They Are What We Feed Them: Understanding the Impact of Forage Quality on Rumen Function

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AB Vista

Making good quality silage is a key factor in ensuring good productivity, health, and performance of the dairy herd. However, there are many factors that can negatively affect silage quality and its nutritive value at feeding. These can range from the quality of the forage that was ensiled in the first place, the ensiling fermentation process itself, and management of the silo both at packing and at feeding out.

There are many factors that can negatively affect the ensiling process leading to poor quality silage. For example, the dry matter content of the crop at cutting, the crop's buffering capacity, the sugar content of the crop, and the level and type of contaminating microorganisms from the field, which can end up dominating the fermentation pathway, may all influence the success of the ensiling process. The consensus of opinion, however, is that one of the most important factors that can influence the efficiency of ensiling and the quality of the resulting silage is the degree of anaerobiosis or lack of oxygen achieved in the silo. Good packing density, efficient sealing of the silo, and good management of the silo when feeding out can help to minimise the proliferation of spoilage organisms and maintain aerobic stability.

AEROBIC STABILITY

Aerobic stability is used to describe the length of time that silage remains cool and does not spoil or start to heat when exposed to air. All silages exposed to air will start to quickly deteriorate and their nutritive value will decrease, leading to losses in cow performance when fed. Of particular concern is the prevalence and proliferation of spoilage yeasts or "bad yeasts" which can reduce aerobic stability. A reduction in aerobic stability also gives the opportunity for secondary fermentations to occur which can affect intakes and further impact performance and health.

GOOD YEASTS VS. BAD YEASTS

The use of active dry yeasts as a direct fed microbial (DFM) to positively impact dairy cow performance and rumen health have been well documented (Chaucheyras Durand et al., 2007). These organisms are generally of the type *Saccharomyces cerevisiae* and as well as being used in animal feed, are also used in the baking, wine, beer, and biofuel industries. Those that are used commercially as DFM have been screened and selected on the basis that they have a positive effect on rumen fermentation, although care should be taken as it has been shown that some strains of *Saccharomyces* may have a negative effect on performance, causing issues with acidosis due to over-stimulation of fermentation and accumulation of VFA (Chung et al., 2011). Those strains of yeast which have been proven to have a beneficial effect on rumen health and productivity are perhaps best considered as "good yeasts." The "bad yeasts" are primarily spoilage yeasts which can degrade lactic acid in the presence of air and are associated with heating and spoilage of silage in the silo or in the total mixed ration (TMR) at feed-out. These organisms tend to be of the genera *Candida*, *Hansenula*, *Pichia*, *Issatchenkia*, and *Saccharomyces* spp. A recent study (Santos et al., 2011), showed that the predominant yeast isolated from high moisture corn (HMC) and corn silage samples were from the *Candida* genera, with *Candida kruesei* being the dominant contaminant. Incubating TMR samples with different doses of *Candida krusei* in rumen in vitro cultures led to a significant reduction in fibre digestion and negative effects on rumen fermentation (Santos et al., 2011).

Spoilage yeasts are relatively acid tolerant and fast growing under aerobic conditions, quickly entering exponential growth leading to a rapid increase in their numbers. Initial yeast counts of 1×10^5 cfu/g can rapidly multiply and increase to 1.6×10^6 cfu/g in several hours. Considering that silages are fed in kg quantities, this means that the levels entering the rumen are in excess of 10 billion cfu and can potentially have a significant effect on the rumen microflora and rumen fermentation. Under anaerobic conditions, these yeast will form ethanol, and the presence of ethanol in silage can be taken as an indicator that there may be a heavy yeast load. The main problem with these spoilage organisms with regard to silage quality is

that they breakdown lactic acid formed during the natural ensiling process, which causes the pH of the silage to rise. The silage starts to heat and volatilisation of the silage acids occurs, causing a further rise in pH, not only leading to increased growth of these primary spoilage yeasts, but also potentially leading to the opportunistic growth of other undesirable secondary organisms (Clostridia, Bacilli, *Enterobacteracae*, *Listeria*) and molds (*Aspergillus*, *Fusarium*, and *Penicillium*). These organisms are generally pathogenic and can impact not only the quality of the silage, leading to nutrient losses and issues with palatability, but also can impact animal health.

UNDESIRABLE PATHOGENS AND MOLDS

Clostridia exist in the soil and, although present at relatively low numbers on standing crops, can be inoculated into the crop during harvest and handling. Clostridia can either be classified as lactate or amino acid utilisers. The lactate utilisers rapidly ferment any residual sugars and lactic acid into butyric acid. Other types of Clostridia are highly proteolytic and asaccharolytic. These organisms use amino acids as a carbon and nitrogen source, deaminating or decarboxylating amino acids resulting in the formation of ammonia, malodourous biogenic amines, carbon dioxide and a variety of acids (McDonald et al., 1991). Some strains of Clostridia are also extremely pathogenic, and have a negative effect on host health. The Clostridia are spore formers and until the growth conditions are right, exist as spores in a dormant state. When exposed to air and at high pH, they quickly wake up and start to grow, leading to the formation of butyric acid, which not only has a strong smell, but as a weaker acid than lactic acid, has a lower acidity and leads to a further increase in pH. Clostridia-infected silages tend to smell very bad and have poor palatability, have low dry matter content, and are greenish in colour. The nutritive quality is also decreased with losses in DM and crude protein due to the high proteolytic activity, thus animal performance can be reduced.

Like Clostridia, Bacilli also are spore formers and exist in the soil. Although some strains of Bacilli have been shown to have anti-fungal properties and as such have been used as inoculants to reduce aerobic spoilage, the majority are considered as undesirable organisms in silage as they can compete with the lactic acid bacteria for sugars and are less efficient at forming lactic acid and acetic acid, thus contributing to aerobic instability if they dominate the fermentation process. A problem also exists if the silage is contaminated with spores of *Bacillus cereus* as this can be a problem not only for silage quality but for milk quality as well, as *B. cereus* is considered one of the main spoilage organisms of pasteurised milk (te Giffel, 1997).

Listeria sp. are perhaps the most serious pathogen to be found in silage, affecting the health of both farm workers and animals. Feeding silages contaminated with *L. monocytogenes* has been shown to cause death in sheep and goats (Vazquez-Boland et al., 1992; Wiedmann et al., 1994). Listeriosis in humans can cause a variety of digestive upsets, and in some cases can be extremely severe. The most effective method to prevent growth of *L. monocytogenes* is to keep the silage anaerobic (McDonald et al., 1991).

Most silage enterobacteria are non-pathogenic, but their presence in silage is undesirable because they compete with the lactic acid bacteria for sugars and are also proteolytic, leading to the production of biogenic amines which can reduce silage palatability (McDonald et al., 1991). Ammonia formed by proteolysis also can increase the buffering capacity of the forage, thus counteracting any decrease in pH. Enterobacteria can also reduce nitrate to nitrite under silage conditions. In silage, nitrite can be further broken down by these organisms to ammonia and nitrous oxide, but in some instances it can be chemically degraded to NO and nitrate (Spoelstra, 1985) which when mixed with air can be oxidised to a mixture of toxic gaseous nitrogen oxides which are given off as a yellow-brown gas. Inhalation of these gases can cause pneumonia-like symptoms and are detrimental to health. Enterobacteria will not proliferate at low pH. Ensiling methods that induce a rapid and sufficient drop in silage pH will therefore help to decrease enterobacterial growth (McDonald et al., 1991).

Molds can develop where oxygen is present, either in the surface layers or where there are issues with aerobic spoilage. They are easily identified under the microscope as they are filamentous fungi, and some produce coloured spores, otherwise known as the "rainbow effect." The most common mold species isolated from silage belong to the genera *Penicillium*, *Fusarium*, and *Aspergillus*. Molds are an issue due not only to their effects on palatability but also because they present a risk to host health and fertility due to the production of mycotoxins. Depending on the type and level of mycotoxin present, health problems can range from minor digestive upsets, issues with fertility, reduced immune function, liver or kidney damage, and abortion in pregnant animals (Scudamore and Livesey, 1998). Mold growth may be limited by improving aerobic stability.

EFFECT OF FEEDING SPOILED SILAGE ON COW HEALTH AND PERFORMANCE

The issue with feeding spoiled silage is the negative effect it can have on health and performance. Problems with palat-

ability will reduce dry matter intakes and lead to sorting. The risk of sub-acute ruminal acidosis (SARA) is increased as a result, leading to a drop in performance and potentially also leading to milk fat depression (MFD). Nutritive losses due to poor fermentation can also affect the energy content of the silage, further reducing performance.

There has been some evidence that in commercial herds there is a strong correlation between the level of spoilage yeast in the silage fed and the incidence of MFD, with high levels of yeast leading to a reduction in milk fat percentage. This has led to several research groups trying to determine whether spoilage yeasts actually contribute to MFD, either by directly changing the rumen bacterial population resulting in an increased flow of fatty acids through alternate pathways of ruminal biohydrogenation (Lock and van Amburgh, 2012), by reducing fibre digestion as evidenced by Santos et al. (2011), or by increasing the risk of acidosis either by over stimulating rumen fermentation (Chung et al., 2011) or affecting intake and eating behaviour.

Milk fat depression can have significant effects on performance and profitability of the dairy herd. Fat content is generally considered the most variable milk component and can be affected by a range of different factors, but mainly by diet. Three main dietary factors can lead to issues with MFD (Lock and Van Amburgh, 2012). These include increased levels of poly unsaturated fatty acids (PUFA) in the diet; deviations from the normal ruminal biohydrogenation pathway via alternate routes, leading to the production of isomers like c10,t12-CLA and the *trans* 10 shift to t10-vaccenic acid as the predominant 18:1 fatty acid, which has been implicated in the onset of MFD; or altered rates of biohydrogenation. Key to all of these factors are changes to the rumen microflora involved in biohydrogenation. Several different microbes have been identified which are involved in the different steps of biohydrogenation (Paillard et al., 2007). *Propionibacterium acnes* (Wallace et al., 2006) and *Megasphaera elsdenii* (Kim et al., 2005) have been identified as organisms which can form t10,c12-CLA from linoleic acid in pure culture. A further study investigating shifts in the bacterial community during the onset of MFD also identified the presence of *M. elsdenii* as a risk factor associated with MFD (Palmonari et al., 2010). The supplementation of rations with yeast of the genera *S. cerevisiae* is known to increase numbers of *M. elsdenii* (Chaucheyras Durand et al., 2007), and it was hypothesised that supplementation with *S. cerevisiae* may lead to the onset of MFD due to this increase in the population of *M. elsdenii* (Troegeler-Meynadier et al., 2006). However, in a study by Julien et al. (2005), supplementation with *S. cerevisiae* instead favoured the main pathway of ruminal biohydrogenation by stimulating the growth of both fibre digesting bacteria and *Butyrivibrio* spp., thus driving the biohydrogenation pathway through the normal route, leading to the formation of c9,t11-CLA and on towards the formation of t11-vaccenic acid. Interestingly, in this study it was t11-vaccenic acid that was accumulated, and the reaction did not proceed to the formation of stearic acid. Although a strain of *S. cerevisiae* has been shown to degrade t10,c 12-CLA in pure culture (Gurvitz et al., 2001), the resulting degradation products were not analysed, and it is not known whether t10-vaccenic was formed, so it is difficult to determine whether the degradation pathway resulted in the formation of an isomer which could potentially be more potent at inhibiting milk fat synthesis than t10,c12-CLA. Thus, there is some evidence to suggest that *S. cerevisiae* may have some impact on ruminal biohydrogenation, although whether positive or negative requires further evaluation. There may also be some different effects depending on the strain or type of yeast. Further work is also necessary to determine whether any of the other spoilage yeasts may also alter the rumen microbiome and affect rumen biohydrogenation or rumen fermentation negatively.

Increased mycotoxin load can have a significantly negative effect on animal performance and health. This is further increased if the animals are experiencing any issues with SARA. Under non-SARA conditions, the normal rumen flora can help to transform mycotoxins into less toxic forms, but these microbes are generally less prevalent under SARA conditions. During SARA, passage rate is also increased meaning less time for the rumen flora to biotransform any mycotoxins, and damage to the rumen wall can also occur, facilitating the passage of mycotoxins across the rumen wall and into the rumen.

USE OF SILAGE INOCULANTS AND ADDITIVES TO IMPROVE SILAGE QUALITY

Silage inoculants are generally added at ensiling in order to optimise the fermentation process and reduce the growth of undesirable spoilage microorganisms, thus leading to improved aerobic stability. By adding in “extra” lactic acid bacteria at ensiling it can help drive the fermentation in the direction needed, thus optimising the ensiling process, and reduces the chance of an uncontrolled wild fermentation occurring.

Traditionally homofermentative bacteria such as *Lactobacillus plantarum*, *Enterococcus faecium*, and *Pediococcus* spp. have been used in silage inoculants as they rapidly convert water soluble carbohydrates into lactic acid, which

acidifies the silage and drops the pH. Lower ethanol and ammonia nitrogen concentrations are also observed, as is improved dry matter content (Weinberg and Muck, 1996). However, these organisms are not effective at improving aerobic stability as lactic acid is not very effective at maintaining aerobic stability or reducing the growth of yeast and molds. Heterofermentative lactic acid bacteria like *Lactobacillus buchneri* are becoming more widely used as these organisms can convert lactic acid produced in the initial phase of the ensiling process to acetic acid. Acetic acid has anti-fungal effects which can inhibit the growth of spoilage yeasts, thus helping to preserve silages susceptible to aerobic spoilage upon exposure to air (Weinberg et al., 2002; Filya, 2003).

ENZYME ADDITIVES

Research has shown that inclusion of enzymes at ensiling may have positive effects on improving the nutritive value of silages, especially if crops are high in NDF and ADF (Dean et al., 2005). However, the effects of enzyme addition at ensiling improving nutrient availability are variable and would appear to be affected by dose rate, enzymes used, dry matter content, and silage type being ensiled. The addition of fibrolytic enzymes such as xylanases and cellulases are thought to improve the NDF and DM digestibility of different silages by helping to break down the plant cell walls, releasing sugars to increase the growth of lactic acid bacteria, thus improving and speeding up the fermentation process. Their addition also appears to pre-solubilise the fibre fraction, leading to improved DMd and NDFd.

Corn silage and HMC may benefit from longer time in the silo to increase DM and starch digestibility (Newbold et al., 2006). Increased starch digestion during prolonged storage was thought initially to be due to solubilisation of prolamins by acids and alcohols, but recent evidence suggested that it may in fact be due to proteolytic activity in the silo (Hoffman et al., 2010). This suggestion led to some recent trials with an experimental acidic protease, which was added at ensiling to potentially increase the breakdown of prolamin. Results demonstrated that starch digestibility of both corn silage (Young et al., 2012; Windle et al., 2014) and HMC (Kung et al., 2014) may be significantly improved and maximised upon addition of an exogenous protease, allowing silos to be opened after a relatively short period.

SILAGE ANALYSIS IN REAL TIME

It has been recognised that to enable accurate precision farming it is necessary to regularly analyse the quality and composition of the silage to allow for any changes in the silo over time, so that the rest of the ration can be nutritionally matched to optimise cow performance. As such, farmers and nutritionists have come to rely on laboratory-based near infra-red technology (NIR) compared to wet chemistry as a fast and cost effective way of checking the quality of their feed and forages. Key in this analysis is the database and calibrations used to derive the predicted values. In order to develop a good database and robust calibrations, a large number of different silage samples over several different seasons and from several different regions must be analysed using both wet chemistry and NIR. The correlation of wet chemistry and NIR spectral analysis allows the derivation of the equations for the calibrations to be made. Further improvements can be made by tying-in digestibility measurements (in vitro, in vivo) to change theoretical digestibility and energy values to actual values, or with other factors affecting performance. One group in Australia has developed a ruminal acidosis index which links the NIR analysis of different grains and their associated risk of causing ruminal acidosis (Lean et al., 2013). In the UK using NIR, grass silages are designated a PAL value (potential acid load) which is linked to the acidity, fermentability, and digestibility of the silage, allowing the identification of problematic silages which may increase the risk of acidosis. By using NIR to analyse feeds and forages, it will soon be possible to determine whether a herd is at risk of SARA and whether a feed additive or buffer to help stabilise rumen pH may be required in the ration. NIR can also be used to measure starch and fibre present in the manure, thus providing a means of not only evaluating the feed and forage entering the animal, but also allowing a means of determining total tract digestibility in a rapid way.

Recent advances in computing power and the ability to miniaturise optical units have led to the development of portable hand held NIR units which can be used on-farm (www.nir4farm.com). This allows the analysis of silage quality in real-time, so it is possible to know exactly what you are feeding on a particular day. Samples need neither be dried nor ground and are instead analysed in their fresh state. Although with a narrower bandwidth than the laboratory based NIR machines, these units have been demonstrated to be able to determine the DM, CP, starch, NDF, ADF, sugar, lactic acid content, D-value, ME value, pH, and ash of a variety of different silages and TMR samples, based on correlations with the same samples being analysed by wet chemistry and laboratory-based NIR machines. Testing of these machines in the field will allow rapid analysis of different forages and determine the degree of variance over time within the silo from previous readings and whether the ration needs to be reformulated to reflect these changes. NIR analysis will prove to be a

useful tool for nutritionists to keep a close eye on silage quality and how it may affect cow performance.

CONCLUSIONS

To conclude, there are a variety of different factors which can affect silage quality leading to a loss in herd performance. Ensuring good silo management both at ensiling and at feeding-out can maintain aerobic stability and reduce the proliferation of spoilage organisms and heating. Different bacterial inoculants and additives have been developed in order to optimise the ensiling process and increase the nutritive value and quality of the silage. New tools are currently being developed which will allow silage analysis to be carried out quickly and in real-time, allowing precision feeding of the dairy herd and optimising performance and rumen health.

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