Effects of spoiled silages on animal performance

Mateus C. Santos[1] and Limin Kung, Jr.[2]

Introduction

Ensiling is the anaerobic process of preserving moist crops by lactic acid fermentation. Compared to other methods of forage preservation, silage retains the largest amount of nutrients from the original material, it can be made from a wide variety of crops and it is less dependent of the weather at harvest (Pahlow et al., 2003). The silage microbiota play a key role in the successful outcome of the conservation process. The biota can basically be divided into two groups, namely the desirable and the undesirable micro-organisms. The desirable microorganisms are lactic acid bacteria (LAB) whereas undesirable organisms include those that can cause poor fermentations (e.g. clostridia and enterobacteria) or aerobic spoilage (e.g. yeasts, bacilli, *Listeria* and moulds). Under optimal ensiling conditions, epiphytic LAB predominantly ferment plant water soluble carbohydrates into lactic acid that acidifies the crop resulting in preservation of nutrients (McDonald et al., 1991). The preservation of these crops as silage can be improved by the utilization of microbial inoculants. Lactic acid bacteria are the most common microorganisms used and they are usually applied at harvesting. Homolactic bacteria improve fermentation efficiency by increasing lactic acid production whereas heterolactic bacteria are used with the objective of preserving forage quality during storage and after silo opening by increasing the production of anti-fungal compounds.

During storage and feed out silage is exposed to oxygen. If populations of lactate-assimilating yeasts are high in silage, exposure to air stimulates their growth and initiates aerobic spoilage of nutrients. The type of yeasts and their metabolism in silage has not been well studied. In addition, cows are often fed silages that have gone through various degrees of aerobic spoilage and can have high numbers of yeasts. To date, the effects of these organisms on normal ruminal metabolism and animal performance are still unknown.

Thus, this review will consider the role of yeasts in silage preservation and the impacts of feeding spoilage silages on animal performance.

[1] PhD. Student. Department of Animal and Food Sciences. University of Delaware.

[2] PhD. Professor. Department of Animal and Food Sciences. University of Delaware.

Yeasts and silage fermentation

All forage crops at ensiling contain aerobic and anaerobic microorganisms and a range of both bacteria and fungi that affect silage quality (Muck, 2010). The surface of plants accommodates a varied microflora and yeasts play an important part in this microflora. The majority of epiphytic yeasts on forage crops belong to genera *Sporobolomyces*, *Cryptococcus*, *Rhodotorula* and *Torulopsis*, which can range in population density from less than 10 to 10^6 or 10^7 colony forming units (cfu)/g of fresh material (Lindgren et al., 1985; Middelhoven and van Baalen, 1988). Middelhoven and van Baalen (1988) reported that these species vanished after two days of ensiling and none of them were able to tolerate acetic acid or assimilated this acid at pH 4.0. Most of the yeasts that are associated with the fresh plant in the field are able to decompose the plant's cuticle, thus promoting leakage of nutrients from which the microflora benefits (Ruinen, 1965). Moreover, yeasts of the genera *Rhodotorula* spp., *Lipomyces* and *Cryptococcus* are classified as obligate aerobes, with exclusively oxidative metabolism (Rosenfeld and Beauvoit, 2003) and may explain why they are vanished after a few days of silage preservation.

After anaerobiosis has been established in the ensiled forage, aerobic fungi are succeeded by a fermentative flora of yeasts (Di Menna et al., 1981; Middlehoven and van Baalen, 1988). These yeasts tend to grow best aerobically but can ferment sugars anaerobically and produce ethanol and CO_2 when trace amounts of oxygen are present (McDonald et al., 1991). During silage preservation, anaerobic as well as aerobic yeast activity is considered undesirable. Ruxton and McDonald (1974) reported that yeasts do not contribute to the preservation of an ensiled forage and actually compete with lactic acid bacteria for available water soluble carbohydrates. The ethanol fermentation in silage not only decreases the amount of sugar for lactic acid production, but it can also decrease the dry matter recovery because of excessive production of CO_2. Bolsen (1997) reported DM losses in the top layer (0.5 m) of bunker silos ranging between 43 to 61% and 24 to 27% in unsealed and sealed silos, respectively.

The two primary factors required in making good silage are the rapid exclusion of air from the forage mass at the start of ensiling and the continued exclusion of air from the silage mass during storage. Excessive exposure to air at the start of fermentation prolongs the metabolism of unwanted microbes that thrive in air and delays the growth of beneficial bacteria that produce lactic acid. This can lead to undesirable fermentations and a loss in nutritive value. The amount of air ingress during silage storage can affect the yeast flora composition. Jonsson and Pahlow (1984) found that if anaerobic conditions were achieved

and maintained in the silage, fermentative, but non-lactate assimilating *Saccharomyces cerevisiae* prevailed, whereas if air penetrated into the silage during storage, lactate assimilating yeasts of the genera *Candida* and *Hansenula* (=*Pichia*) predominated.

The low pH achieved by the lactic acid fermentation, together with anaerobiosis usually provides conditions adverse to microorganisms. The degree of anaerobiosis during storage period and the concentration of organic acids are important factors that affect the survival of yeasts. Jonsson and Pahlow (1984) stated that the presence of oxygen enhances survival and growth of yeasts, whereas a high level of acetic acid reduces their viability during the storage period.

Yeasts and aerobic deterioration of silages

When silage is exposed to air, during feed-out in the silo or in the feed bunk, aerobic microorganisms can become active, causing heating and spoilage. It is generally acknowledged that the population of yeasts present upon exposure to air governs the vulnerability of silage to aerobic deterioration. In a summary of 2009 corn silage analysis, Cumberland Valley Analytical (Maugansville, MD) reported that more than 50% of samples analyzed (n = 121) had a population of yeasts with > 1×10^7 cfu/g (Figure 1).

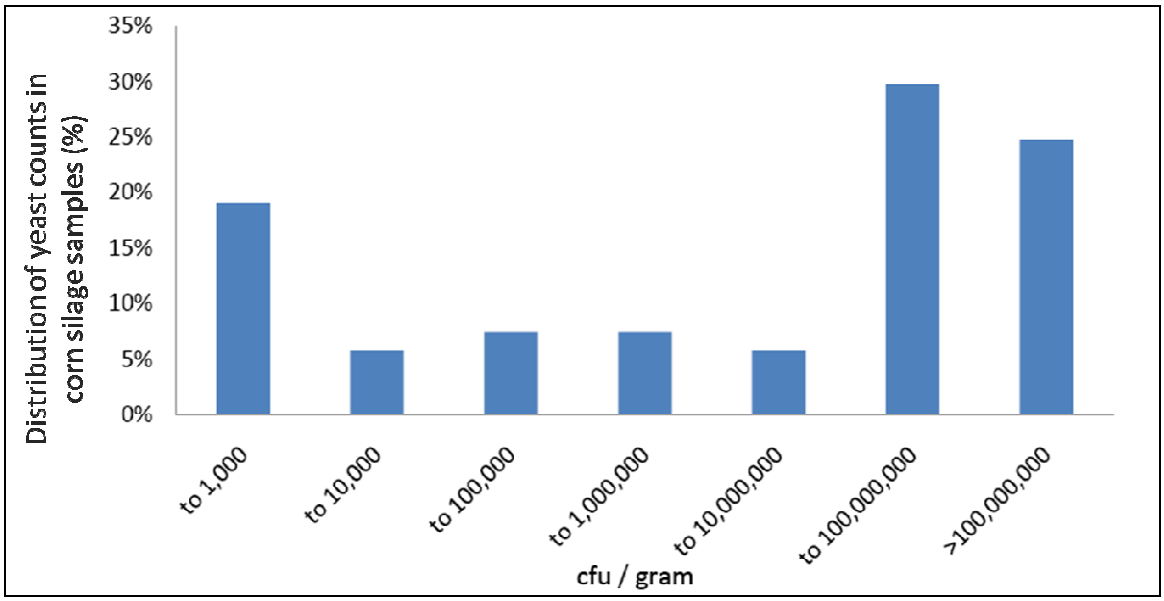

Figure 1. Distribution of yeast counts in 121 samples of corn silage analyzed by Cumberland Valley Analytical Services (Cumberland Valley Analytical Services, 2009).

Daniel et al. (1970) reported that silages having a population in excess of 10^5 cfu/g DM are particularly prone to aerobic deterioration. Middelhoven (1998) also reported that

silage with a large yeast population, e.g. 10^5 cfu/g, will be spoiled by these organisms upon exposure to air. Kung et al. (1998) reported that aerobic stability was negatively correlated to the number of yeasts present at the time the silo was opened (Figure 2). Corn silage with low yeast populations (10^3 cfu/g) remained cool for up to 3 times longer than silages with high initial yeast populations (10^6 cfu/g). Preventing silage from spoiling when it is exposed to air can improve the efficiency of a farm by preserving forage as high quality silage that is palatable to cows.

In silages, the population of yeasts associated with aerobic spoilage can be divided into two physiological groups. First, there are the yeasts with a high fermentative ability for sugars but a variable ability to assimilate lactic acid. These organisms include *Saccharomyces cerevisiae* and species of *Torulopsis*. The second group is composed of yeasts that have a high respiratory affinity for lactic acid. These organisms include species of *Candida*, *Issatchenkia*, *Pichia*, *Hansenula*, and *Endomycopsis* (Moon and Ely, 1979; Jonsson and Pahlow, 1984; McDonald et al., 1991).

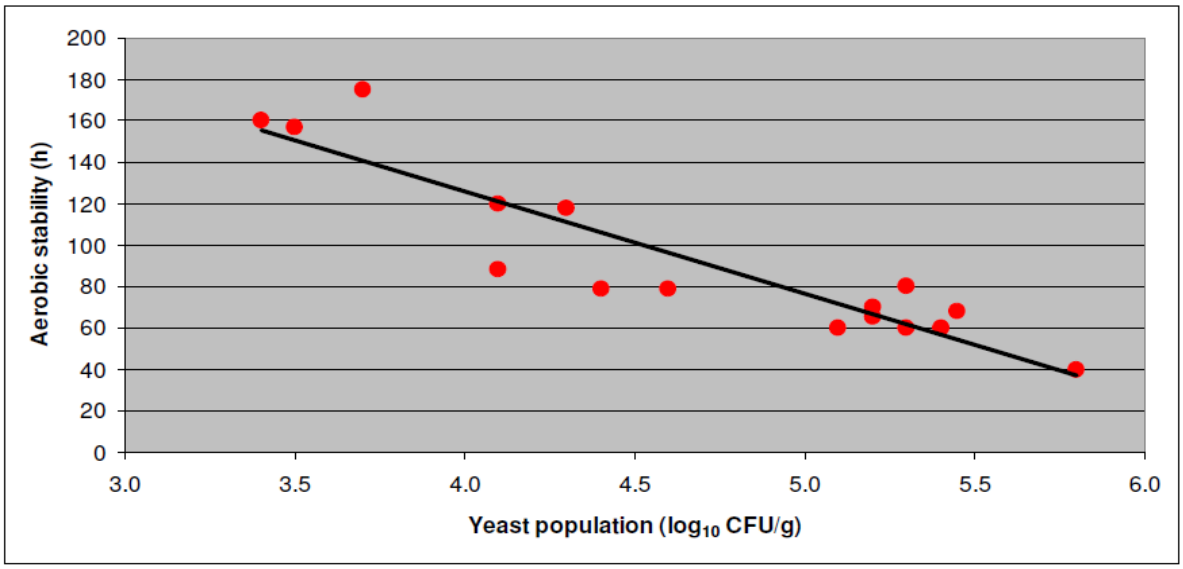

Figure 2. The effect of yeast population on the aerobic stability of corn silage (Kung et al., 1998).

Pahlow et al. (2003) reported that yeasts of the genera *Candida* and *Hansenula* (*Pichia*) are particularly prolific during the aerobic phase because of their lactate assimilating ability and their strong affinity for glucose. *Candida valida* (including its teleomorph *Pichia membranifaciens*) shows notable resistance towards acidic pH and high concentrations of preservatives (Deak et al., 1992). Moon (1983) used a growth medium containing 2% of glucose to study the effects of acetate, lactate and propionate on the growth of acid tolerant

yeasts isolated from deteriorated silages and/or spoiled lactic acid fermented foods. The author observed that all of the yeasts (*Saccharomyces uvarum*, *Geotrichum candidum*, *Endomycopsis burtonii* and *Hansenula canadensis*) grew well with glucose or 1% lactate but not 1% propionate as an energy source. All yeasts except *S. uvarum* could grow in the presence of 1% acetate after a two week incubation period. When acids were present in the growth medium, an increase in turbidity was considerably delayed when compared with the control medium containing glucose. After two weeks incubation, final turbidities were similar between all tubes suggesting that at these concentrations of the acids delayed growth rather than caused cell death.

Under aerobic conditions, the metabolism of sugars and lactic acid by yeasts results in the production CO_2, heat and H_2O. This inevitable oxidation of sugars usually results in high loss of dry matter (Woolford, 1990) and the loss of important nutritional components (Kung et al., 1998). In addition, the degradation of lactic acid causes a rise in silage pH, which in turn triggers the growth of many other spoilage organisms (McDonald et al., 1991). Kung (2005) reported that exposure to air initiates a chain of reactions that ultimately results in spoiled feed (Figure 3). Specifically, yeasts that degrade lactic acid in the presence of air are the primary microbes that cause spoilage in silages. Degradation of lactic acid also increases the pH of the silage to a level that allows opportunistic bacteria (e.g. Bacilli) and molds (e.g. *Aspergillus*, *Fusarium*, and *Pencillium*) to grow and further reduce silage quality (McDonald et al., 1991). In some cases bacteria from the genus *Acetobacter* may initiate aerobic spoilage in corn silages but their role is less understood (Muck and Pitt, 1994). The authors conducted six trials to evaluate the microbial development at 5, 20, 35, and 50 cm from the exposed face of corn silages and observed that most microbial activity occurred at the 5-cm level in all six trials. Acetic acid bacteria initiated heating in all trials although yeasts were significant in two trials. Because silages are often incorporated into total mixed rations (TMR), the stability of the TMR may also be an issue on farms. In a small survey of TMR sampled in DE, PA and MD over two years, more than 50% of 30 TMR that were sampled within 1 hour of being made spoiled in less than 12 h when incubated at a controlled laboratory temperature of about 23°C (Kung, Mulrooney and Morges, unpublished data Univ. of Delaware).

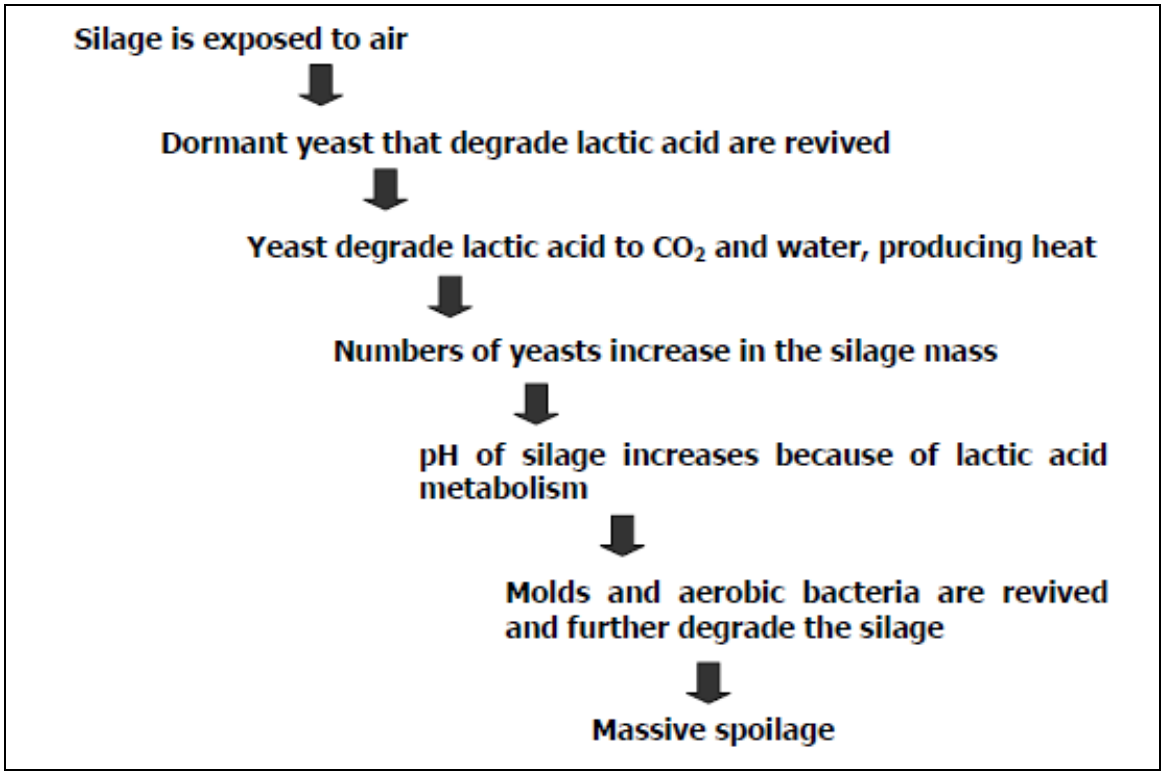

Figure 3. The "domino effect" of air causing aerobic spoilage in silages (Kung, 2005)**.**

Impacts of feeding spoilage silages on animal performance

When animals consume spoiled silages, the exact causes of reduced intake and/or performance are not fully understood. Detrimental yeasts may affect rumen microorganisms or produce end products that might alter rumen fermentation, the direct consumption of spoiled of nutrients may reduce performance, and the production of undesirable end products (e.g. mycotoxins) from further spoilage by molds and other organisms may also be problem.

Yeasts occur naturally in the microbial community of the rumen (Lund, 1974). Up to 1.3 x 10^5 yeasts/ml grew when dilutions of bovine rumen fluid were incubated at 25°C, but only 3.5 x 10^3/ml grew at 39°C, suggesting that the yeasts present normally are essentially transient members of the community, entering with the fodder (Lund, 1974). Kung et al. (1997) added a direct fed microbial strain of *S. cerevisiae* to sterile ruminal fluid and reported that this organism was able to maintain viable counts through 24 h of incubation but some cell death apparently took place by 48 h. Thus yeasts, and particularly *S. cerevisiae*, are not normally significant members of the rumen microbial community (Wallace and Newbold, 1995). The temperature and chemical composition of rumen fluid tended to be inhibitory to growth of these microorganisms.

It is well known that yeasts have the potential to alter the fermentation process in the rumen (Wiedmeier et al., 1987; Harrison et al., 1988), and yeast products based on *Saccharomyces cerevisiae* are increasingly used in ruminant diets to improve animal performance (Desnoyers et al., 2009; Robinson and Erasmus, 2009). However, improvements in animal performance are not always achieved, and in fact publications have reported that these effects are strain specific. Chung et al. (2011) investigated whether the risk of subacute ruminal acidosis (SARA) in dairy cows would be decreased by feeding diets with selected yeast strains based on *S. cerevisiae*. The authors compared a commercial product widely used in dairy production (strain 1) with a novel strain that is not used commercially (strain 2) and with a control diet. They observed that the concentration of lactate and total VFA in the rumen fluid were similar among treatments, however yeast strain 2 decreased ($P = 0.04$) the proportion of acetate in rumen fluid compared with yeast strain 1 and increased ($P < 0.01$) the proportion of propionate in ruminal fluid compared with the control or yeast strain 1. Cows that received yeast strain 2 experienced lower ($P \leq 0.03$) average daily minimum, mean, and maximum pH compared with cows that received no yeast or yeast strain 1. The authors concluded that yeast strain 2 increased the risk of acidosis when compared with the commercial product containing a different strain of *S. cerevisiae*.

When spoiled silages are fed to animals, depression in intake and performance has been observed in dairy cattle (Hoffman and Ocker, 1997) and in beef cattle (Whitlock et al., 2000). In these cases, the high number of spoilage yeasts that is ingested by the animal may also affects ruminal fermentation parameters, resulting in poor animal performance. In the study conducted by Hoffman and Ocker (1997), 18 mid-lactation cows were fed a total mixed ration containing fresh or aerobically deteriorated high-moisture corn silage. The fresh material was removed from the silo each day before being fed to the herd and the aerobically deteriorated silage was removed from the silo and piled on a concrete floor at the beginning of each experimental period (14 d). The researchers found significant differences in milk yield ($P < 0.01$). Cows that were fed a total mixed ration containing the aerobically deteriorated high-moisture corn produced 3.2 kg less milk per cow/day when compared to cows fed aerobically stable silages (Figure 4).

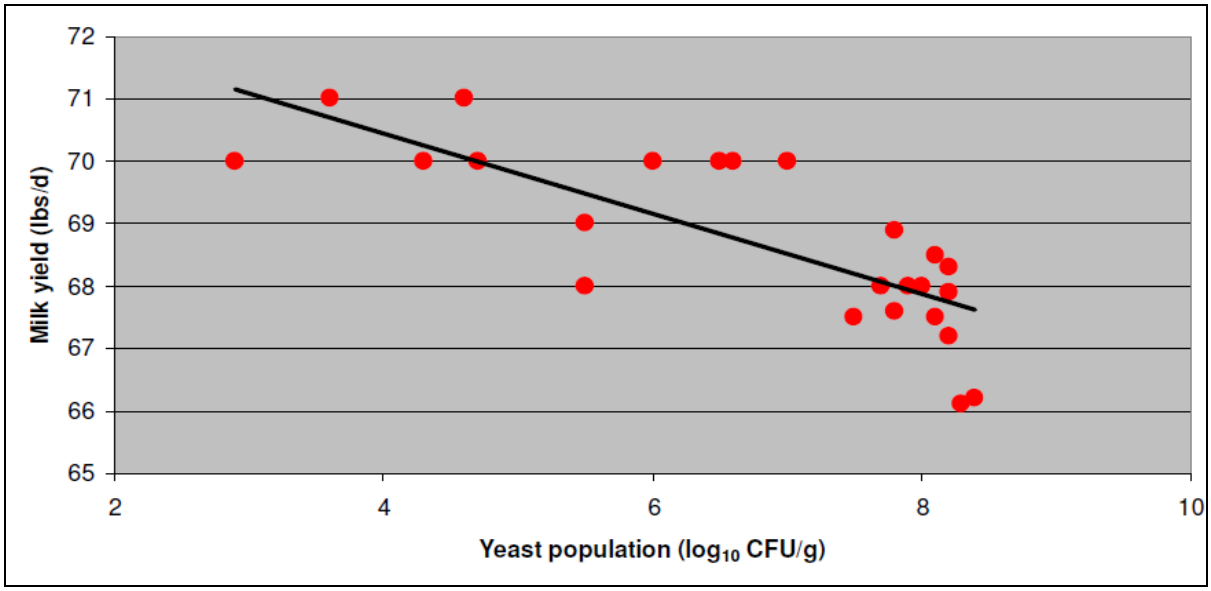

Figure 4. Effect of aerobically unstable high moisture corn on milk yield (Hoffman and Ocker, 1997).

Whitlock et al. (2000) reported that feeding surface spoilage had a significant negative impact on the nutritive value of a whole-plant corn silage-based ration. There was a depression in DM intake of steers when aerobically deteriorated silage was incorporated in the diet. In addition, feeding cannulated steers with a 3:1 ratio of normal:spoiled corn silage reduced organic matter, crude protein, neutral detergent fiber (NDF) and acid detergent fiber (ADF) digestibility by 5.0, 4.1, 7.2 and 9.9 percentage units, respectively. These researchers reported that feeding 25% spoiled silage partially or totally destroyed the mat phase in the rumen. The percentage of digestible NDF in a silage or TMR is often associated with feed intake and rumen fill in high-producing cows. Oba and Allen (1999) evaluated the relationship of NDF-D and animal performance and estimated that a 1-unit increase in forage NDF-D in vitro or in situ was associated with increases of 0.17 kg/d of DMI, 0.23 kg/d of milk yield, and 0.25 kg/d of 4.0% fat-corrected milk.

Conclusions

The presence of spoilage yeasts in silages can affect silage quality and animal performance. These microorganisms become active when silage is exposed to air and they can achieve high concentration during the feedout phase and in the feedbunk. The ingestion of these yeasts by ruminants can affect rumen fermentation resulting in poor intake and animal performance. Good silo management not only minimizes the aerobic activity of spoilage yeasts but also reduces dry-matter losses and maximizes the anaerobic conversion of

water-soluble carbohydrate to organic acids, resulting in silages with good nutritive value and with a beneficial population of microorganisms.

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