



Managing High Moisture Co-product Feeds for Improved Aerobic Stability

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Summary

High moisture, co-product feeds such as brewer's and distiller's grains can be economical feeds for ruminants. However, two major factors limit the use of these types of feeds. First, their high moisture content (approximately 50-70%) limits the distance that they can be economically shipped from their sources of production (perhaps 75 to 100 miles). Second, the aerobic stability of these feeds can be extremely poor (two to seven days) in warm weather limiting delivery to small quantities that must be fed quickly. Spoiled grains are extremely unpalatable and cause reductions in nutrient intake when fed to livestock. This paper will describe the microbial ecology of wet grains and discuss various methods to improve their aerobic stability.

Introduction

Wet co-product feeds such as brewer's and distiller's grains can be economical feeds for ruminant because they are high in protein, fat, and digestible fiber. Specifically, the supply of distiller's grain is increasing with the opening of more plants for the production of ethanol as an alternative source of fuel. For simplicity, we will generally center on the use wet distiller's grains (WDG) as an example of all high moisture co-product feeds for the remainder of this paper.

During the production of ethanol a significant amount of heat is used to gelatinize the starch that, also kills many undesirable microorganisms. Distiller's grains are essentially sterile after processing but may have low numbers of residual yeasts. After processing, the grains are hot (70-80°C), have a low pH (< 4) and have low residual amounts of soluble sugars and starch.

Microbial Ecology of WDG

There is little published research on the microbial ecology of wet grains. Thus, some of the following discussion is based on internal research studies from Kemin Industries and on known information on silages.

Inoculation of the WDG by spoilage microorganisms occurs quickly and comes from a variety of sources including processing equipment, spoiled feed around the storage pad, the general environment, and transportation equipment. The initiators of spoilage are usually yeasts that are not readily discernable by eye. Even a low number of yeasts can result in poor aerobic stability because they often have generation times of about one hour. If WDG were contaminated with 100 (10^2) yeasts per gram of wet grain, under optimal conditions, their population could increase to more than 100,000 (10^5) cfu/g in less than ten hours. In an additional four hours, the population could increase to more than 1,600,000 (10^6) cfu/g. Species of yeasts that have been identified specifically in WDG include *Kluveromyces*, *Pichia*, *Candida*, and *Debrayomyces* (Kemin Industries, 2003 unpublished data). Species of the later three are also often responsible for initiating aerobic spoilage in silages. For example, in corn silage, numbers of yeasts are highly and negatively correlated with aerobic stability. In a summary of published studies (Figure 1), the data show that when there are more than 10^6 cfu of yeast per gram of corn silage, the aerobic stability of that silage is extremely poor (Kleinschmit, 2003 MS Thesis University of Delaware). In contrast, if no yeasts were present, corn silage would be stable for more than 150 hours. As spoilage progresses for WDG, molds that are often easily identified by

eye, continue the process followed by further spoilage by bacteria. Species of molds that have been identified in WDG include *Aspergillus* sp., *Geotrichum* sp., *Mucor* sp., *Paeliomyces* sp., *Fusarium* sp., *Botrytis* sp., and *Penicillium* sp. (Kemin Industries, 2003 unpublished data). Several of these species have been implicated in the production of a variety of toxins that could have negative effects on animal health. Wadhwa (1995) reported mycotoxicosis in buffalo fed brewer's grains contaminated with *Aspergillus flavus*.

The rapidity of growth for yeasts and molds on WDG is dictated by a variety of factors. Temperature can have a profound effect on the growth of yeasts because most grow well between temperatures of 25 to 40°C. In cool weather, their metabolism is slowed. For example, Savard et al. (2002) reported that a species of *Saccharomyces* from fermented vegetables increased in numbers from 10³ cfu/ml to 10⁵ cfu/ml in less than 3 days in juice with a temperature of 30°C, where as it took about 30 days for a similar increase if the temperature was at 3°C. Reports from the field generally agree that the problem of poor aerobic stability for WDG is greatly reduced during cold weather.

Water available for metabolic activity (a_w) is also a factor that affects the growth of spoilage microorganisms. The fact that is a_w limiting in dry feeds is the reason that they resist spoilage. Dried distiller's grains can be stored for prolonged

periods of time with little fear of spoilage. In contrast, WDG have sufficient A_w that allows for rapid growth of microbes. Yeasts are also primarily aerobic microorganisms and thus exposure to air stimulates their growth. Residual amounts of sugars and some starch in distiller's grains provide sufficient nutrients for this to occur. Unlike many bacteria, yeasts are very tolerant of low pH. In fact, depending on the acidifying agent, some yeast can grow well even when the pH is below 2. Thus, although WDG has a pH below 4, yeasts and molds can thrive in this environment.

A variety of factors can affect the population of spoilage organisms on WDG by the time it reaches the farm. For example, the grains may sit on a pad at the ethanol plant for 30 min up to 3 days prior to delivery to the farm. The numbers of yeasts and molds on WDG sampled at ethanol plants vary based on the location and time of sampling relative to it leaving the process line. If sampled after storage on a pad, numbers of yeasts may be as high as 10⁴ to 10⁵ cfu/g. In contrast, in eight samples of WDG treated with a preservative during the summer in Midwest ethanol plants, there were less than 10 cfu/g and 10³ cfu/g of molds and yeasts, respectively in these freshly treated samples (Kemin Industries, 2003 unpublished data).

Methods to Improve the Aerobic Stability of WDG

Organic Acids

Weak organic acids such as propionic, sorbic, and benzoic acids act as uncoupling agents in fungal cells. Of the three acids, sorbic and benzoic acid are effective at inhibiting the growth of yeasts and molds. Propionic acid is more effective at inhibiting the growth of molds than yeasts although its ammoniated form is relatively active in inhibiting the later. Undissociated acids are more potent because they readily pass through the cell membranes and liberate their protons, thus acidifying the cytoplasm and decreasing the proton gradient. Thus, the majority of acids have poor antifungal activity at a neutral pH. Note that in the common acids listed below (Table 1) lactic acid that is commonly the major acid in silages is only mildly dissociated (15%) at pH of 3.5. This explains why a low pH in silages is not a good indicator of the degree to which silage will be stable when exposed to air.

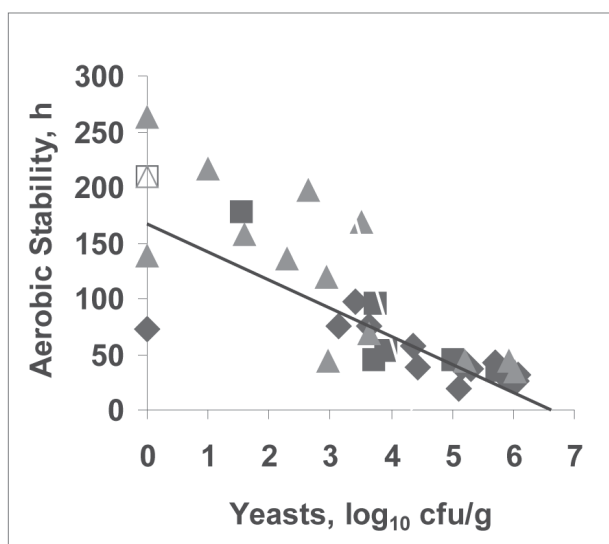


Figure 1. The relationship between yeasts and aerobic stability in corn silage. (Kleinschmit, 2004 M.S. Thesis University of Delaware.

Table 1. Undissociation of acids as affected by pH.
(Modified from DeBoer and Nielsen (1995).

Preservative	pKa	% undissociated acid at pH			
		2.5	3.5	4.5	5.0
Acetic acid	4.74	99	95	63	33
Lactic acid	2.74	64	15	1.7	0.5
Benzoic acid	4.19	98	83	33	13
Propionic acid	4.87	100	96	70	43
Sorbic acid	4.76	99	95	65	37

In contrast, acetic, propionic and sorbic acids are highly undissociated at a pH of 3.5 (close to the pH of wet distiller's grains as it leaves the plant). Anion toxicity has also been suggested as an explanation for antifungal properties of weak acids. In addition, alteration of cell membrane transport and inhibition of NADH oxidation have also been suggested as mechanisms for antifungal activity. The degree of antifungal strength is also based on solubility. For example, potassium sorbate and sodium benzoate are more soluble than their respective counterparts. Propionic acid and potassium sorbate have both been used to stabilize wet brewer's grains (Schneider et al., 1995; Wyss, 2002). Other compounds such as antibiotics, herbs, antioxidants, spices, essential oils and other chemicals have been used with varying success to control yeasts and molds in foods. However, there is little information on their use to stabilize WDG.

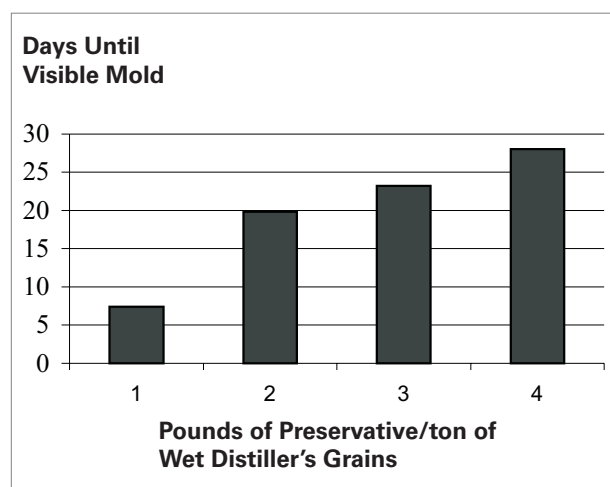


Figure 2. Days to mold in WDG treated with an organic acid preservative. Kemin Industries. Fact Sheet 42003.

Combinations of antifungal agents have been shown to sometimes be more effective than single agents alone. Thus, many aerobic stabilizers used for silages have multiple active ingredients (Kung et al., 2003). A preservative designed specifically for use on high moisture grains was tested in a laboratory study (Figure 2). The preservative contained a blend of organic acids and antioxidants. Treatments were with 0, 6, 12, or 18 lb of preservative/ton of a modified WDG (50% moisture). Samples were placed in Styrofoam coolers and were exposed to air at room temperature ($\sim 25^{\circ}\text{C}$) for 28 days. Visible mold was observed within 7 days on untreated WDG but increasing amounts of treatment resulted in less rapid development of molds. These data show that the aerobic stability of WDG can be improved with organic acid-based preservatives.

Ensiling and Storing

Wet distiller's grains alone do not undergo a robust ferment because low numbers of naturally occurring lactic acid bacteria, a low pH that retards the growth of fermentative bacteria and sometimes marginal amounts of fermentable substrates. Variable results were obtained when wet brewer's grains were ensiled with bacterial inoculants (Schneider et al. 1995). Wet brewer's grains have been combined with other feeds that have supplied more fermentable substrates and ensiled well. Combining wet grains with drier feeds also decreases the moisture content and helps to decrease the potential for excessive amounts of seepage. Mills and Grant (2002) reported that it was practical for wet corn gluten feed to replace from 10 to 30% of corn forage (DM basis) at the time of ensiling and that there were no negative effects on the subsequent silage fermentation. Partial replacement of corn forage with wet corn gluten feed also increased packing density of the silage. Kalchauer et al. (2003) reported that a 50:50 mixture of corn forage and WDG ensiled in bag silos fermented well and had a low pH and moderate amounts of acetic acid. Schneider et al. (1995) successfully ensiled wet brewer's grains with beet pulp.

In warm weather, bagging or covering WDG with a tarp on a pad to minimize its exposure to air can improve its aerobic stability (perhaps an added 7 to 14 d or more). However, care must be taken to not over pack bag silos or they will split. In addition, bags must be managed during feed out to minimize the contact between feed and air.

Storing WDG in a location out of direct sunlight to minimize heating may also help to retard spoilage during warm weather.

Conclusions

High moisture co product grains are excellent feeds for ruminants. However, their short shelf life in warm weather is a drawback to their use. Only a

limited amount of research has been undertaken to understand the microbial ecology of wet distiller's grains and factors affecting its aerobic stability on farm. Use of organic acid-based preservatives to improve the aerobic stability of high moisture grains appears promising. Overall, more research is needed in order to better understand the factors related to enhancing the aerobic stability of high moisture co product grains.

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