

UNDERSTANDING THE BIOLOGY OF SILAGE PRESERVATION TO MAXIMIZE QUALITY AND PROTECT THE ENVIRONMENT

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ABSTRACT

The goal of making silage is to preserve as much as the original nutritive value of the crop as possible so that it can be fed throughout the year. Many factors affect the fermentation process and unfortunately the end result is not always good. Poor fermentations can lead to excessive run off, loss of nutrients, and production of spoiled silage that is no longer fit for feeding and must be disposed of. Understanding the fermentation process and how it interacts with management factors such as silo packing speed, silage pack density, type of additive used, chop length, silo management during storage, and silo management during feed-out should help us to minimize nutritive losses during fermentation. In many, but not all cases, the fermentation that a crop undergoes can be explained by how microbes interact with factors such as air, moisture content, buffering capacity, and sugar content.

THE ENSILING PROCESS

From a practical view, the three most important things that must occur in order to make good silage are 1) the rapid removal of air, 2) the rapid production of lactic acid that results in a rapid drop in pH, and 3) continued exclusion of air from the silage mass during storage and feedout.

Rapid removal of air is important because it prevents the growth of unwanted aerobic bacteria, yeasts, and molds that compete with beneficial bacteria for substrate. Air can be eliminated by wilting plant material to recommended dry matters (DM) for the specific crop and storage structure, chopping forage to a correct length, quick packing, good compacting, even distribution of forage in the storage structure, and immediately sealing the silo. After chopping, plant respiration continues for several hours (and perhaps days if silage is poorly packed) and plant enzymes (e.g., proteases) are active until air is used up. Air must be removed before optimal fermentation can take place. If air is not removed quickly, high temperatures and prolonged heating are commonly observed in the silage mass.

Once air is removed, fermentation can begin. Lactic acid bacteria (LAB) utilize water-soluble carbohydrates to produce lactic acid, the primary acid, responsible for increasing the acidity and decreasing the pH in silage. The strength of silage acids can be determined by measuring silage pH. A pH above 7 is considered basic whereas a pH below 7 is acidic. A pH of 7 is neutral and means that a product is neither acidic nor basic. Depending on the crop, plant material in the field can range from a pH of about 5 to 6 and decrease to a pH of 3.6 to 4.5 after acid is produced. A quick reduction in silage pH will help to limit the breakdown of protein in the silo

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by inactivating plant proteases. In addition, a rapid decrease in pH will inhibit the growth of undesirable anaerobic microorganisms such as enterobacteria and clostridia. Eventually, continued production of lactic acid and a decrease in pH inhibits growth of all bacteria. Good fermentations probably result in total dry matter losses of less than 10 to 12%. Poor fermentations coupled with poor storage conditions may drive total dry matter losses to greater than 20%. Losses of DM may come from run off, oxidation and loss of volatile organic compounds.

In general, once fermentation is complete, good silage will remain stable and not change in composition or heat. This is why filling silos quickly and sealing of silos immediately after filling is so important. However, depending on the mixture of fermentation end products, silage can spoil rapidly if exposed to air during storage and feed out. A common misconception is that molds are responsible for spoilage of silage when it is exposed to air. However, yeasts (not molds) are the primary microorganisms that cause aerobic spoilage and heating. When exposed to air, yeast metabolize lactic acid that causes the pH of the silage to increase, thus allowing bacteria that were inhibited by low pH to grow and further spoil the mass. Airtight silos and removal of sufficient silage during feed-out can help to prevent aerobic spoilage.

Although the ensiling process appears quite simple, many factors can affect what type of fermentation takes place in a silo and thus, the mixture of end products (Figure 1). For example, the buffering content of a forage mass can have an effect on silage fermentation. Alfalfa has a high buffering capacity in comparison to corn. Thus, it takes more acid production to lower the pH in alfalfa than in corn silage, resulting in the former being more difficult to make. The dry matter content of the forage can also have major effects on the ensiling process via a number of different mechanisms. First, drier silages do not pack well and thus it is difficult to exclude all of the air from the forage mass. Second, as the dry matter content increases, growth of lactic acid bacteria is curtailed and the rate and extent of fermentation is reduced. (For example, acidification occurs at a slower rate and the amount of total acid produced is less). Thirdly, undesirable bacteria called clostridia tend to thrive in very wet silages and can result in excessive protein degradation, DM loss, and production of toxins. Where weather permits, wilting forage above 30-35% DM prior to ensiling can reduce the incidence of clostridia because these organisms are not very osmotolerant (they do not like dry conditions). Delayed filling, which, results in excessive amounts of air trapped in the forage mass can have detrimental effects on the ensiling process. Another factor that can affect the ensiling process is the amount of water-soluble carbohydrates present for good fermentation to take place. Hirsch and Kung (unpublished data, University of Delaware) showed that WSC dramatically decreased and DM losses increased when corn forage was not immediately packed into silos after chopping (Figure 2). Losses increased with prolonged times of delay. The types and numbers of bacteria on the plant also have profound effects on silage fermentation. Natural populations of lactic acid bacteria (LAB) on plant material are often low in number and heterofermentative (produce end products other than lactic acid). In addition, if air is not removed from the silage mass, other types of fermentation can occur. Some important management practices that will help in making high quality silage are listed in Table 1.

MONITORING THE END PRODUCTS OF SILAGE FERMENTATION

The end products of silage fermentation are often monitored to assess silage quality and the composition of “normal silages” is presented in Table 2 (Kung and Shaver, 2001). High concentrations of ammonia-N, acetic acid, butyric acids, and ethanol are undesirable (Table 3). Many commercial laboratories offer analytical services for silage end products and such analyses can help to when evaluating silage quality. Collectively, data from a fermentation analyses can tell us whether an excellent, average, or poor fermentation has occurred. Based on these analyses, we can usually make some educated assumptions about the kinds of microorganisms that controlled the ensiling process. In many, but not all cases, the fermentation that a crop undergoes can be explained by various crop factors such as moisture content, buffering capacity, and sugar content. However, management factors such as silo packing speed, silage pack density, type of additive used, chop length, silo management during storage, and silo management during feed-out can affect fermentation analyses. In some cases fermentation analyses can qualitatively explain poor silage nutritive value or low intakes, but they cannot be used to balance diets for cattle. Thus, they should always be used in conjunction with other standard chemical analyses (i.e. ADF, NDF, CP, RDP/RUP, NE_L , NDF digestion, etc.).

Silage pH. The pH of an ensiled sample is a measure of its acidity, but is also affected by the buffering capacity of the crop. Two samples may have the same pH, but different concentrations of acids. In general, legume silages have a higher pH than corn or other grass silages and take longer to ensile because of their higher buffering capacity. Seldom do corn silages have a pH higher than 4.2. Such cases may be associated with extremely dry (> 42% dry matter) silages that are overly mature or drought stricken. Because of its normally low pH (3.8), corn silage intake usually benefits from the addition of sodium bicarbonate prior to feeding to neutralize its acidity.

Common reasons for legume silages having a pH higher than 4.6 to 4.8 include: ensiling at < 30% dry matter (DM) which causes a clostridial fermentation, and ensiling at > 45-50% DM, which restricts fermentation. In the first example, a high pH due to clostridia is a definite indicator of an undesirable fermentation that has led to poor quality silage. However, in the second example, a high pH due to restricted fermentation is not always indicative of a poor fermentation or poor silage. But, silage from a restricted fermentation usually is unstable when exposed to air because insufficient amounts of acid were produced to inhibit secondary microbial growth.

Some common reasons for a high silage pH are as follows:

- dry silage (> 50% DM)
- silage not fully fermented due to early sampling time relative to harvest, cold weather during harvest, and slow or poor packing
- legume silages with extremely high ash contents (> 15% of DM) and (or) high protein content (> 23-24% CP)
- silage with excess ammonia or urea
- clostridial silages
- spoiled or moldy silages

- silages containing manure.

Buffering Capacity. Buffering capacity measures to what degree a forage sample will resist a change in pH. All forages have different buffering capacities. Fresh forage with a high buffering capacity will require more acid to reduce its pH than forage with a low buffering capacity. In general, fresh legumes have a higher buffering capacity than do fresh grasses or corn.

Lactic Acid. Lactic acid should be the primary acid in good silages. This acid is stronger than other acids in silage (acetic, propionic and butyric) and thus usually responsible for most of the drop in silage pH. Fermentations that produce lactic acid result in the fewest losses of dry matter and energy from the crop during storage.

Some common reasons for low lactic acid content are as follows:

- restricted fermentation due to high DM content (especially legumes and grasses with > 50% DM).
- restricted fermentation due to cold weather.
- sample taken after considerable aerobic exposure that has degraded lactic acid.
- silages high in butyric acid (Clostridial silages) are usually low in lactic acid.

Lactic acid should be at least 65 to 70% of the total silage acids in good silage.

Acetic Acid. Extremely wet silages (< 25-30% DM), prolonged fermentations (due to high buffering capacity), loose packing, or slow silo filling can result in silages with high concentrations of acetic acid (>3 to 4% of DM). In such silages, energy and DM recovery are probably less than ideal. Silages treated with ammonia also tend to have higher concentrations of acetic acid than untreated silage, because the fermentation is prolonged by the addition of the ammonia that raises pH.

The effect of high concentrations of acetic acid (> 4 to 6% of DM) in silages fed to animals is unclear at this time. In the past, some studies can be found where DM intake was depressed when silage high in acetic acid concentration was fed to ruminants. However, the depression in intake to high acetic acid in the diet has not been consistent. There has been speculation that decreased intake may be actually due to unidentified negative factors associated with a poor fermentation and not to acetic acid itself. A new microbial inoculant (*Lactobacillus buchneri*) designed for improving the aerobic stability of silages causes higher than normal concentrations of acetic acid in silages. However, production of acetic acid from this organism should not be mistaken for a poor fermentation and feeding treated silages with a high concentration of acetic acid does not appear to have a negative effect on animal intake.

If a producer has intake problems due to silages with excessively high acetic acid (> 5 to 6% of DM), the amount of that silage should be reduced in the TMR. Other alternatives for managing these silages include: aerating the silage for a day to volatilize the acetic acid, removing the silage and then gradually reincorporating it back into the diet over a 2 –3 week period, and/or

partially neutralizing the silage with sodium bicarbonate prior to feeding (about 0.5 to 1% addition on DM basis).

Propionic Acid. Most silage contains very low concentrations of propionic acid (< 0.1 to 0.2%) unless the silage is very wet (< 25% DM). In silages with more typical concentrations of DM (35 to 45% DM), concentrations of propionic acid may be undetectable. Concentrations of propionic acid that are higher than 0.3 to 0.5% are usually associated with poor fermentations.

Butyric Acid. A high concentration of butyric acid (> 0.5% of DM) indicates that the silage has undergone clostridial fermentation, which is one of the poorest fermentations. Silages high in butyric acid are usually low in nutritive value and have higher ADF and NDF levels because many of the soluble nutrients have been degraded. Such silages may also be high in concentrations of soluble proteins and may contain small protein compounds called amines that have sometimes shown to adversely affect animal performance.

High concentrations of butyric acid have sometimes induced ketosis in lactating cows and because the energy value of silage is low, intake and production can suffer. As with other poor quality silages, total removal or dilution of the poor silage is advised.

Ammonia-N. High concentrations of ammonia (> 10 to 15% of CP) are a result of excessive protein breakdown in the silo caused by a slow drop in pH or clostridial action. In general, wetter silages have higher concentrations of ammonia. Extremely wet silages (< 30% DM) have even higher ammonia concentrations because of the potential for clostridial fermentation. Silages packed too loosely and filled too slowly also tend to have high ammonia concentrations.

Theoretically, high amounts of ammonia (by itself) in silage should not have negative effects on animal performance if the total dietary nitrogen fractions are in balance. However, if the high ammonia contributes to an excess of ruminal-degraded protein (RDP), this could have negative consequences on milk and reproductive performances. Blood or milk urea nitrogen can be used as an indicator of excess RDP. Often times, silage with high concentrations of ammonia coupled with butyric acid may also have significant concentrations of other undesirable end products, such as amines, that may reduce animal performance.

Ethanol. High concentrations of ethanol are usually an indicator of excessive metabolism by yeasts. Dry matter recovery is usually worse in silages with large numbers of yeasts. These silages are also usually very prone to spoilage when the silage is exposed to air. Usual amounts of ethanol in silages are low (< 1 to 2% of DM). We do not know the level at which ethanol becomes a problem in dairy cattle diets. Most ethanol that is consumed is probably converted to acetic acid in the rumen. Extremely high amounts of ethanol (> 3 to 4% of DM) in silages may cause off flavors in milk. Ethanol is also an issue as it is classified as a dominant volatile organic compound in silage.

THE SIGNIFICANCE AND FATE OF SILAGE FERMENTATION END PRODUCTS

There have been many attempts to correlate the end products of silage fermentation with animal performance. The results of these studies have not always been in total agreement (Rook and

Gill, 1990; Steen et al., 1998) relative to the effects that silage acids may have on affecting DM intake. Eisner et al. (2006) reported that the concentration of acetic acid in silage was negatively correlated with intake when silage and concentrates were fed separately. However, total acid concentration was the best factor that increased the fit of a model for the prediction of DM intake prediction when animals were fed a total mixed ration. In a more recent summary, Huhtanen et al. (2007) reported that only total acid concentration and propionic acid concentration were negatively correlated with intakes in lactating cows. In steers Krizsan et al. (2007) reported that 71% of the variance of intake of 24 low DM grass silages was best explained by total acids, total volatile fatty acids, lactic acid/total acid ratio and propionic acid. Total acid concentration in silages is negatively correlated to dry matter content of the crop. In drier silages, fermentation becomes restricted because water activity limits the growth of microbes responsible for fermentation. Overall, these studies tend to support the anecdotal reports from the field that intakes are depressed when lactating cows are fed wet silages that tend to have high concentrations of total acids.

Ingested lactic acid from silage is rapidly converted to propionic acid in the rumen by *Selenomonas ruminantium*, *Megasphaera elsdenii* or Propionibacteria. This is especially true if the rumen microbes are well adapted to metabolize lactic acid. Propionic acid is then absorbed from the rumen and is converted to glucose by the liver of the cow. Under certain conditions, extremely high levels of lactic acid (and total acids) in silages may contribute to sub acute acidosis and may affect intakes.

Acetic acid ingested from silages is absorbed from the rumen and ultimately contributes to milk fat production and energy metabolism in the cow. Feedback from the field suggesting that silages high in acetic acid depress intake must be interpreted with caution because this acid can be produced from a variety of organisms (e.g., enterobacteria, lactic and acetic acid bacteria, clostridia and bacilli). Other negative end products from these organisms may also play a role in reduced intake from silages and thus acetic acid may only be a marker for poor fermentations. For example, Figueiredo et al. (2007) reported finding at least 168 volatile compounds in red clover silage and the effect(s) of most of these compounds on DM intake in ruminants has not been studied. Silages treated with the heterolactic acid bacterium *L. buchneri* have moderately higher concentrations of acetic acid than untreated silages, but because this is a “controlled” acetic acid fermentation, depressions in intake when feeding these treated silages have not been observed (Dreihuis et al., 1999; Kendall et al, 2002; Kung et al., 2003; Ranjit et al., 2002; Taylor et al., 2002).

Propionic acid is seldom found in well fermented silages. Although Propionibacteria are able to produce this acid from glucose and lactic acid it is doubtful if this occurs in good silage because these organisms are very intolerant of a low pH. It is more common to observe high levels of propionic acid (> 0.3 to 0.5%) in poorly fermented silages, especially because it can be an end product from some strains of Clostridia. Added amounts of propionic acid would be extremely difficult to detect. For example, if one added 2 lb (about 60% propionic acid) of a propionic acid-based additive to 35% DM corn silage, this would increase the concentration of propionic acid in that silage by less than 0.2% (DM basis).

Ethanol is a high-energy compound and much of it is converted to acetic acid in the rumen. Moderate levels of ethanol in the diet do not adversely affect ruminal fermentation. However, high levels of ethanol (>4-5%) may be a problem because of some direct absorption which can cause off flavors in milk in addition to wobbly cows.

Butyric acid from silages adds to the ketogenic load of ruminants as it is converted to beta-hydroxybutyrate and acetoacetate (ketone bodies) in the rumen wall before entering the general circulation. Oetzel (University of Wisconsin, personal communication) recommends limiting the intake of butyric acid from silages to 50 g/d to prevent the possibility of ketosis. If possible, clostridial silages should not be fed to high producing cows in early lactation or to cows in the transition period. Clostridial silages also tend to become worse the longer they remain in the silo. Thus, feeding these silages out as fast as possible is recommended. If you have a silage that could be susceptible to clostridial fermentation such as hay crop silage ensiled on the wet side or after significant rain damage during wilting, feed that silage out early before it has a chance to go clostridial.

High concentrations of ammonia (>12 to 15% of CP) are a result of excessive protein breakdown in the silo caused by a slow drop in pH or excessive growth by clostridia or enterobacteria. In general, wetter silages have higher concentrations of ammonia. Extremely wet silages (< 30% DM) have even higher ammonia concentrations because of the potential for clostridial fermentation. Theoretically, high amounts of ammonia (by itself) in silage should not have negative effects on animal performance if the total dietary nitrogen fractions are in balance. However, if the high ammonia contributes to an excess of ruminally-degraded protein (RDP), this could have negative consequences on milk and reproductive performances. Blood or milk urea nitrogen can be used as an indicator of excess RDP. Often times, silage with high concentrations of ammonia coupled with butyric acid may also have significant concentrations of other undesirable end products, such as amines, that may reduce animal performance.

Some strategies for managing silages with high (>5%) acetic acid, high ethanol (> 3-4%), high butyric acid (> 0.5%) include: reducing the amount of that silage fed, aerating the silage to volatilize the acids, removing the silage and then gradually reincorporating it back into the diet over a 2–3 week period, and partially neutralizing the silage with sodium bicarbonate prior to feeding (about 0.5 to 1% addition on DM basis).

CONCLUSIONS

Various microorganisms interact during the ensiling process and affect the nutritive value of silages for ruminants. Managing the forage crop in the silo has profound effects on the ensuing silage fermentation. Quick packing to eliminate air and use of microbial inoculants to dominate the ensiling process can help to assure a more desirable fermentation. Silage fermentation analyses can help to describe the type of fermentation that occurred in the silo. If questionable silage is being fed, an assessment should be made of general harvest and silo management to prevent similar problems with the next crop (e.g. is the silo face too wide, is the silage packed tight enough, is the silage too dry/wet, is the particle length too long, should one use an additive specifically designed to improve aerobic stability, etc.). Many basic management tools are

available that can help to reduce loss of DM that potentially harm the environment and lower nutritive value.

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Figure 1. The three major events that make good silage and factors that can affect the silage fermentation process.

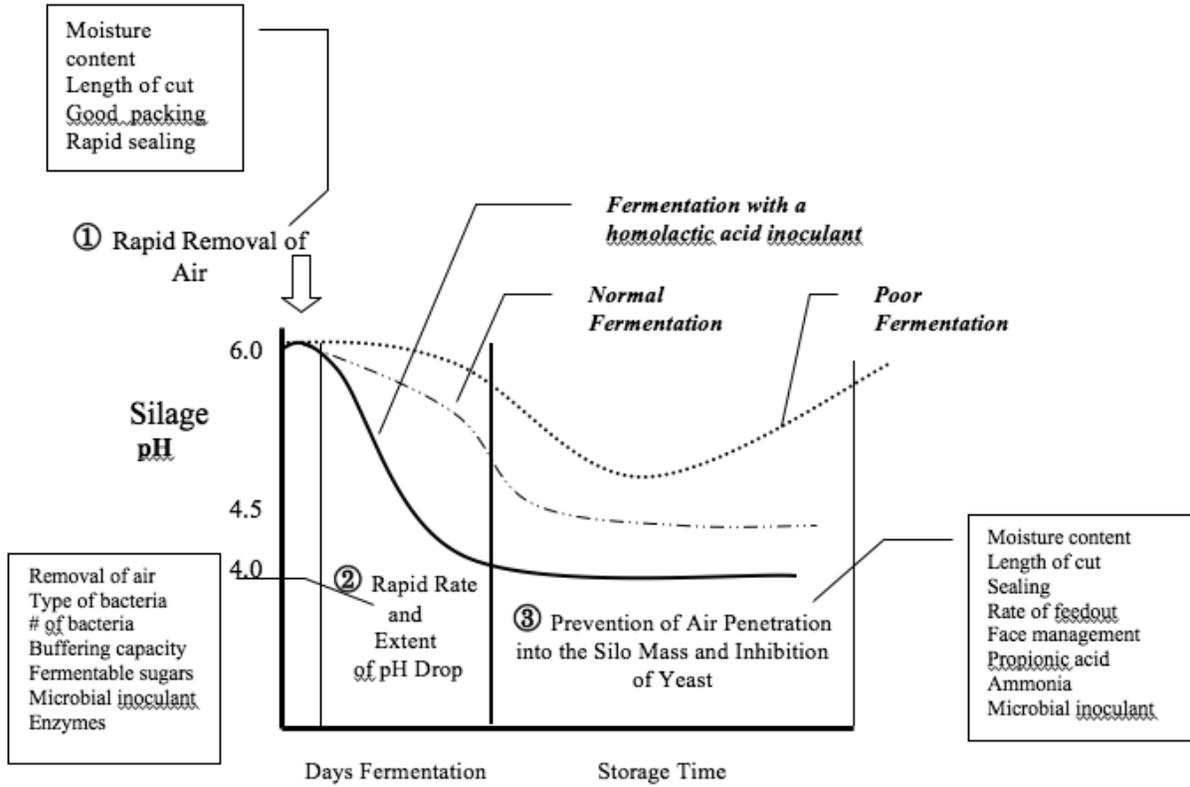


Figure 2. Effect of delayed filling on (A) water soluble carbohydrate (WSC) and (B) dry matter (DM) loss in corn silage. Hirsch and Kung, Univ. of Delaware, unpublished data.

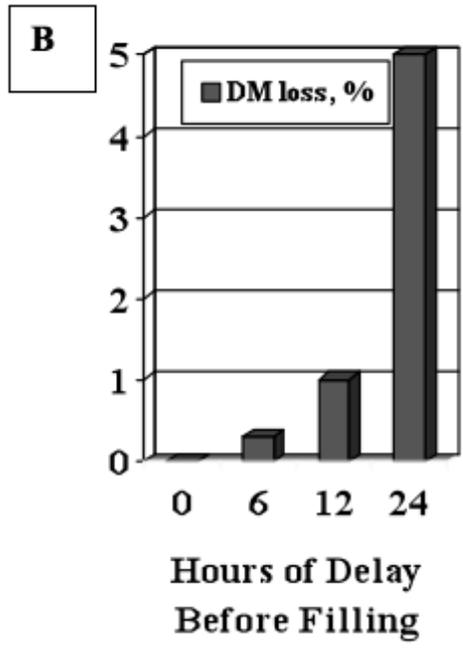
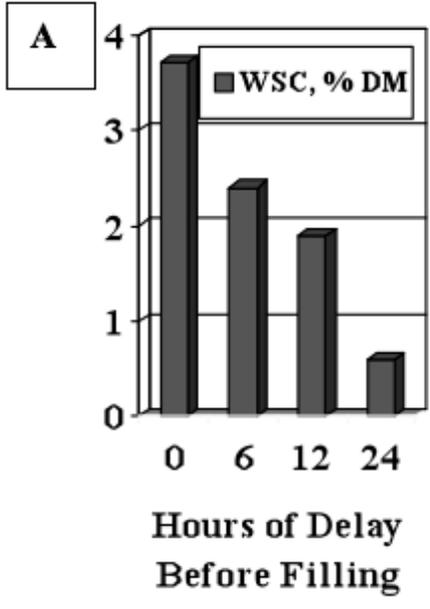


Table 1. Some good silage management practices.

Silage Practice	Reasoning
<u>Harvest crop at correct maturity and DM</u>	<ul style="list-style-type: none"> ● Optimizes nutritive value (protein, fiber, energy, etc.) ● In some cases optimizes DM content ● Ensures good packing, elimination of excess oxygen ● Minimizes seepage losses ● Prevents clostridial (butyric acid) fermentation
<u>Check that all equipment is in good working order</u>	<ul style="list-style-type: none"> ● Sharpen knives ● Be sure that silos are free from leaks ● In upright silos, a good distributor helps to distribute and pack silage
<u>Chop material to correct length:</u> theoretical of about 3/8 to 1/2 inch (as needed for the specific crop, conditions, silo)	<ul style="list-style-type: none"> ● Promotes good packing and elimination of oxygen ● Promotes cud chewing by cow
<u>Wilt and chop during dry weather</u>	<ul style="list-style-type: none"> ● Prevents extensive DM losses from rained on forage ● Promotes rapid drying
<u>Harvest, fill, and seal quickly</u>	<ul style="list-style-type: none"> ● Quick elimination of oxygen reduces DM losses from respiration and prevents growth of undesirable aerobic organisms ● Pack in 6 inch layers ● Fill bunks as progressive wedges ● Sealing minimizes exposure to air (tarps and sheeting) ● Pack to proper density (~15 to 17 lb DM/cu ft) to eliminate air
<u>Allow silage to ferment for at least 30-60 days</u> (maybe longer depending on the crop)	<ul style="list-style-type: none"> ● Properly ensiled silage will minimize production losses during silage changeover

Table 2. Amounts of common fermentation end products in various silages.

Item	Alfalfa Silage, 30 - 35% DM	Alfalfa Silage, 45 - 55% DM	Grass Silage, 25 - 35% DM	Corn Silage, 35 - 40% DM	High Moisture Corn, 70 - 73% DM
pH	4.3 - 4.5	4.7 - 5.0	4.3 - 4.7	3.7 - 4.2	4.0 - 4.5
Lactic acid, %	5 - 7	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, %	< 0.2	< 0.1	< 0.1	< 0.1	< 0.1
Butyric acid, %	< 0.5	0	<0.5	0	0
Ethanol, %	0.5 - 1.0	0.5	0.5 - 1.0	1 - 3	0.2 - 2.0
Ammonia-N, % of CP	10 - 15	< 12	8 - 12	5 - 7	< 10

Table 3. Common end products of silage fermentation.

Item	Positive or Negative Effect	Action(s)
pH	+	Low pH inhibits bacterial activity.
Lactic acid	+	Inhibits bacterial activity by lowering pH.
Acetic acid	-	Associated with undesirable fermentations.
	+	Inhibits yeasts responsible for aerobic spoilage.
Butyric acid	-	Associated with protein degradation, toxin formation, and large losses of DM and energy.
Ethanol	-	Indicator of undesirable yeast fermentation and high DM losses.
Ammonia	-	High levels indicate excessive protein breakdown
Acid detergent insoluble nitrogen (ADIN)	-	High levels indicate heat-damaged protein and low energy content.