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Silage review: Interpretation of chemical, microbial, and organoleptic components of silages[1]

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ABSTRACT

The goal of making silage is to produce a stable feed with a high recovery of dry matter, energy, and highly digestible nutrients compared with the fresh crop. Microbial fermentation in the silo produces an array of end products and can change many nutritive aspects of a forage. High-quality silage should be void of undesirable compounds that could negatively affect animal performance, the environment, or net farm income. This review discusses the interpretation of the common fermentation end products, microbial populations, organoleptic properties, and changes in nutritive aspects of silages during storage of silages with emphasis on a North American perspective.
Key words: silage, fermentation

INTRODUCTION

In the absence of air, the fermentation of soluble carbohydrates in forages results in a variety of end products, ultimately resulting in the preservation of a forage crop as silage. Measuring the pH and quantifying the production of organic acids and alcohols are the main basis of evaluating silage fermentations. When deemed necessary, other components that are commonly quantified in silages include mycotoxins and a variety of nitrogenous compounds. Organoleptic characteristics can be used to assess silage quality because the volatile nature of many fermentation end products produces a variety of distinct odors. Cherney and Cherney (2003) provide a detailed summary of laboratory methods used to assess silage quality.

Although the chemical processes occurring in a silo have generally been thought to quickly reach a steady state after a few weeks of fermentation, it is clear that small but significant changes in some components continue to take place for months, and such processes can affect silage quality. In general, data from silage fermentation analyses can be used to determine whether an excellent, average, or poor fermentation has occurred. Based on these analyses, educated assumptions can be made that can be used to explain various outcomes. For example, the fermentation that a crop undergoes often can be explained by factors including moisture content, buffering capacity, sugar content, and types of organisms that dominated the process. Management factors such as the speed of packing, pack density, type of additive used, chop length, covering management, and silo management during feed-out can also affect silage fermentation and its subsequent quality. In some cases, fermentation analyses can qualitatively explain poor nutritive value or low intakes. The best way to evaluate the quality of silage is by sampling it appropriately and requesting both fermentative and nutritive analyses from an endorsed analytical laboratory. Cherney and Cherney (2003) provide recommendations for sample collection and shipment of forage samples to analytical laboratories. Our objective was to review the common chemical, microbial, and organoleptic properties of silages and the factors affecting them as they relate to the efficiency of silage fermentation, aerobic stability, nutritive value, animal performance, and potential effects on the environment, with emphasis primarily on a North American perspective.

INTERPRETATION OF DATA FROM CHEMICAL AND MICROBIAL ANALYSES OF SILAGES

The most common measurements used for evaluating silage fermentation include pH; the concentrations of organic acids, alcohols, and NH_3-N; and the size of various microbial populations. In an ideal fermentation, homolactic acid bacteria use water-soluble carbohydrates (e.g., glucose) for growth and produce only lactic acid, resulting in a relatively high recovery of DM and energy (Pahlow et al., 2003). However, the fermenta-

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Table 1. Typical suggested concentrations of common fermentation end products in various silages

Item	Legume silage <30–35% DM	Legume silage 45–55% DM	Grass silage 25–35% DM	Corn silage 30–40% DM	High-moisture corn 70–75% DM
pH	4.3–4.5	4.7–5.0	4.3–4.7	3.7–4.0	4.0–4.5
Lactic acid, %	6–8	2–4	6–10	3–6	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	<0.5–1.0	0	0
Ethanol, %	0.5–1.0	0.5	0.5–1.0	1–3	0.2–2.0
NH_3-N, % of total N	10–15	<12	8–12	5–7	<10

tion of forage crops is very complex and involves many types of microorganisms, resulting in variety of different end products. Table 1 shows typical recommended values for common fermentation end products from the primary types of silages in the United States (Kung and Shaver, 2001). Figures 1, 2, and 3 show trends in concentrations for these end products based on the DM of the crop for corn silage, legume silage, and high-moisture corn (**HMC**). The values in these figures must be viewed with caution because many silages sent in for analyses are "problem samples" and the potential exists for samples to be compromised during shipment to the laboratory, which may result in the analyses not truly reflecting the composition of the sample at the farm. For example, not all legume silages below 30% DM are clostridial, as shown in Figure 2; however, the probability of this happening certainly is greater when the moisture content is very high in these silages.

Silage pH and Lactic Acid

The pH of an ensiled sample is a measure of its acidity. Whole-plant corn and alfalfa (the primary forage crops for dairy cows in the United States) have pH levels that range from about 5.5 to 6 immediately after chopping. During ensiling, lactic acid (pK_a of 3.86), produced by lactic acid bacteria (**LAB**), is usually the acid found in the highest concentration in silages, and it contributes the most to the decline in pH during fermentation because it is about 10 to 12 times stronger than any of the other major acids [e.g., acetic acid (pK_a of 4.75) and propionic acid (pK_a of 4.87)] found in silages. Typical

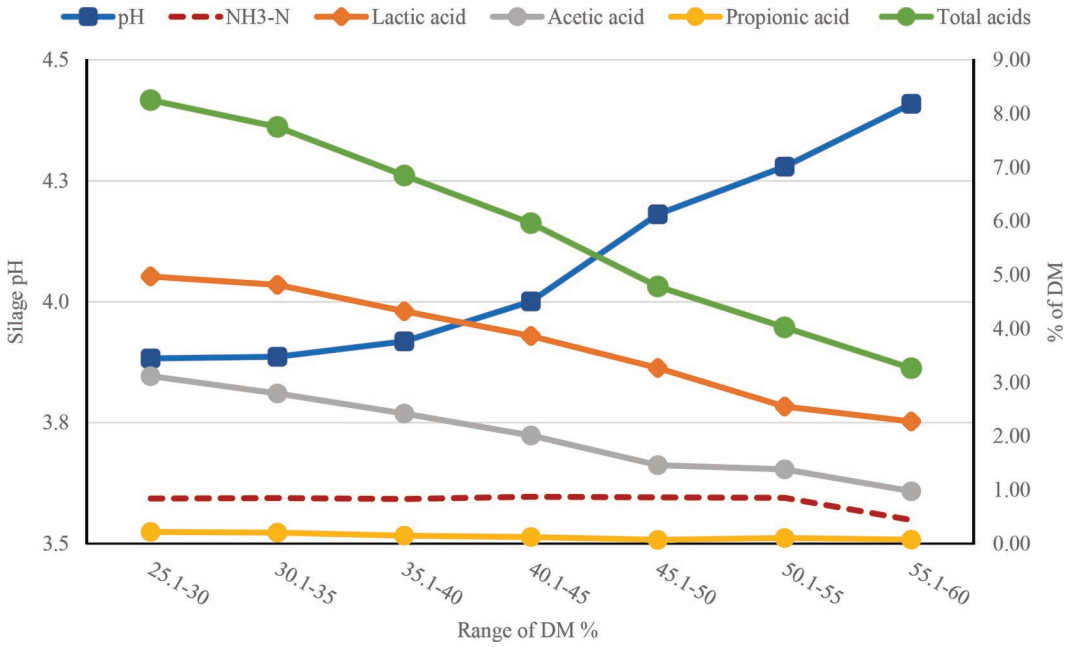

Figure 1. The pH and common fermentation end products of corn silage in the United States as affected by DM content. Ammonia-N is presented on a CP equivalent basis. Butyric acid was generally not found in corn silage samples, so values are not shown. Values are from samples analyzed by Cumberland Valley Analytical Services (Waynesboro, PA) between January 1, 2012, and August 31, 2017. Values are from wet chemical analyses. Color version available online.

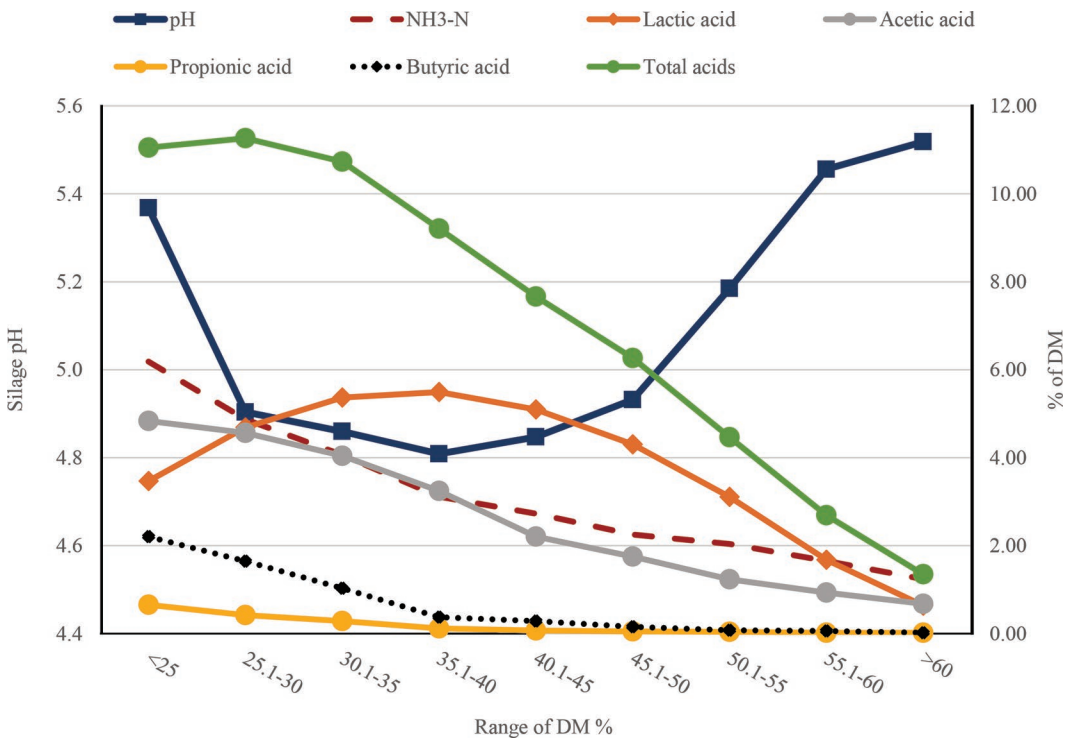

Figure 2. The pH and common fermentation end products of legume silage in the United States as affected by DM content. Ammonia-N is presented on a CP equivalent basis. Values are from samples analyzed by Cumberland Valley Analytical Services (Waynesboro, PA) between January 1, 2012, and August 31, 2017. Values are from wet chemical analyses. Color version available online.

concentrations of lactic acid in commonly fed silages range from 2 to 4% of the DM but can be considerably higher in silages with low concentrations of DM (<30%; Table 1). Under normal feeding conditions, lactic acid from silage is converted to propionic acid in the rumen. The final pH of silage is affected by many factors

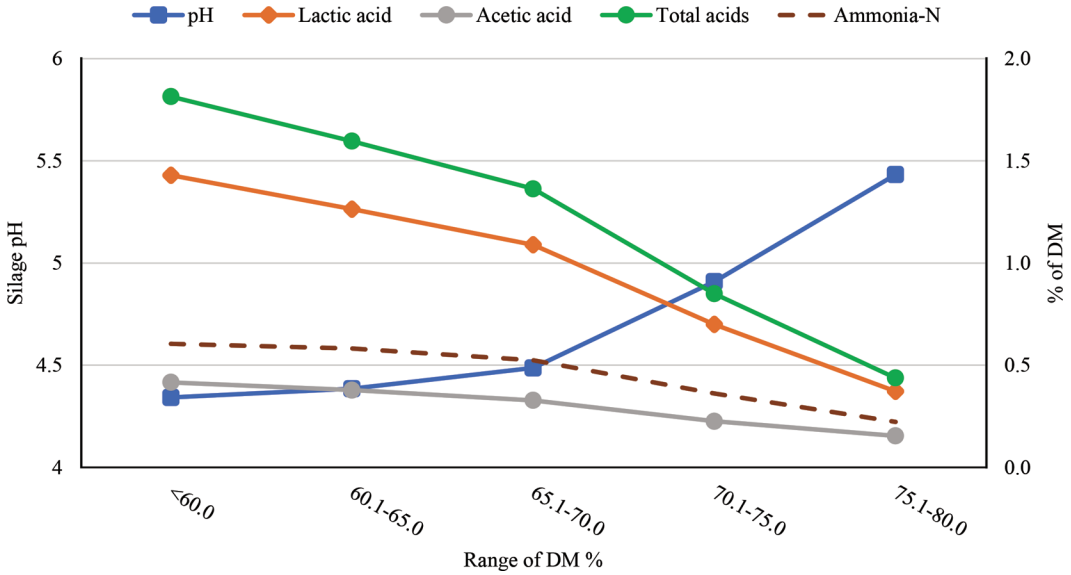

Figure 3. The pH and common fermentation end products of high-moisture corn in the United States as affected by DM content. Values are from samples analyzed by Cumberland Valley Analytical Services (Waynesboro, PA) between January 1, 2012, and August 31, 2017. Values are from wet chemical analyses. Color version available online.

but is most related to the concentration of lactic acid and buffering capacity of the crop. Corn silages have a lower final pH (3.7–4.0) than legume silages (4.3–5.0; Table 1) because they have lower buffering capacities (buffering capacity of 200–250 mE/kg of DM for corn and 500–550 mE/kg of DM for legumes; McDonald et al., 1991). In Figures 1 and 2, silage pH is at its lowest in corn and legume silages at about 30 to 35% DM. The low pH from lactic acid stabilizes silage fermentation by inhibiting the growth of or killing microbes intolerant of a low pH. However, as DM increases above 40 to 45%, silage pH increases. This occurs because metabolic water available for growth of lactic acid bacteria starts to become limiting as silage DM increases (Whiter and Kung, 2001). Drier silages that ensile well may spoil quickly when exposed to air because these silages tend to be more porous in the silo than wetter silages and they lack sufficient amounts of organic acids (e.g., acetic acid) with antifungal activity to suppress the growth of lactate-assimilating yeasts that initiate aerobic spoilage. Treatment with a homolactic acid inoculant can result in a lower silage pH compared with an untreated silage because of the greater production of lactic acid and may be more evident in legume than corn silage (Muck and Kung, 1997).

For legumes, the concentration of lactic acid has the potential to decrease as silage DM decreases below 35 to 40% (see Figure 2) because clostridial organisms can thrive in wet conditions (<30–35% DM) and convert lactic to butyric acid. However, this is not the case for corn silages (Figure 1) because the lower pH of this crop, compared with legumes, prevents the growth of clostridia. As previously noted, not all wet legume silages succumb to clostridial fermentations, although the probability of this occurrence is increased when the moisture content is above 70%. Thus, the recommended values for lactic acid for crops below 30 to 35% DM, as presented in Table 1, are higher than what is observed in Figure 1.

Several factors can be responsible for silages that present a pH that is higher than normal. For example, an abnormally high buffering capacity (e.g., in legume silages with very high protein and ash contents) or a restricted fermentation (e.g., cold climatic conditions) may be the cause of a higher than expected pH. Silages inoculated with *Lactobacillus buchneri* often will be 0.1 to 0.2 pH units higher than untreated silage (Kleinschmit and Kung, 2006) because of the moderate conversion of lactic acid to acetic acid, 1,2-propanediol (**1,2PD**), and ethanol (Oude Elferink et al., 2001). Aerobic spoilage initiated by lactate-assimilating yeasts can also be responsible for higher than normal pH values in silages (McDonald et al., 1991). For example, Ranjit and Kung (2000) reported that corn silage increased from pH 3.8 to pH 5 after exposure to air for 3 d. Aerobic spoilage of silage is accentuated in warm weather. Ammoniated corn silages typically have higher than normal pH (~4.0) because of the alkalizing effect of the added ammonia (Kung et al., 2000). Clostridial metabolism of lactic to butyric acid often explains why some legume and grass silages with less than 30 to 35% DM have higher than normal silage pH. In contrast, extremely low pH (<3–3.5) has been observed in some wet corn and grass silages and may be the result of the reaction of NO_2 and water being converted to nitric acid. Enterobacteria can convert nitrate to nitrite, which under acidic conditions can be converted to NO and NO_3 in a 2:1 ratio. In the presence of oxygen, NO rapidly converts to NO_2 and NO_x (Pahlow et al., 2003).

Volatile Acids

Acetic acid is the acid found in the second highest concentration in silage, usually ranging from 1 to 3% of DM (Table 1). Similar to lactic acid, the concentration of acetic acid is usually inversely related to DM content (Figures 1–3). When acetic acid in silage is consumed by a ruminant, it can be absorbed from the rumen and used for energy or be incorporated into milk or body fat. Moderate concentrations of acetic acid in silage can be beneficial because they inhibit yeasts, resulting in improved stability when silage is exposed to air. In fact, silages with very low concentrations of acetic acid may be unstable when exposed to air. Moderately higher than normal concentrations of acetic acid (~3–4%) are often found in silages treated with *L. buchneri* because of the conversion of lactic to acetic acid as previously discussed. The increase in acetic acid leads to an improvement in aerobic stability because acetic acid has strong antifungal characteristics. Silages treated with anhydrous or aqueous ammonia tend to have higher concentrations of acetic acid than untreated silage because the fermentation is prolonged by its buffering (Kung et al., 2000). Excessively high concentrations of acetic acid (>4–6%) are most often detected in extremely wet (>70% moisture) silages characterized by unwanted (but natural) fermentations dominated by enterobacteria, clostridia, or heterolactic acid bacteria (McDonald et al., 1991). Legume silages with high ash contents (>15%) also are sometimes very high in acetic acid because of prolonged fermentations.

Propionic acid is usually undetectable (especially in drier silages) or in very low concentrations (<0.1%) in good silages. Propionibacteria that convert glucose and lactic acid to propionic and acetic acid have been found in silages, but it is doubtful that natural populations can flourish in most silages. High concentrations of propionic acid (>0.3–0.5%) are more commonly found in

clostridial fermentations, likely a result of *Clostridium propionicum.* Use of additives containing propionic acid to improve the aerobic stability of silages can increase its concentration at ensiling by about 0.15 to 0.30% (DM basis) when added at 1 to 2 kg/t of wet (~35% DM) forage, but this is dependent on the proportion of the acid in the additive. Silages treated with *L. buchneri* sometimes have higher concentrations of propionic acid because 1,2PD can be converted to this acid by *Lactobacillus diolivorans* (Krooneman et al., 2002). Consumed propionic acid is absorbed by the rumen and converted to glucose by the cow's liver.

Butyric acid should not be detectable in well-fermented silages. The presence of this acid indicates metabolic activity from clostridial organisms, which leads to large losses of DM and poor recovery of energy (Pahlow et al., 2003). Some clostridia are able to ferment sugars to butyric acid (saccharolytic), some can convert lactic to butyric acid, and some species are highly proteolytic. Besides the presence of butyric acid and lower than normal concentrations of lactic acid, clostridial silages are often characterized by a higher than normal pH and higher than normal concentrations of acetic acid, NH_3-N, and soluble protein (Figure 2 shows some of these trends). Clostridial silages also tend to have high concentrations of fiber and low DM digestibility because much of the readily available soluble nutrients have been degraded (Mills and Kung, 2002). High levels of biological amines (varying by type of silage; e.g., putrescine, cadaverine, tyramine, and histamine) can sometimes be found in clostridial silages, which can adversely affect animal performance (Scherer et al., 2015), but these compounds are not routinely analyzed by commercial feed laboratories. The chances of a clostridial fermentation can be minimized by ensiling forages above 30 to 35% DM and inducing a rapid production of lactic acid because clostridia are intolerant of both high osmotic pressure and low pH. Delayed silo filling or prolonged wilting periods, resulting in a substantial reduction in fermentable sugars, can also result in a clostridial fermentation (Mills and Kung, 2002). Minimizing contamination of forage from soil and manure during harvest should also be practiced because they are the primary source of clostridial spores. Intake of high concentrations of butyric acid (more than 50–100 g/d) can induce ketosis in lactating cows, and because the energy value of the silage is low, intake and production can suffer (Oetzel, 2007). Paradoxically, silages with butyric acid tend to be stable when exposed to air because this acid has strong antifungal characteristics.

There have been many attempts to correlate the concentrations of silage acids with effects on animal performance. For example, total acids and acetic acid have been commonly implicated as factors that negatively affect intake in ruminants (Rook and Gill, 1990; Steen et al., 1998), but the results have been equivocal (Huhtanen et al., 2007; Krizsan et al., 2007). Eisner et al. (2006) reported that the concentration of acetic acid in silage was negatively correlated with intake when silage and concentrates where fed separately, but not when the silage was fed as part of a TMR. There has been speculation that decreased intake with silages high in acetic acid may be due to organoleptic factors or unidentified negative factors associated with a poor fermentation rather than due only to the high concentrations of acetic acid itself. Huhtanen et al. (2007) reported that total acid concentration and propionic acid concentration of silages were negatively correlated with intakes in lactating cows. They pointed out that the negative correlation between propionic acid and intake was probably not due directly to the acid itself but rather to other effects associated with poor fermentation that produce this acid.

Alcohols and Esters

Ethanol is the alcohol most commonly found in silages. It can be produced by a variety of microbes (heterolactic acid bacteria, enterobacteria, and yeasts) and is usually low in whole-plant corn and legume silages (0.5–1.5%). Ingested ethanol is converted to acetic acid in the rumen or absorbed by the rumen wall (Bruning and Yokoyama, 1988) and subsequently can be converted to milk fat or is available for body metabolism or growth. Driehuis and van Wikselaar (2000) reported that concentrations of ethanol as high as 5 to 6% could be found in some Dutch grass silages. Sugarcane silage is not fed in North America, but such silages can contain in excess of 15% ethanol on a DM basis (Kung and Stanley, 1982; Daniel et al., 2013) because high numbers of epiphytic yeasts convert sucrose to ethanol. High concentrations of ethanol in silages (>3–4%) are often associated with high numbers of yeasts, and such silages usually spoil readily when exposed to air because some yeasts can assimilate lactic acid under these conditions. High amounts of ethanol are also associated with high losses of DM and when fed in large quantities can cause off flavors in milk. Although cases of ethanol poisoning have been reported in ruminants (Peixoto et al., 2011), this is unlikely to occur in most commonly fed silages even with high concentrations of ethanol.

A variety of microorganisms can produce 1,2PD, including some species of clostridia and yeasts (Suzuki and Onishi, 1968; Sanchez et al., 1987). However, in typical silages, 1,2PD is most likely the result of metabolism of lactic acid by *L. buchneri.* Naturally occurring populations of *L. buchneri* can sometimes result in low concentrations of 1,2PD (0.1–0.3%) in silages,

especially if stored for prolonged periods of time (>6–8 mo). In silages inoculated with this organism, 1,2PD usually can be detected only after 30 to 60 d of fermentation because the degradation of lactic acid is not fully activated before this time. Silages inoculated with *L. buchneri* to improve aerobic stability may contain between 0.25 and 1.5% 1,2PD (but concentrations as high as 3% have been observed). Detection of 1,2PD can be loosely used to indicate whether inoculation with *L. buchneri* dominated the fermentation, but the naturally occurring microbe, *L. diolivorans*, that is sometimes present in the silage can metabolize this compound producing propanol and propionic acid (Krooneman et al., 2002). When consumed by the cow, 1,2PD can be absorbed and converted to glucose in the liver or converted to propionic acid in the rumen. Although ingestion of 1,2PD in silage ultimately leads to the production of glucose in a ruminant, it will not likely play a role in the prevention of ketosis. Feeding silage with 1,2PD would also not be a viable treatment for ketosis because cows would likely not consume enough 1,2PD to match the 250- to 400-g bolus treatment of 1,2PD that is commonly recommended for treatment of ketosis. Furthermore, a cow that is already ketotic would most likely be off feed, and thus intake of 1,2PD would be even low.

A variety of other volatile organic compounds (**VOC**) can be found in silages (Krizsan et al., 2007; Daniel et al., 2013; Weiss, 2017) Recently, some commercial feed testing laboratories in the United States have offered tests to determine a variety of alcohols (e.g., methanol, propanol) and esters (ethyl acetate, ethyl lactate) in silages. Free methanol should not exceed 150 mg/kg as a food additive in animal feeds (Sellers, 2008), but higher concentrations than this have been detected in a variety of silages (583–878 mg/kg in grass silages; Weiss, 2017). Methanol has been shown to be converted to methane in the rumen (Pol and Demeyer, 1988), and it has been infused into the rumen of Holstein steers (210 g/d to steers weighing 399 kg) with no ill effects on intake or OM and total-tract starch digestion (Winsco et al., 2011). Concentrations of 1-propanol appear low in most silages but can be high in corn silages treated with *L. buchneri* (1.02% of DM; Hafner et al., 2014) and averaged 0.44% of DM in grass silage samples, with a maximum concentration of 2.17% (Kristensen et al., 2010). Randby (2007) reported that feeding 200 g of propanol to cows decreased silage but not total DMI. He also reported that feeding propanol decreased the percentage of milk fat and protein as well as ECM but not total milk production. Furthermore, he found that feeding propanol changed the organoleptic properties of milk in the evening but not morning milk. In contrast, feeding 300 to 400 g of propanol/d to cows had no effects on intake or milk production or composition (Raun and Kristensen, 2011). Weiss (2017) reported concentrations of ethyl lactate as high as 1,305 mg/kg and ethyl acetate as high as 1,109 mg/kg in corn silage. Ethyl lactate was found to have a weak negative correlation with DMI in goats (Gerlach et al., 2013), but clear links to effects on intake in lactating dairy cows are lacking.

Lactic Acid:Acetic Acid Ratio

The ratio of lactic acid to acetic acid is commonly used as a qualitative indicator of fermentation. Good silage fermentations usually have a ratio of these acids of about 2.5 to 3.0. In silages treated with a homolactic acid inoculant, especially legume silages, one can find a slightly higher ratio of lactic acid to acetic acid (Muck and Kung, 1997) because homolactic lactic acid bacteria produce only lactic acid. In contrast, silages treated with *L. buchneri* will have higher concentrations of acetic acid and a lower ratio of lactic acid to acetic acid than untreated silages (Kleinschmit and Kung, 2006) because of the metabolism of some lactic acid to acetic acid; this should not be taken as an indicator of a poor fermentation. The moderate increase in production of acetic acid by *L. buchneri* is within normal ranges, and feeding silages treated with this inoculant has not negatively affected intake (Schmidt et al., 2017). Silages with very high levels of lactic acid:acetic acid ratio may sometimes be more aerobically unstable than those with normal ratios because low concentrations of acetic acid may not be sufficient to inhibit lactate-assimilating yeasts. Lactic acid:acetic acid ratios below 1 are usually an indication of abnormal fermentations.

Soluble N and Ammonia N

Plant and microbial proteolytic processes lead to changes in nitrogenous compounds in silages. Briefly, fermentation results in an increase in soluble N [between 55 and 60% of total N (data not shown) and NH_3-N (usually less than 10–15% of total N; Figures 1–3)]. In general, high-moisture silages have higher concentrations of soluble N and NH_3-N than drier silages because of the overall more robust fermentation in the former. Higher than normal levels of soluble N and NH_3-N in wet legume silages are usually a result of proteolytic activity from clostridia (Figure 1).

Microbial Populations

Enumeration of yeasts and molds in silages may be useful because, as previously mentioned, high numbers of yeasts in silages are usually associated with high

concentrations of ethanol, and their numbers are often inversely related to the aerobic stability of silages. This is especially so in corn-based crops. For example, Kung et al. (1998) published the negative relationship $y = 315.4 - 45.7x$, $r = 0.79$, where x is the $\log_{10}$ total number of yeasts in corn silage and y is the predicted hours of aerobic stability (defined as >2°C increase in the temperature of a silage mass after exposure to air). This equation predicts that aerobic stability is zero when there are ≥ 6 $\log_{10}$ cfu of yeasts/g of wet corn silage for samples in that study; thus, good silages should contain less than this number of yeasts per gram of wet silage. Knowing the numbers of yeasts on the fresh crop is of limited value because data from the senior author's laboratory (data not shown) have yielded no correlation between the numbers of total yeasts on freshly chopped whole-plant corn plants and the final number of yeasts in the resulting (untreated) silage. This is not surprising because ensiling conditions and various treatments can alter the resulting fermentation.

Although anecdotal evidence from the field suggests that high numbers of yeasts in silage are associated with poor animal performance, an exact link of cause and effect has not been made. Santos et al. (2015) isolated a spoilage yeast from HMC, which when added to rumen fluid decreased in vitro fiber digestibility, but this has not been proven to occur in vivo. Windle and Kung (2013) reported that heifers ate less when offered a spoiling TMR (with silage) that contained high yeast counts (7.82 log cfu/g of fresh weight) compared with a fresh TMR (5.03 log cfu/g of fresh weight). In that study, elevated numbers of yeasts were also found in the ruminal fluid of those heifers fed the spoiled TMR. It is unclear whether potential negative effects of feeding silages or TMR with high yeasts are due to a direct effect of the yeasts themselves, to changes in the organoleptic properties of the silage, production of toxic compounds, or a combination of factors. The total number of molds in silage should not be used as an indicator of mycotoxins (Gotlieb, 2016), but high numbers (>6 $\log_{10}$ cfu/g of wet silage) are also usually associated with aerobically spoiled silages. Hoffman and Ocker (1997) reported a negative correlation between molds and milk production from HMC that had been removed from a silo all at once and fed over a 14-d period. Care should be taken when interpreting the numbers of yeasts and molds in silages for several reasons. First, analytical laboratories enumerate the total number of yeasts but do not differentiate between those that are lactate assimilators and those that are not. Second, yeast may be able to grow on selective agar during enumeration, but this does not necessarily reflect their metabolic capabilities in silage. Thus, a silage with a moderate amount of yeasts can still be relatively aerobically stable. Third, especially in corn silages, *Acetobacter* can initiate aerobic spoilage (Spoelstra et al., 1988), and thus silages with low yeast numbers can be aerobically unstable. Furthermore, silages that have undergone extensive spoilage may have very low numbers of yeasts and molds because these organisms have died due to the lack of substrate. Finally, numbers of yeasts and molds can increase markedly from the time of sampling to arrival at the laboratory, especially in warm weather.

Analyses for clostridia in silage is questionable because an accumulation of butyric acid is more than enough to confirm their presence.

ORGANOLEPTIC CHARACTERISTICS OF SILAGE FERMENTATIONS

Running a complete analysis of chemical and nutritional profiles for a silage sample can be expensive. Some clues can be drawn about what happened during the fermentation from the appearance of the silage and what it smells like.

Smells from Silages

Well-fermented silages should not have a strong, particular odor because lactic acid—the main organic acid from the fermentation—is nearly odorless. However, most silages tend to have a mild odor of vinegar (acetic acid) because this acid is produced in the second highest concentration after lactic acid and is very volatile. Smelling silages with very high concentrations of acetic acid will often leave a burning sensation in one's eyes and nose. Besides the smell of vinegar, wet silage with excessive acetic acid also presents a yellow color, especially at the bottom of a silo because the influence of compaction will further increase the moisture content in this area.

Silages with a fruity, sweet odor are mistakenly associated with being a well-fermented, stable feed. In reality, these smells are generally due to high alcohol (ethanol) concentrations that are produced mainly by yeasts but also by many bacteria (McDonald et al., 1991). Ethanol is considered one of the most significant VOC from corn silages to the atmosphere (Hafner et al., 2013). Furthermore, the alcohols may react with acids in the silage, producing esters and adding to the fruity aroma. Some well-fermented, stable corn silages with no signs of fungal contamination or deterioration have been described to smell like nail polish or nail polish remover with acetone-like overtones. The limited research available indicates a correlation of these odors with the levels of ethyl and propyl esters of lactate and acetate and possibly phenyl acetic acid.

Bacilli are found in soil rather than in the fresh plant material, and their number of spores can increase if manure is used as fertilizer. The facultative anaerobic bacilli can use different sugars, which they convert to organic acids, ethanol, 2,3-butanediol, and glycerol (Pahlow et al., 2003). Bacilli are one of the first groups of microorganisms to develop in silages after the aerobic spoilage process is initiated by yeasts. This type of material undergoes a different form of aerobic spoilage, involving severe heating, driven by the growth of thermogenic bacilli. An earthy odor in silages is a sign of bacillus growth, and the silage likely presents a high pH.

Silages that are aerobically unstable may also present a musty or moldy smell and may have visible mold growth. Moldy silage should be discarded because it could be contaminated with mycotoxins, which can cause serious production, health, and fertility issues (Korosteleva et al., 2009) in addition to already being lower in nutritive value due to the prior growth of yeasts (Santos et al., 2015).

Saccharolytic clostridia (*Clostridium tyrobutyricum* and *Clostridium butyricum*) are usually found in silage and utilize soluble sugars or organic (lactic) acids to produce acetic acid and butyric acid, which has a strong, foul rancid-butter smell. Other species of clostridia (e.g., *Clostridium sporogenes*) can ferment both carbohydrates and proteins, with the latter being converted to ammonia and biogenic amines. The excessive proteolysis can give a putrid, fishy or ammonia-like odor. Clostridial silages often have a slimy, olive-green appearance. Furthermore, these silages have a low level of energy and high soluble protein, so feed intake will be low (Muck, 2011); thus, forcing a higher intake using flavor- and aroma-masking agents can increase production, health, and fertility problems.

Alfalfa haylage ensiled at high DM contents (in particular those with >50–55% DM) may present a tobacco smell that is associated with changes in the characteristics of the protein fractions, producing heat-damaged proteins as a result of the Maillard reaction (Goering et al., 1973). In this reaction, proteins bind with sugars in the presence of high temperature. Oxygen that is excessively trapped in the forage mass stimulates plant respiration and metabolic activity of aerobic microorganisms, leading to the production of heat. In the early stages of this process the odor is more like sweet tobacco, which is less of a concern. This type of feed presents a dry appearance and a brownish coloration and should be tested for ADIN to check the level of bound or unavailable protein in case dietary adjustments are necessary. Silages that are extremely heat damaged may actually smell burnt and look black in color. It is worth noting that although reduction of the bioavailability of protein and AA has been the focus from Maillard reactions, energy (TDN) losses are perhaps more important (Coblentz et al., 2011).

Silage Temperatures

The production of heat is a normal occurrence during silage fermentation. If silage is well packed and sealed immediately, the average temperature of the forage mass should not increase to more than about 5 to 8°C above the ambient temperature at filling. However, temperatures in the uppermost layers (top 5–10 cm) of a forage mass may reach as high as 45 to 60°C, especially if left overnight in warm weather for several days due to equipment breakdowns or packing delays because of bad weather. These high temperatures are a result of excessive amounts of air trapped in the forage mass leading to oxidation from aerobic microorganisms. The key is that these temperatures should decrease quickly as further packing removes air from the mass and fermentation takes place. Prolonged high temperatures above 45 to 50°C can lead to heat-damaged protein and increases in ADIN as previously noted. Temperatures in this range may also be detrimental to many lactic acid bacteria that are needed to achieve a successful fermentation. Thus, forage should be chopped adequately, packed quickly, and sealed tightly as soon as possible to remove and keep the air out of the forage mass.

When the active phase of fermentation is complete, temperatures in the core of the silo often decrease slowly to 25 to 30°C. Small silos (including bag silos and large bales) should cool more quickly than larger silos. Retained heat should seldom register above 35°C, especially after several months of storage. Core silage temperatures often remain high for prolonged periods of time in large silos because the large forage mass acts as insulation, resulting in a very slow dissipation of heat.

In some instances, silages may be relatively hot (>30–35°C) even after 4 to 6 wk (or more) in the silo. This finding may be more common in silages that have been harvested dry (>40–45% DM) and poorly packed. In these instances, prolonged heat may be the result of a slow fermentation or aerobic oxidation. Dry silages tend to be more porous when packed, and the slow fermentation prolongs total microbial activity and is unable to suppress the metabolism of yeasts and molds because low amounts of antifungal acids are produced and the pH is rather high. If the silo in question is being fed from, this may exacerbate the problem because the silo face is constantly being disturbed, allowing air to penetrate into the mass. Decreasing the length of

chop for high-DM forages (and mechanical processing of corn silage) may be warranted as these practices help increase packing density.

High temperatures in a silo, especially after months of fermentation and during feed-out, are most likely a result of aerobic deterioration. Penetration of air into the silage mass results in growth of lactate-assimilating yeasts and an increase in silage temperature and is followed by an increase in silage pH. The latter ultimately results in the growth of opportunistic bacteria and molds that thrive in oxygen and causes more heating and spoilage. In some cases, temperatures in silage faces may exceed 50°C. In goats, Gerlach et al. (2013) reported that the change in corn silage temperature over ambient temperature was negatively correlated ($r = -0.835$, $P < 0.0001$) with DMI. During cool weather, steam often is released during feed-out from the face of large silos because of the difference between retained heat and the ambient temperature. The presence of steam does not always mean that silage is spoiling, especially in the winter months. Signs that silage is aerobically spoiling include measuring temperatures in excess of 40°C 10 to 20 cm in back of the silo face at feed-out, reheating in the bunk, visible mold, lack of a sharp or sweet smell to the silage, or a flat or moldy, musty smell. If a pH meter is available, a moldy smell coupled with a high pH may also be a good indicator that the silage has undergone aerobic deterioration. Aerobic deterioration of silages is of course more common during warmer weather. Silage that causes the TMR to reheat quickly in the feed bunk most likely has a high concentration of yeasts that are causing further spoilage in the feed bunk. Relatively inexpensive probes can be used to monitor temperatures in silage piles and bunkers, but extreme care should be taken to not endanger one's self when taking the temperature measurements.

EFFECTS OF ENSILING TIME ON SILAGE FERMENTATION

Overall, the fermentation phase of the ensiling process is thought to last 7 to 45 d (Pahlow et al., 2003). However, recent research indicates that fermentation continues for much longer in whole-plant corn silage (**WPCS**; Der Bedrosian et al., 2012; Windle et al., 2014) and HMC (Kung et al., 2014).

The decrease in silage pH generally is more rapid in whole-plant corn than in legume silage because the latter has a higher buffering capacity. Within legume silages, the decrease in silage pH is more rapid in forages with low DM (<30%) compared with those with high DM (>40%) because more metabolic water is available in the former. Windle et al. (2014) and Der Bedrosian et al. (2012) reported pH decline in WPCS over time in storage for up to 150 d (45-, 90-, and 150-d fermentation times) and even after 180 d (45-, 90-, 180-, 270-, and 365-d fermentation times). Furthermore, both research reports noted a gradual increase in lactate and acetate concentrations as storage length progressed. Similar results have been reported in HMC trials. Decreased pH was reported by Baron et al. (1986) and Wardynski et al. (1993) when HMC was ensiled for up to 90 or 165 d, respectively, in relationship to increased concentrations of lactate and acetate over time in storage. Likewise, a gradual decrease in pH was reported from 120 to 365 d of storage by Stock et al. (1991). Decreased pH over extended periods of storage is in agreement with bacterial activity in HMC when the fermentation process was evaluated for up to 200 d (Burmeister et al., 1966; Bothast et al., 1975).

Hoffman et al. (2011) 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. This could explain reports of greater ruminal in situ starch digestibility with ensiling in corn kernels harvested at half milk line stage (Philippeau and Michalet-Doreau, 1998) or greater ruminal in situ DM degradability for HMC with lower DM contents and longer duration of silage fermentation (Benton et al., 2005). Furthermore, ammonia N content increased as zein-protein subunits in HMC decreased in the study by Hoffman et al. (2011). Greater soluble N concentration was observed when HMC was ensiled up to 90, 165, or 365 d in the trials of Baron et al. (1986), Wardyinski et al. (1993), and Benton et al. (2005), respectively. Likewise, prolonged ensiling time increased concentrations of ammonia N and soluble CP in WPCS trials (Der Bedrosian et al., 2012; Windle et al., 2014; Ferraretto et al., 2015a,b; Ferraretto et al., 2016).

Ensiling time effects on WPCS ruminal in vitro starch digestibility (**ivSD**) are summarized in Figure 4. Although some variation in response across trials has been observed, generally there is about a 5 to 10 percentage unit increase in ivSD within the 45-d fermentation phase (Pahlow et al., 2003) followed by a similar magnitude of increase between 45 and 120 d of ensiling. Maximal WPCS ivSD may not be reached until about 9 mo in storage. Similar results were reported for HMC (Kung et al., 2014), with an 8 percentage unit increase in ivSD from 0 to 70 d of ensiling followed by another 8 percentage unit increase after 140 d. Correlation coefficients for relationships between ivSD and ammonia N or soluble CP were, respectively, 0.82 and 0.70 in WPCS (Ferraretto et al., 2015b) and 0.78 and 0.74 in HMC (Ferraretto et al., 2014).

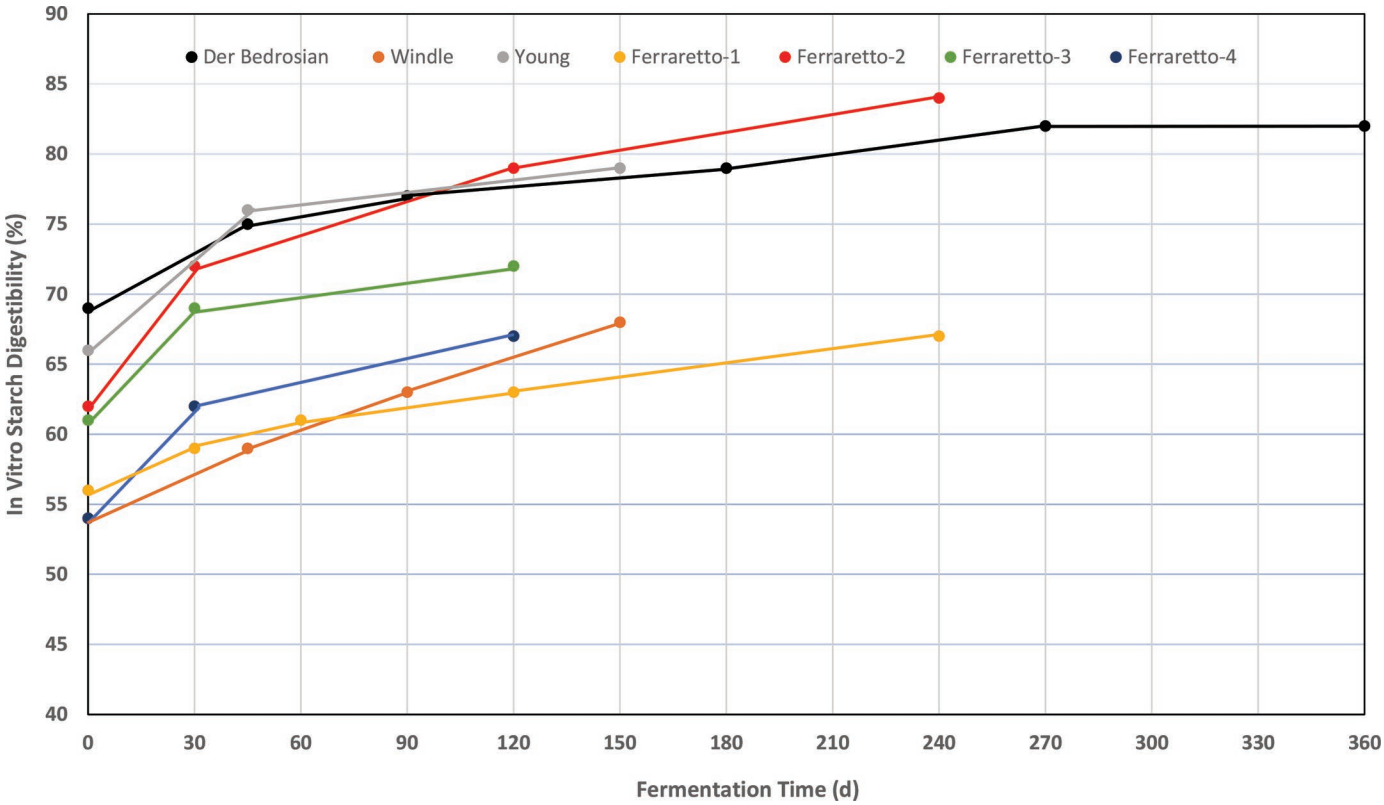

Figure 4. Effect of days of ensiling on ruminal in vitro starch digestibility. Data from Der Bedrosian et al. (2012), Windle et al. (2014), Young et al. (2012), Ferraretto et al. (2015a; referred to as Ferraretto-1), Ferraretto et al. (2015b; referred to as Ferraretto-2), and Ferraretto et al. (2016; referred to as Ferraretto-3 and -4). Color version available online.

The main mechanisms (solubilization and proteolysis) responsible for the disruption of the zein-proteins cross-linked to starch granules occur under acidic conditions, which suggests that continuous alterations in fermentation profile as storage time progresses may directly affect ivSD. First, zein-proteins are soluble in acetic and lactic acids (Lawton, 2002), the 2 main fermentation end products of ensiling (Muck, 2010). Furthermore, proteolytic activity, either from microbial or plant proteases, occurs more extensively during the anaerobic fermentation process (Baron et al., 1986). The anaerobic phase is characterized by a drastic decrease in pH (Muck, 2010), which favors the activity of plant proteases specific to the endosperm of cereal grains (Simpson, 2001) even though the activity of plant proteases is typically reduced under low pH (Muck, 1988). Junges et al. (2017) evaluated the contribution of proteolytic sources on protein solubilization in rehydrated corn ensiled for 90 d. These authors reported that bacterial proteases are responsible for 60% of the increase in soluble CP concentrations, followed by kernel enzymes (30%) and fungi and fermentation end products (5% each).

Trials evaluating the effects of ensiling time on ruminal in vitro NDF digestibility (**ivNDFD**) in WPCS are summarized in Table 2. Cherney et al. (2007) and Der Bedrosian et al. (2012) observed decreases in ivNDFD of 6 and 2 percentage units after 30 and 45 d of ensiling, respectively. These changes were thought to be related to solubilization of fiber early during ensiling. Furthermore, a minor 1-percentage-unit lower ivNDFD was observed for 45 d compared with 150 d of ensiling (Young et al., 2012). No effects were observed in the trials by Hunt et al. (1993), Sanderson (1993), or Ferraretto et al. (2015b). Overall, these results emphasize that ivNDFD is minimally affected by ensiling and that hybrid selection and ranking hybrids based on ivNDFD of unensiled samples is valid (Lauer et al., 2013).

POTENTIAL ENVIRONMENTAL CONCERNS OF SILAGE FERMENTATION END PRODUCTS

Under certain conditions, silages may contain potentially toxic compounds such as nitrates, prussic acid, and mycotoxins. Previous reviews and reports have thoroughly covered the topics of mold and mycotoxin

Table 2. Effects of ensiling time on ruminal in vitro NDF digestibility (% of NDF) in whole-plant corn silage

	Days ensiled											
Source	0	30	45	60	90	120	150	180	240	270	360	*P*-value
Cherney et al. (2007)[1]	56	50	—	—	—	—	—	—	—	—	—	0.001
Der Bedrosian et al. (2012)[2]	62	—	60	—	60	—	—	59	—	59	60	0.01
Ferraretto et al. (2015b)[3]	57	56	—	—	—	55	—	—	56	—	—	NS[4]
Hunt et al. (1993)[5]	73	—	—	71	—	—	—	—	—	—	—	NS
Sanderson (1993)[5]	66	—	62	—	—	—	—	63	—	—	—	NS
Young et al. (2012)[3]	64	—	61	—	—	—	60	—	—	—	—	0.02

[1]Ruminal in vitro NDF digestibility at 48 h on samples ground through a 1-mm screen.
[2]Ruminal in vitro NDF digestibility at 30 h on samples ground through a 2-mm screen.
[3]Ruminal in vitro NDF digestibility at 30 h on samples ground through a 1-mm screen.
[4]Nonsignificant.
[5]Ruminal in vitro NDF digestibility at 48 h on samples ground through a 2-mm screen.

concerns in silage (Mahanna and Chase, 2003; Alonso et al., 2013), nitrates and silo gas considerations when feeding silage to livestock (Mahanna and Chase, 2003; Rasby et al., 2007), and prussic acid concerns (Collins and Hannaway, 2003; Whittier, 2011). This section focuses on the relatively new environmental concern surrounding VOC in silages.

Most research on agricultural air quality from livestock operations has focused on odor, ammonia, or particulate material (Hafner et al., 2013). However, emissions of VOC from silage have been identified as a significant contributor to poor air quality in regions of concentrated agriculture such as the San Joaquin Valley in central California (Howard et al., 2010). In the future, measurement of VOC in silage will become increasingly important.

Silages emit substantial amounts of VOC, which are precursors to tropospheric ozone (Bonifacio et al., 2017). In the presence of sunlight, VOC emitted into the atmosphere react with oxides of N to form ground-level ozone, which is regulated by the US Environmental Protection Agency (USEPA, 2016). The 2 greatest contributors to VOC emissions are TMR in the mangers or feed lanes followed by silage storage systems (Chung et al., 2010).

Silage emits more than 50 VOC (Howard et al., 2010) that can be grouped as acids, alcohols, ketones, esters, and aldehydes. A review by Hafner et al. (2013) identified the most important VOC deriving from corn silage. Alcohols compose the largest mass of VOC, with ethanol being the predominant alcohol. Excluding acids, ethanol composes more than half of the mean mass of VOC from corn silage. When emission is high and all VOC are nearly depleted, then acids such as acetic acid may be important. Aldehydes and esters are more volatile than either acids or alcohols and can be important when exposure time of the silage to air is short.

Management practices may have a substantial effect on VOC concentrations. Effective control of VOC from silage would involve reductions in both VOC production and emission. A greater understanding of the process of VOC emissions on farm is required for best management, but to date substantial research has focused on development and use of silage additives and inoculants.

The majority of VOC are likely produced directly or indirectly by heterofermentative lactic acid bacteria or undesirable microbes such as enterobacteria, clostridia, or yeasts (Hafner et al., 2013). Silage additives may directly inhibit the activity of specific microbial groups or elicit environmental conditions that inhibit them. However, it may well be difficult to reduce production of all VOC, or even a few, without increasing others (Driehuis and van Wikselaar, 2000; Hafner et al., 2013).

Studies that have evaluated bacterial inoculants on corn silage have shown inconsistent results. Some studies such as Filya and Sucu (2010) found that *Lactobacillus plantarum* and *L. buchneri*, alone or in combination with other bacteria, decreased ethanol production in corn silage by 30 to 40%. In contrast, Contreras-Govea et al. (2011) observed no effect of inoculation with *L. plantarum* and other lactic acid bacteria on ethanol content of corn silage. Finally, some studies such as Steidlová and Kalac (2003) actually measured up to 3-fold increases in ethanol concentration of corn silage in response to inoculation with *L. plantarum* or *L. buchneri*. A meta-analysis showed that inoculation with *L. buchneri* had no consistent influence on ethanol in corn silage (Kleinschmit and Kung, 2006). For corn silages, bacterial inoculants do not appear to result in consistent reductions in ethanol (Hafner et al., 2014, 2015).

Though less researched, chemical additives designed to inhibit fungi or undesirable bacteria have promise for reducing ethanol in silage (Hafner et al., 2014). Propionic acid appears to have no effect on ethanol produc-

tion by corn silage (Kleinschmit et al., 2005), but a 1:1 mixture of potassium sorbate and EDTA depressed ethanol by 80% (Kleinschmit et al., 2005). Similarly, Teller et al. (2012) observed a 20 to 90% reduction in ethanol with potassium sorbate applied to corn silage. However, Knicky and Sporndly (2011) observed that a blend of potassium sorbate, sodium benzoate, and sodium nitrite reduced ethanol in legume and grass silage but not in higher DM grass silage or corn silage. In the future, a mixture of homofermentative inoculant to minimize acetic acid and ethanol plus a chemical additive to suppress yeasts may be a fruitful approach to reduce VOC (Hafner et al., 2015). Whatever strategies are developed, they must be consistent with well-accepted practices that minimize silage storage losses.

A process-based model for predicting VOC emissions has been developed by Bonifacio et al. (2017) and incorporated into the Integrated Farm System Model (version 4.3; Rotz et al., 2016). The model performance was evaluated using measures of ethanol and methanol emissions from conventional silage piles, silage bags, TMR, and loose corn silage. The model simulations showed that the greatest silage VOC emissions came from the TMR lying in the feed mangers or feed lanes and not from exposed silage faces. Based on this model, mitigation efforts should focus on VOC emissions associated with feeding. For the farm simulation, VOC emissions were reduced by approximately 30% when cows were housed indoors versus an open lot and by 23% if feed was delivered 4 times versus once daily. Even though this model indicates that feeding represents the greatest concern for VOC emissions, reducing the exposed face of silage during storage is also important. For example, use of silage bags reduced emissions from the silage face by 90% compared with silage piles.

CONCLUSIONS

Chemical, microbial, and organoleptic components of silage can be used to explain the type of fermentation that occurred and may explain or predict poor animal performance when fed to ruminants when these components deviate far from the norm. Increasingly, measurement of VOC from silages, along with odor, ammonia, and particulate matter, will be an environmental concern for farms. There is a need for establishing more predictive use of chemical data and for identifying other measurable factors that might be used to better explain animal performance.

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