DEALING WITH PROBLEM SILAGES: FOCUS ON CLOSTRIDIUM

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The dairy industry realizes the importance of forage quality, and puts a lot of effort into producing a feed that is highly digestible, properly preserved, and palatable. Unfortunately, the end result is not always what was intended. Environmental conditions and delayed harvest timing can lower silage digestibility. Excessive growth of molds and yeasts, along with inappropriate silage fermentations, can result in silage with poor aerobic stability and decreased palatability. This paper will focus on clostridial silage fermentations. The causes of these problem fermentations, methods to decrease their risk, consequences they may cause on the dairy, and strategies to handle this type of silage will be discussed.

CAUSES AND CHARACTERISTICS OF CLOSTRIDIAL FERMENTATIONS

Silage that goes through a clostridial fermentation stinks! Clostridial fermentations are so malodorous because of the high levels of butyric acid, amines (e.g. putrescine and cadavarine), and ammonia that typify these fermentations. Clostridia silage is typically wet (< 32% DM) and often olive or greenish in color.

Clostridia are naturally present in the soil. Although their numbers are very low on standing forage, inoculation occurs during mechanical handling of the crop. The bacteria prefer growth conditions that are wet (< 30% DM) and at a higher pH, although growth can still occur at slightly higher DM and down to a pH of 4.2. They typically take over the fermentation when the lactobacilli have used up available substrate, the silage is wet, and the pH is still above ~4.8 (McDonald, 1991). Although the clostridia genus contains over 60 species, it appears that only about seven play a predominant role in silage fermentation (Gibson, 1965). *Clostridium tyrobutyricum* is the species most frequently isolated from silage (McDonald, 1991). Botulism is caused by ingestion of the botulinum toxin, produced by *Clostridium botulinum*. Botulism toxicosis has been reviewed elsewhere (Whitlock and Williams, 1999), and will not be discussed here.

Clostridia have been classified as either lacate or amino acid fermenters. Lactate fermenting clostridia bacteria ferment any remaining sugars and lactic acid, increasing silage pH and producing butyric acid as a fermentative end-product. Amino acid fermenting clostridia deaminate or decarboxylate amino acids, resulting in the production of ammonia, amines, CO2, and a variety of acids (McDonald, 1991). The pH of a clostridial fermentation increases, since butyrate is a much weaker acid than lactate, and since it takes two moles of lactate to produce one mole of butyrate.

Kemble (1956) inoculated ryegrass silage with clostridia or lactobacilli. Ammonia levels increased markedly in the silage inoculated with clostridia, with the largest increase occurring between 21 and 57 days post-ensiling. The amount of ammonia present in silages has been used to estimate the degree of proteolytic clostridial activity since it had been thought that little ammonia was produced by other silage microbes. However, large amounts of ammonia can also be produced by enterobacteria (McDonald, 1991). A guideline used in the evaluations of silage fermentations is that ammonia nitrogen should be less than 10% of the total nitrogen (Mahanna and Chase, 2003).

The importance of low DM silage in the evolvement of a clostridial fermentation can be seen from the data of Ward (2000). Fermentation profiles from approximately 1200 alfalfa and 400 grass silages indicate that butyrate and ammonia nitrogen levels increased, and lactate decreased, as silage DM decreased (Figures 1 and 2). These data corroborate that compiled by Pitt (1990), which indicate that alfalfa typically has inadequate levels of sugar to complete the fermentation at DM less than 34%, while grasses, due to their lower natural buffering capacity and higher sugar levels, may still have a complete fermentation at DM as low as 17%. Evidence of a clostridial fermentation (increased butyrate and ammonia, decreased lactate) in grasses begins at the 28-32% DM range, and becomes more pronounced as DM further decreases (Figure 2).

The amino acid composition of silage is dramatically altered in a clostridial fermentation. Mead (1971) grew three species of clostridia (*Cl. bifermentans, Cl. sporogenes, and Cl. tetanomorphum*) in pure culture to evaluate their relative effects on various amino acids. Both the direction and degree of change in amino acid levels varied with the species of clostridia. Their results indicated that the amino acid fermenting clostridia will certainly change the amino acid composition of silage, the degree and direction of the alteration varying with the species of clostridium predominating in the fermentation.

ANIMAL TRIALS WITH CLOSTRIDIAL SILAGES OR THEIR FERMENTATION END-PRODUCTS

Thomas et al. (1961) fed heifers silage of varying dry matters. They found that intake was linearly and positively related to the dry matter content of the silage. The authors concluded that the dry matter content of the silage at ensiling, and the resultant fermentation process, was very important in determining the rate of silage consumption.

Since then many trials have evaluated animal responses when silage juice, or a purified component of something found in silage juice, was orally intubated or ruminally infused. Most of these trials used sheep, steers, or heifers; few have used high-producing dairy cows. The silage juice infusion generally has a greater negative effect on intake than the infusion of purified or a laboratory prepared mix of silage juice components.

Clancy et al. (1977) treated alfalfa at ensiling with formic acid. Pressed silage juice from the control and formic acid treated silages, and a synthetic juice with many of the

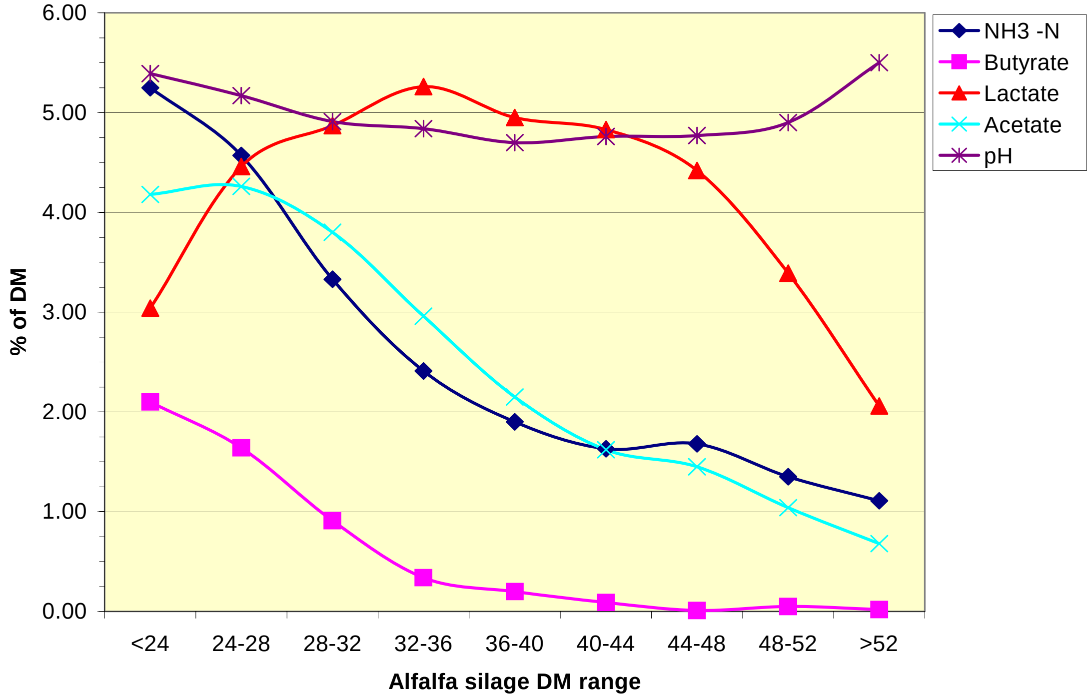

Figure 1. The relationship between alfalfa silage DM and the resultant fermentation (Ward, 2000).

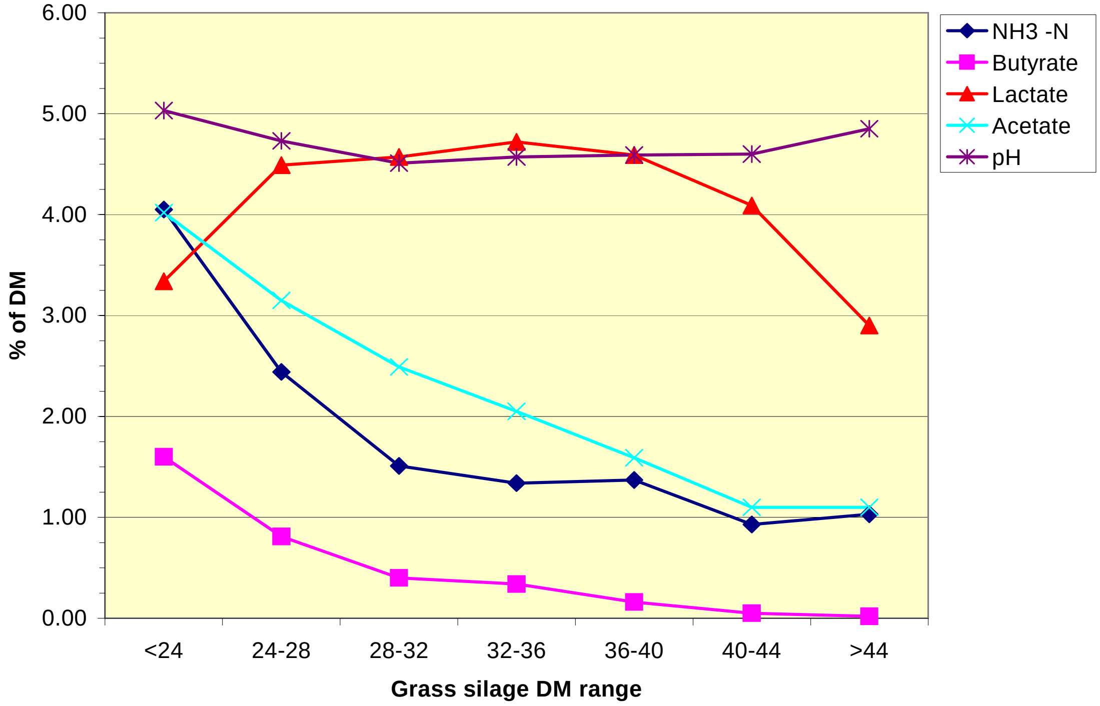

Figure 2. The relationship between grass silage DM and the resultant fermentation (Ward, 2000).

same characteristics as the silage juice, was ruminally infused into sheep. Intakes were depressed for a longer time period in sheep infused with the control silage juice than with that obtained from formic acid treated silage. Intakes were depressed less when sheep were infused with the synthetic juice. The authors concluded that other factors, such as the amines, may have resulted in the greater intake depressing effects seen with the control silage juice.

Barry et al. (1978a) preserved fresh-cut lucerne with formic acid or formaldehyde, and then fed the silage to sheep in a nitrogen balance trial. Voluntary intake decreased linearly with increasing degradation of silage proteins and the formation of acetic acid and ammonia. They concluded that the control of proteolytic clostridial activity was more critical than the control of plant respiration following ensiling in avoiding restrictions to intake and nitrogen retention. In a companion paper, Barry et al. (1978b) evaluated the influence of silage amino acid composition on the nutritive value of the silage. They reported that intake and nitrogen utilization were related to the net changes in amino acids involved with decarboxylation reactions, while those involved with deamination reactions appeared to be of much less importance. Formaldehyde treatment of fresh forage reduces protein degradation in the silo, thus dramatically reducing the potential development of biogenic amines (Barry et al., 1978a).

Each of the amines listed in Table 1 are formed from decarboxylation reactions. For example, lysine is catabolized to form cadaverine and CO_2, while arginine forms putrescine and CO_2. Lysine and arginine can also be deaminated, resulting in the formation of acetate, butyrate and ammonia (from lysine), and ornithine, ammonia, and CO_2 (from arginine) (MacDonald et al. 1991).

Lingass and Tveit (1992) ruminally intubated cows with butyric acid (200 g split into 2 doses given 2 h apart) for three consecutive days. Blood acetoacetate levels significantly increased in both early and late lactation cows. Acetoacetate levels rose higher, and remained elevated for a longer period of time, in early lactation as compared to later lactation cows. It would require the consumption of 2.85 kg DM of 7% butyric acid haylage (the level found in the silages described in Table 1) to equal this quantity of infused butyrate.

Lingass and Tveit (1992) also ruminally intubated cows with putrescine (100 g in 1 dose) for three consecutive days (Figure 3). The putrescine infusate resulted in significant decreases in feed intake and milk yield, with marked individual variation in response. For example, one of the cows consumed very little feed on the last experimental day, although she recovered completely after the experimental period. The quantity of putrescine infused was much greater than cows would normally consume, even if the feeds had undergone a clostridial fermentation. For example, it would take approximately 100 kg DM of the silage described in Table 1 to provide 100 g of putrescine, or approximately 20 kg DM of the silage to provide 100 g of all of the measured amines. Lingass and Tveit (1992) recognized this, noting that the effect of the amines when fed in combination and for a longer time period, as when feeding silage, may well be different than when infusing only one amine for a relatively short time period. Support for this observation is seen in Tveit et al. (1992), who fed cows haylage that had undergone a clostridial fermentation. They found that the ruminal concentrations of amines increased throughout the seven day experimental period. The authors speculated that the amine degradation capacity of rumen microflora was reduced over time, leading to an increase in ruminal amine concentrations and thus an increased risk to the “possible toxic effects” of the amines.

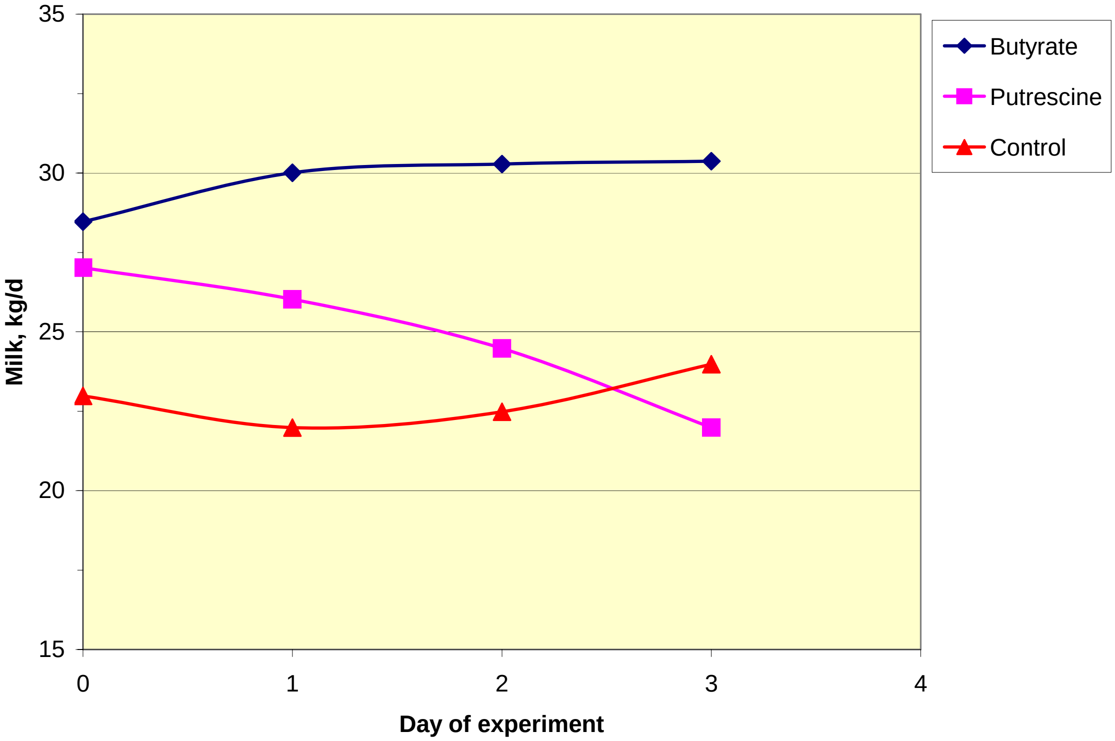

Figure 3. The effect of ruminal intubation of butyrate, putrescine, or no treatment on milk production (Lingaas and Tveit, 1992). Infusions occurred on days 1-3.

Anderson and Lundstrom (1985) fed early lactation Swedish Red and White cows varying amounts a grass silage containing approximately 2% butyric acid. They reported a significantly negative correlation between ketone bodies and milk yield, and a trend for ketone values to increase when butyrate intake exceeded 100 g. Milk yield averaged about 25 kg during the eight week trial.

Tveit and Lingaas (1992) found that while the concentration of amines from eleven samples collected from two silos were highly correlated (~.7), the correlation between the amines and butyrate was only about .2. These results seem logical, since the clostridia involved with the fermentation could have been butyrate or amino acid fermenters. The weak correlation between the amines and butyrate could explain the variability in performance when animals are fed clostridial silages. Several commercial laboratories measure silage VFA levels, while there are very few labs that measure the amines. Additionally, it currently costs approximately $20 for a VFA analysis, while the amine analysis costs in excess of $100. Largely because of these reasons, feeding recommendations for clostridial silages have sometimes been made based on an estimated level of butyric acid intake (Anderson and Lundstrom, 1985). Since the amines have had more pronounced deleterious effects on performance than butyrate, and the two may not be strongly correlated, it could be problematic to base feeding recommendations of clostridial silages on the predicted level of butyric acid intake. However, the critical levels of an amine, or combination of amines, or amines and butyrate, for causing problems in a given species and in a given class of animal are unknown. Thus, the safest approach is to try to minimize exposure of clostridial silage to classes of animal that may be at a higher risk of developing health problems (e.g. recently fresh and high DMI cows).

MANAGEMENT OF CLOSTRIDIAL SILAGE

Since intake can be reduced and ketone levels elevated when clostridial silage is fed, it makes sense to try to avoid exposure of transition and high DMI animals to this silage.

Haylage was collected from the upper, middle, and lower thirds of nine bunker silos in New York. The silage was analyzed for VFA and other typical forage components (Stone et al., 2003). Butyrate levels varied between and within silos (Figure 4). Some of the silo regions could really cause some problems, especially if the feeder obtained all of the silage for the prefresh or fresh groups from a region that had underwent a clostridial fermentation. Nutritionists and producers should constantly evaluate bunker silos for layers and regions that have gone through a bad fermentation. These regions can typically be identified by critically using our visual and olfactory senses. Objective VFA measurements can be readily obtained from commercial feed laboratories. Ideally, silage defacers would be used to remove silage from these regions, either discarding it or feeding to a less susceptible class of animal (e.g. bred heifers).

Two samples of wet, greenish-brown, foul-smelling silage were used in an experiment to evaluate the effect of aeration and mixing on the levels of VFA and amines. Silage from both bunkers was spread out on the floor in layers 2.5-5.0 cm deep. The silage was allowed to aerate, and samples were collected at the start of the study, and 2, 4, 8, and 26 hours later. Additionally, each of the silages was mixed in a portable cement mixer, with samples collected after 2 and 4 hours of mixing. The temperature averaged approximately 21° C throughout aeration time where the floor samples were located, and 26° C during operation of the cement mixer. The VFA were measured by Dairy One (Ithaca, NY) using a gas chromatograph, while the amines were measured by the toxicology lab at Mississippi State University using the procedure of Tamim et al. (2002).

Both silages were characteristic of a clostridial fermentation – low DM, with high levels of butyrate, the amines, and ammonia, and low levels of lactate (Tables 1 and 2). The alfalfa silage that was spread out on the floor dried out much more than the other samples, simply because there was less of this silage and the layer was only about 2.5 cm deep. Interestingly, the reduction in ammonia was also the greatest in this treatment

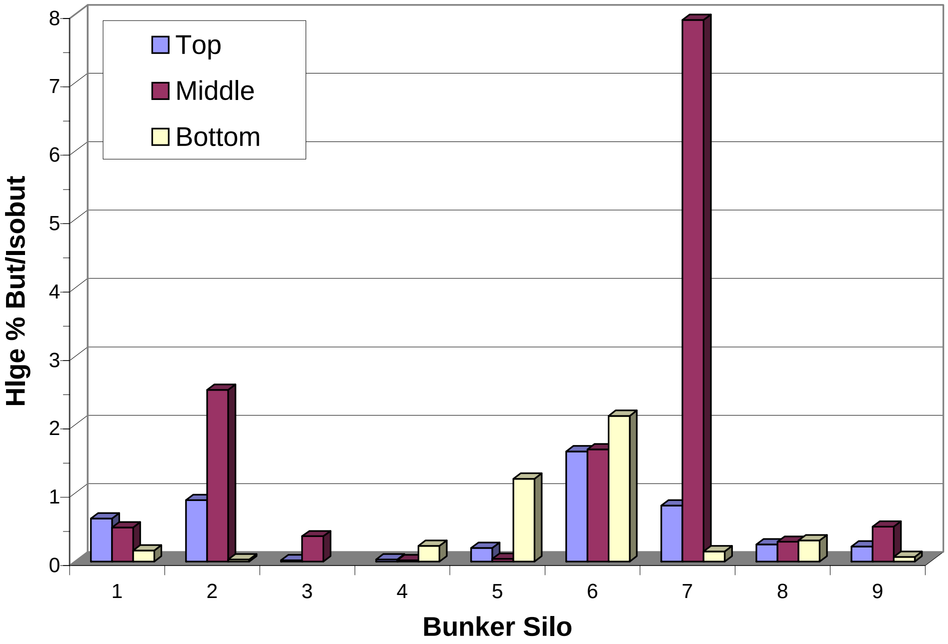

Figure 4. Levels of butyric and isobutyric acid (% DM) in bunker silos of haylage at nine New York dairies. Samples were collected with a backhoe from the upper, middle, and bottom thirds of most silos.

(Table 1). Crude protein levels in feed dried for four hours at 60° C were about ten percentage points lower than the measured CP in the wet samples. This represents the large quantity of ammonia nitrogen volatized during the drying process. Since feed proteins typically average about 16% nitrogen, CP levels are obtained by multiplying the nitrogen level by 6.25. Ammonia is 82% nitrogen. Thus, the volatized nitrogen should technically be multiplied by a factor of 1.2, resulting in an increase in CP in the wet samples of about two percentage points over the dry CP measurements. Butyrate levels were significantly reduced with aeration, with the greatest reduction occurring in the alfalfa silage that dried out the most. The decrease in butyrate in the other samples was significant, although the slight decrease in these samples may not have clinical or practical relevance. Mixing did not result in any significant loss of VFA or ammonia.

Neither aeration or mixing affected amine levels if all results were included in the statistical analysis. However, results from the alfalfa haylage that was placed in the cement mixer were inconsistent with the other sample results; the amine levels in this sample increased during mixing time. Dr. Lloyd Bennett, who ran the amine analyses and co-developed the analytical procedure, states that the relative standard deviation of the procedure is small (approximately 10%), but that variations in amine levels throughout the silo can be large (personal communication). Despite the samples being well-mixed, it is likely that the results from this treatment were a sampling artifact. If these results are excluded from the statistical analysis, then amine concentrations decreased over time (Table 2). In conclusion from this study, aeration appears to

Table 1. The effect of aeration and mixing on VFA and ammonia-N levels[a].

Hour	DM (b)	NH3 Nitrogen as % of Total N	pH	Lactate	Acetate	Propionate (c)	Butyrate (d)	Total VFA	CP, DM	CP, AF (e)
Alfalfa Silage - Floor										
0	25.9	54.0	6.2	0.1	3.7	1.3	7.6	13.3	19.0	29.2
2	27.2	56.7	6.2	0.1	3.7	1.4	7.7	13.2	18.4	29.9
4	30.2	52.0	6.3	0.0	3.9	1.4	7.5	13.2	19.6	26.8
8	35.7	49.0	6.5	0.1	3.8	1.4	7.2	12.9	20.0	28.1
26	68.4	27.2	6.4	0.1	2.7	0.9	4.3	8.1	18.1	
Alfalfa Silage - Cement Mixer										
0	25.9	54.0	6.2	0.1	3.7	1.3	7.6	13.3	19.0	29.2
2	28.1	53.0	6.3	0.0	3.5	1.3	7.5	12.8	19.1	28.8
4	31.7	54.6	6.4	0.1	3.7	1.3	7.2	12.8	18.0	24.9
Grass Silage - Floor										
0	23.7	96.0	6.1	0.1	7.4	1.7	7.3	17.1	13.7	26.1
2	24.5	85.2	6.1	0.1	7.2	1.6	7.1	16.5	14.3	26.0
4	25.6	99.8	6.2	0.1	7.8	1.7	7.5	17.5	13.9	25.9
8	29.4	88.2	6.2	0.1	6.6	1.5	6.5	15.1	13.6	25.7
26	33.9	84.7	6.4	0.0	6.2	1.4	5.8	13.8	12.9	23.1
Grass Silage - Cement Mixer										
0	23.7	96.0	6.1	0.1	7.4	1.7	7.3	17.1	13.7	26.1
2	24.0	96.0	6.2	0.1	7.1	1.6	7.1	16.5	13.4	26.1
4	24.8	98.6	6.3	0.1	7.1	1.6	7.1	16.5	13.1	27.4

Unless noted, results are on a DM basis.

[a] Samples were allowed to aerate by either spreading them out on the floor or mixing in a cement mixer.

[b] DM increased with aeration ($P < .01$)

[c] Propionate tended to decrease with aeration ($P = .07$).

[d] Butyrate decreased with aeration ($P < .0001$).

[e] As fed CP levels tended to decrease with aeration ($P = .06$).

reduce butyrate and amine concentrations, the level of reduction probably being correlated to the length of time the samples are aerated. Drying can further help to reduce butyrate and ammonia levels.

PREVENTION OF CLOSTRIDIAL FERMENTATIONS

The primary means to reduce the risk of a clostridial fermentation is to harvest haylage at DM above 34%. Increasing sugar levels at ensiling and promoting a rapid decrease in silage pH will also minimize the risk.

Alfalfa and grass will dry out more quickly, and will have higher sugar levels at ensiling, if windrows are simply made wider. Wider windrows increase the crop’s surface area that is exposed to sun and air, resulting in substantial reductions in required drying times. This is critical in New York, where the number of consecutively sunny days in May and June is often very few. Some producers have begun to replace their existing harvesting equipment with newer models that are more amenable to wider windrows. Others have purchased smaller haybines (e.g. 14’ versus 18’) so that less material is in a windrow.

Table 2. The effect of aeration and mixing on amine concentrations[a].

Hour	DM	Tryptamine ppm	Phenethylamine ppm	Putrescine ppm	Cadaverine ppm	Histamine ppm	Tyramine ppm
Alfalfa Silage - Floor							
0	25.9	467.3	1245.2	1074.7	1725.1	207.1	897.2
2	27.2	439.5	1472.5	1321.2	2197.7	113.0	1543.9
4	30.2	449.7	1348.7	1037.5	1602.4	183.8	982.1
8	35.7	377.0	1198.6	1041.7	1656.9	167.2	862.7
26	68.4	317.7	847.5	680.2	1111.0	133.7	622.8
Alfalfa Silage - cement mixer							
0	25.9	467.3	1245.2	1074.7	1725.1	207.1	897.2
2	28.1	525.1	1363.0	1189.7	1920.5	152.6	971.6
4	31.7	645.6	1508.5	1183.1	2100.6	181.1	1208.8
Grass Silage - Floor							
0	23.7	397.7	751.2	913.2	1825.7	108.5	1210.5
2	24.5	324.8	667.0	733.8	1369.0	105.0	1032.2
4	25.6	338.8	714.9	677.8	1552.6	133.6	780.2
8	29.4	415.0	828.3	794.3	1639.0	123.6	1153.8
26	33.9	313.7	644.6	650.9	1273.4	94.6	722.5
Grass Silage - Cement Mixer							
0	23.7	397.7	751.2	913.2	1825.7	108.5	1210.5
2	24.0	172.9	482.2	497.4	940.4	71.7	63.6
4	24.8	38.5	383.0	331.2	643.7	52.3	385.5
[bc]Significance, P =		0.02	0.07	0.04	0.03	0.06	0.07

Results are on a DM basis.

[a] Samples were allowed to aerate by either spreading them out on the floor or mixing in a cement mixer.

[b] Aeration time did not affect amine concentrations when all treatments were included.

[c] Amine concentrations were reduced at the noted significance level if results from the alfalfa silage - cement mixer treatment were excluded.

Contamination of haylage with soil should be minimized, since it harbors clostridium and other unfavorable silage bacteria (McDonald et al., 1991).

Management techniques (e.g. aggressive packing) and additives (e.g. lactobacillus inoculant) that aid in a rapid reduction in silage pH should also help to minimize the risk of a clostridial fermentation, since these bacteria prefer to grow at a higher pH.

CONCLUSION

Silage that has underwent a clostridial fermentation can reduce intake and increase blood ketone levels. The main management approach to minimize the risk of a clostridial fermentation is to harvest haylage at DM above 34%. Nutritionists and feeders should regularly inspect haylage for evidence of a clostridial fermentation. Regions of poor haylage within the bunker should be directed away from transition and high DMI cows. Aeration can aid in decreasing butyrate, ammonia, and amine levels typically found in clostridial silage.

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