal performance if the total dietary nitrogen fractions are in balance. However, if the high ammonia contributes to an excess of ruminally-degraded protein (RDP), this could have negative consequences on milk and reproductive performances. Blood or milk urea nitrogen can be used as an indicator of excess RDP. Often times, silage with high concentrations of ammonia coupled with butyric acid may also have significant concentrations of other undesirable end products, such as amines, that may reduce animal performance.

Some strategies for managing silages with high (>5%) acetic acid, high ethanol (> 3-4%), high butyric acid (> 0.5%) include: reducing the amount of that silage fed, aerating the silage to volatilize the acids, removing the silage and then gradually reincorporating it back into the diet over a 2–3 week period, and partially neutralizing the silage with sodium bicarbonate prior to feeding (about 0.5 to 1% addition on DM basis).

Table 1. Amounts of common fermentation end products in various silages.

Item	Alfalfa Silage, 30 - 35% DM	Alfalfa Silage, 45 - 55% DM	Grass Silage, 25 - 35% DM	Corn Silage, 35 - 40% DM	HM Corn, 75% DM
pH	4.3 - 4.5	4.7 - 5.0	4.3 - 4.7	3.7 - 4.2	4.0 - 4.5
Lactic acid, %	7 - 8	2 - 4	6 - 10	4 - 7	0.5 - 2.0
Acetic acid, %	2 - 3	0.5 - 2.0	1 - 3	1 - 3	< 0.5
Propionic acid, %	< 0.5	< 0.1	< 0.1	< 0.1	< 0.1
Butyric acid, %	< 0.5	0	< 0.5	0	0
Ethanol, %	0.5 - 1.0	0.5	0.5 - 1.0	1 - 3	0.2 - 2.0
Ammonia-N, % of CP	10 - 15	< 12	8 - 12	5 - 7	< 10

Conclusions

Various microorganisms interact during the ensiling process which may affect the nutritive value of silages for ruminants. Managing the forage crop in the silo has profound effects on the ensuing silage fermentation. Quick packing to eliminate air and use of microbial inoculants to dominate the ensiling process can help to assure a more desirable fermentation. Silage fermentation analyses can help to describe the type of fermentation that occurred in the silo. If questionable silage is being fed, an assessment should be made of general harvest and silo management to prevent similar problems with the next crop (e.g. is the silo face too wide, is the silage packed tight enough, is the silage too dry/wet, is the particle length too long, should one use an additive specifically designed to improve aerobic stability, etc.)

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