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Chapter 1

Importance of the Fermentation to Produce High-Quality Silage

Thiago Carvalho da Silva, Leandro Diego da Silva, Edson Mauro Santos, Juliana Silva Oliveira and Alexandre Fernandes Perazzo

Additional information is available at the end of the chapter

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Abstract

The objective of this chapter was to discuss the importance of the fermentation processes for silage making and how it affects the final quality of the silage. The preservation of the forage crops as silage is based on a fermentation process that lowers the pH and preserves the nutritive value of the fresh crop. The main principle is the production of lactic acid by the lactic acid bacteria from the metabolism of the water-soluble carbohydrates in the fresh crop. However, different fermentations may occur into the silo environment and it depends on the availability of substrate, the microbial populations, the moisture content, and the buffering capacity of the crop at the ensiling. The fermentation is quite important in the ensiling process because it affects the nutritional quality of the silage and the animal performance. If the fermentation does not occur as recommended and the undesirable fermentations will take place, which will result in a total spoiled feed that is potentially risky for animals and human’s health. Well-fermented silage can be used in diets for ruminant animals without any risk for their health and without compromise the productive performance.

Keywords: additives, ammonia nitrogen, mycotoxins, lactic acid, organic acids, pH

1. Introduction—silage production and utilization

Grazing is the most common and economical way to feed cattle; however, it is cannot be done over the entire year, due to the climatic conditions that limit the grasses growth. The availability of pastures in livestock systems depends on the seasons because the factors that affect plant
growth (e.g., temperature, luminosity, and rainfall) are different for each season, which leads to periods with high forage production and periods of its shortage. In the winter, for example, there is no forage production enough to feed the animals [1].

The choice of suitable forage conservation process to provide constantly feed, essentially depends of the climatic conditions at harvest. In hot areas with dry seasons, probably the haymaking is the best choice for forage preservation, because it is a simple technology, where the fresh crop is dehydrated after cutting and the material is stable and preserved after reach an adequate moisture content.

In tropical regions with hot and humid climates, it is difficult to produce high-quality hay, due to high humidity and frequent rainfall at the optimum stage of maturity for crop with better nutritional value. In this context, ensiling is an important method of forage preservation because it is not too dependent on weather as the haymaking. In addition, in many parts of world, the silage is the major source of energy in the total mixed rations of ruminants [2, 3]. Thus, the objective of this chapter was to describe the fermentation processes for silage making and its manipulation and how it affects the final quality of the silage, which includes the effects on animal performance and health.

2. Importance of the fermentation for silage making

According to [2], in short, the silage is made by keeping chopped crop air-tight in a silo, as follows: (1) the crops are harvesting and chopping in a specific length at the better nutritional value and proper moisture content; (2) application of continuously heavy weights to pack at adequate densities; (3) and complete sealing. The preservation of the forage crops as silage depends of anaerobic environment, because it is based on a lactic acid fermentation that decreases the pH and associated with high osmotic pressure that inactivates the microorganisms preserving the nutritive value of the fresh crop (Figure 1). Even the presence of some mycotoxins in the fresh crop may be denatured due to the acid pH of silage.

The main principle of silage is anaerobic environment and fermentation of the water-soluble carbohydrates in the fresh crop by the epiphytic lactic acid bacteria (LAB) and production of lactic acid. However, different fermentation pathways may occur into the silo environment, depending on the availability of substrate, the predominant microbial populations, the dry matter (DM) content, and the buffering capacity of the crop at the ensiling (Figure 2). In addition, the fermentation must be limited to a certain extent, because it alters the chemical composition of the feed. This process may last for days or months, which may result in silage containing high levels of alcohols, butyric acid, ammonia, amines, and acetic acid that represent the major silage losses. Generally, the epiphytic microbial populations found in growing crops include pseudomonas, actinomyces, listeria, and mainly the LAB that we expect to dominate the fermentation process to produce high-quality silage (Table 1) [4].
High-quality silages are resulted of a fast and efficient fermentation preserving the crop nutrients, which depends if the fresh crop has high nutritional value and good characteristics for the ensiling process, as described before. In addition, the fermentation process cannot improve the crop nutritive value, but in some cases occur an increase on digestibility, always with energy losses. Efficient fermentation ensures a more palatable and digestible feed, which improves the animal performance. As noted above, the most important factors related to the crop characteristics to ensiling are adequate dry matter content, sufficient water-soluble carbohydrates for fermentation, and low buffering capacity.

The dry matter content affects directly the microbial activity, specific density, and effluent losses. Crops with dry matter content below 25% at ensiling show high effluent losses and high activity of undesirable microorganisms such as the genus *Clostridium* [6]. In addition, the LAB are more tolerant to low moisture conditions (low water activity) than other undesirable anaerobic microorganisms. However, dry matter content above 45% difficult the process of
forage packing, resulting in high porosity, which may cause losses by the development of aerobic microorganisms [7].

<table>
<thead>
<tr>
<th>Group</th>
<th>Population, colony-forming units/g of fresh forage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aerobic bacteria</td>
<td>&gt;10,000,000</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>10–1,000,000</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>1000–1,000,000</td>
</tr>
<tr>
<td>Yeasts</td>
<td>1000–100,000</td>
</tr>
<tr>
<td>Molds</td>
<td>1000–10,000</td>
</tr>
<tr>
<td>Clostridia</td>
<td>100–1000</td>
</tr>
<tr>
<td>Bacilli</td>
<td>100–1000</td>
</tr>
<tr>
<td>Acetic acid bacteria</td>
<td>100–1000</td>
</tr>
<tr>
<td>Propionic acid bacteria</td>
<td>10–1000</td>
</tr>
</tbody>
</table>

Table 1. Typical microbial populations on crops before ensiling [4].

About the amount of water-soluble carbohydrates, they present a narrow range of optimum values (60–80 g/kg of dry matter), because they are readily available substrates for the LAB and other microorganisms [6]. Furthermore, the excess sugar can stimulate the growth of anaerobic yeasts that are not fully inhibited by the low pH, as occurs in sugarcane silage, which results in high DM losses because the fermentation goes to the ethanol pathway [8].

The silage resistance to the pH lowering is named buffering capacity. This is exerted by compounds present in the crop, as the crude protein, inorganic ions, organic acids, and others. The greater buffering capacity needs more water-soluble carbohydrates content for an effective fermentation by reducing pH and inhibiting undesirable fermentations [9].

The fermentation coefficient (FC) was developed to predict if the crop is suitable to ensiling or not, as follows [10]:

$$\text{FC} = \text{DM} (%) + 8 \frac{\text{WSC}}{\text{BC}}$$

where FC = fermentation coefficient, DM = dry matter content, WSC = water-soluble carbohydrates, and BC = buffer capacity.

The forage crops with FC < 35 can result in undesirable fermentations and high dry matter losses, requiring additive application to control silage fermentation. If the FC ≥ 35, sufficient fermentable substrates are available. However, in high DM, crops are used microbial inoculants to ensure the presence of osmotolerant LAB to dominate the fermentation process.

3. Silage fermentation processes

The ensiling process, didactically, is divided into four principal phases [4]:

1. Initial aerobic phase since the harvest to the oxygen exhaustion in the silo. This phase is characterized by crop respiration and activity of all obligate and facultative aerobic organisms such as molds, yeasts, and some bacteria until finish up all the oxygen (Figure 1). In addition, the plant enzymes such as proteases and carbohydrases remain active. This phase must be short, because the sugars are converted to CO$_2$ and water with heat release, representing dry matter losses, increased Maillard products, and drops in the silage quality. This phase is also important because of CO$_2$, hydrogen peroxide, and other compounds that are produced with antimicrobial effect.

2. The main fermentation phase started with a short lag phase followed by rapid growth of facultative and obligate anaerobic microorganisms. The undesirable microorganisms as enterobacteria, clostridia, and yeasts compete with the desirable genera of LAB by the substrates. The main genera of LAB commonly associated with silage are Lactobacillus, Pediococcus, Leuconostoc, Enterococcus, Lactococcus, Streptococcus, and Weissella. The lactic acid production and the rate of pH decline are responsible for the disappearance of enterobacterial and clostridial secondary fermentations. Obtaining a well-fermented silage depends on the fresh crop characteristics as the adequate dry matter content (300–500 g/kg of fresh matter), water-soluble carbohydrates (60–120 g/kg of dry matter), and low buffering capacity. In addition to speed of harvesting, length of chop and silage distribution and compaction will be responsible for the successful conservation of feed nutrients.

3. Stable phase: In the acidic environment and without oxygen, the activity of microorganisms decreases substantially, and only acid-tolerant enzymes keeps a slow hydrolysis of carbohydrates and protein. The final pH of the ensiled forage depends on the ensiled crop. Theoretically, under ideal conditions, silage can be stored indefinitely if those conditions are maintained, because the losses are minimal. However, in the farm, it is usually stored for a maximum of 1 year or until the next harvest season. In arid and semiarid regions, farmers can store silage for longer periods because the dry period can comprehend two or more years.

4. Feed-out phase is very critical, because the undesirable microorganisms consume the compounds that make the silage stable in the silo (lactic acid) in the presence of oxygen and can produce many compounds decreasing the silage quality. Well-fermented silage with high lactic acid content and residual carbohydrates are more susceptible to aerobic deterioration, because they are the main substrates for the yeasts that initiate the deterioration process. The molds, yeasts, and acetic acid bacteria consume the acids, sugars, and protein for growth releasing heat and can cause considerable changes in the chemical composition in addition with rise in pH other microorganisms that were inhibited can proliferate and lead to a massive spoilage. Because this, care must be taken of removing a uniform layer of silage every day to not provide sufficient time for the undesirable microorganism’s proliferation. Normally, silage can stay stable when exposed to air for approximately 30–40 h, but it depends of environmental conditions and silage characteristics.
3.1. Substrates

The most important substrates for the fermentation are the water-soluble carbohydrates and various amino acids and vitamins of the crop. In addition, after chopping the enzymes, plants can hydrolyze starch and hemicelluloses providing more hexoses and pentoses to microbial growth. Hexose monosaccharides, oligosaccharides, and polysaccharides, such as glucose, fructose, sucrose, and fructans, are the main water-soluble carbohydrates readily available for fermentation. Other important carbohydrate is the starch, which is the main storage polysaccharide in some crops, but it is practically not used in the fermentation because it is insoluble in water [9].

3.2. Types of fermentations

In silage fermentation, several pathways occur simultaneously; the fermentation type depends on the environmental conditions, microorganism species, and substrate availability. The LAB show two basic types of hexose fermentation to lactic acid. The most efficient pathway in energy conservation is the obligate homofermentative, which produces almost exclusively lactic acid (>85%). The facultative heterofermentative lactic acid bacteria show besides the homolactic pathway; they present ability to ferment pentoses, because they have both enzymes aldolase and phosphoketolase. The obligate heterofermentative lactic acid bacteria present DM loss from hexose fermentation due the CO$_2$ production as well as lactic acid, and acetic acid or ethanol [4]. The acetate or ethanol production depends on the fermentation substrate: if the fermentation substrate is a hexose, the end-product is acetic acid, and if it is a pentose, the end-product is ethanol [6]. Although heterolactic pathway causes DM loss, a partial increase in acetic acid concentration improves the aerobic stability of silage, because the acetic acid inhibits the activity of yeasts during the feed-out phase [11]. The end-products of well-fermented silages are presented in Table 2.

<table>
<thead>
<tr>
<th>Item, % of dry matter</th>
<th>Silage and dry matter contents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alfalfa, 32.5%</td>
</tr>
<tr>
<td>pH</td>
<td>4.3–4.5</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>7.0–8.0</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>2.0–3.0</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td>Ammonia N$^1$</td>
<td>10.0–15.0</td>
</tr>
</tbody>
</table>

$^1$% of total nitrogen.

Table 2. Amounts of common fermentation end products in various silages [12].

When the acidification is not fast and adequate and/or with high moisture content, undesirable secondary fermentations can occur by other microorganisms, which are able to compete for nutrients with the LAB. Enterobacterial fermentation pathway is similar to the heterofermen-
tative LAB and ferments glucose to acetic acid, formic acid, and alcohol. In addition, enterobacteria can decarboxylate and deaminate amino acids and reduce NO$_3^-$. Other undesirable is the clostridial fermentations, which derive their energy from organic compounds such as carbohydrates and proteins producing butyric acid, acetic acid, propionic acid, ethanol, biogenic amines, and CO$_2$. These processes represent major losses that decrease silage quality and increase the production cost because of the low DM recovery. In addition, other smaller fermentations like the Propionibacterium can ferment glucose, fructose, glycerol, lactate, lactose, sucrose, xylose, and starch producing propionic acid, acetic acid, CO$_2$, and formic acid or isovaleric acid. The facultative anaerobic yeasts can ferment glucose, maltose, and sucrose with the main products such as ethanol, CO$_2$, and others compounds (alcohols, volatile fatty acids and lactate). The facultative anaerobic bacilli can ferment carbohydrates to organic acids or ethanol, 2,3-butandiol, and glycerol [4].

The secondary fermentations are undesirable because they preserve less energy in its end-products compared to the lactic acid fermentation, which is explained by the production of CO$_2$. These fermentations can also produce toxic compounds that impair the animal health and performance.

3.3. Efficiency of the fermentation process

The prevalent fermentation pathways in the ensiling process depend on several factors. They are related to the fresh crop and are basically the contents of DM and water-soluble carbohydrates. In addition, there are some characteristics related to the process techniques such as particle size, specific density, and especially the length time until the installation of anaerobic conditions in the silo. According to [13], the homofermentative LAB pathway results in only 0.7% of energy loss and it can be described as follows:

$$\text{Glucose or fructose} + 2 \text{ADP} + 2 \text{Pi} = 2 \text{lactate} + 2 \text{ATP} + 2 \text{H}_2\text{O}.$$ 

The heterofermentative LAB pathway from glucose results in 24% of DM loss and 1.7% of energy loss. When they ferment, fructose results in 4.8% of DM loss and 1.0% of energy loss, and it can be described as follows:

$$\text{Glucose} + \text{ADP} + \text{Pi} = \text{lactate} + \text{ethanol} + \text{CO}_2 + \text{ATP} + \text{H}_2\text{O}, \text{ or}$$
$$\text{Fructose} + 2 \text{ADP} + 2 \text{Pi} = \text{lactate} + \text{acetate} + 2 \text{mannitol} + 2 \text{CO}_2 + 2 \text{ATP} + \text{H}_2\text{O}.$$ 

In the clostridial fermentations, DM loss is 51.1% and the energy loss is 18.4%, and it can be described as follows:

$$2 \text{lactate} + \text{ADP} + \text{Pi} = \text{butyrate} + 2 \text{CO}_2 + 2 \text{H}_2 + \text{ATP} + \text{H}_2\text{O}.$$ 

In the yeasts’ fermentation, the DM loss is 48% and the energy loss is 0.2%, and it can be described as follows:

$$\text{Glucose} + 2 \text{ADP} + 2 \text{Pi} = 2 \text{ethanol} + 2 \text{CO}_2 + 2 \text{ATP} + 2 \text{H}_2\text{O}.$$
4. Manipulating silage fermentation

The knowledge about silage fermentation provides technology improvement to produce high-quality silages. In addition, crops that were once considered inappropriate to ensiling, mainly legumes, are routinely ensiled in many farms nowadays. Theoretically, all forage crops can be conserved as silage, if the ensiling techniques such as the finely chopped, well packed in the silo, and complete sealed through of plastic sheet are done carefully to promote adequate anaerobic condition. However, the crop intrinsic characteristics will direct the fermentation pathway and affect the final silage quality.

4.1. Changing the harvest time

Each crop, depending on environment, has the ideal stage of maturity for silage production considering the yield due to the profitability, dry matter, and fermentable sugar contents for bacteria and maximum nutritional value for livestock (Table 3). Practically, all factors involving the fermentation will change with crop maturity stage. In addition, the water-soluble carbohydrates have a diurnal fluctuation cycle, and their concentrations are highest at 18:00 h and lowest at 06:00 h. Generally, advancing crop maturity results in increases in dry matter, carbohydrates, and LAB population as well as total microorganism number. In addition, decreases in buffering capacity and crude protein concentration are observed, and some crops have showed a decrease in digestibility with advancing maturity [9].

<table>
<thead>
<tr>
<th>Crop</th>
<th>Maturity</th>
<th>Dry matter (%)</th>
<th>Cut length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>Milk line 1/2–2/3 down the kernel</td>
<td>28–37</td>
<td>9.5–12.7</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>Mid-bud 1/10 bloom, wilt to</td>
<td>30–40</td>
<td>6.4–9.5</td>
</tr>
<tr>
<td>Cereal</td>
<td>Milk or soft dough, wilt to</td>
<td>28–37</td>
<td>6.4–9.5</td>
</tr>
<tr>
<td>Grasses</td>
<td>When the first stems head out</td>
<td>28–37</td>
<td>6.4–9.5</td>
</tr>
<tr>
<td>Clover</td>
<td>1/4-1/2 bloom, wilt to</td>
<td>28–37</td>
<td>6.4–9.5</td>
</tr>
<tr>
<td>Sorghum</td>
<td>Grain medium to hard dough</td>
<td>30–35</td>
<td>9.5–12.7</td>
</tr>
</tbody>
</table>

Table 3. Harvest and dry matter recommendation for main crops conserved as silage [14].

4.2. Wilting

Some crops, like tropical grasses and some legume such as alfalfa and forage soybean (Table 4), have a quite low DM content at the same time when the nutritive value is high. Obtaining a good fermentation and eliminating the effluent losses must increase the dry matter content prior to chopping and ensiling. Generally, those crops need be wilted at harvest with a mower-conditioner to increase DM content and to enhance the lactic fermentation. Mowing and conditioning can increase the leaves losses and affect the microbial populations on the crop. The plant juice released can increase the nutrients losses and bacterial population and cause a shift in the microbial species present [15].
Table 4. Effect of crop vegetative stage and preharvest wilting time on ensiling parameters and in vitro rumen degradability of forage soybean silage [16].

<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>Wilting</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R4</td>
<td>R5</td>
<td>R6</td>
<td>20 h</td>
</tr>
<tr>
<td>Crop dry matter, g/kg of fresh matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After cutting</td>
<td>244</td>
<td>266</td>
<td>282</td>
<td>–</td>
</tr>
<tr>
<td>Wilted</td>
<td>449&lt;sup&gt;a&lt;/sup&gt;</td>
<td>471&lt;sup&gt;b&lt;/sup&gt;</td>
<td>529&lt;sup&gt;c&lt;/sup&gt;</td>
<td>438</td>
</tr>
<tr>
<td>Ensiled</td>
<td>454&lt;sup&gt;c&lt;/sup&gt;</td>
<td>485&lt;sup&gt;b&lt;/sup&gt;</td>
<td>518&lt;sup&gt;a&lt;/sup&gt;</td>
<td>444</td>
</tr>
<tr>
<td>pH</td>
<td>5.19</td>
<td>5.23</td>
<td>5.10</td>
<td>5.11</td>
</tr>
<tr>
<td>Chemical composition, g/kg of dry matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonia-N</td>
<td>3.3</td>
<td>2.5</td>
<td>2.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>32.7</td>
<td>29.9</td>
<td>29.3</td>
<td>32.0&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>In vitro rumen degradability</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fiber</td>
<td>0.319&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.388&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.465&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.399</td>
</tr>
<tr>
<td>Crude protein</td>
<td>0.391&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.503&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.548&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.495&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a–c</sup>,<sup>*</sup> P < 0.05.

The wilting before ensiling is more common in regions with dry weather or with well-defined seasons, because the rainfall during the wilting period may cause significative losses than the ensiling wet crop. During the wilting, the crop remains metabolically active, and the cell respiration and proteolysis cause losses, the most important factor is the time until reaching the desired DM. The fast dehydration decreases plant carbon losses and protein degradation. The respiration loss is unavoidable, and its intensity depends on the oxygen, DM, and water-soluble carbohydrate contents. Depending on environmental conditions, the crop containing high level of crude protein may have high proteolysis during wilting, which decreases the silage quality [15].

4.3. Silage additives

In specific cases, when all ensiling techniques and fermentation process are understood and managed properly, the use of additives is necessary to regulate the fermentation process and to obtain high-quality silages. Silage additives can be used to help fixing some historic problems of the crops (low LAB epiphytic, and low DM and soluble sugars contents), oversized silos, silage storage for prolonged time, or silage moved from silo to another structure [17]. In addition, the additives are used to reduce heating and DM losses improving the silage fermentation quality and profitability. Most commercial additives contain more than one active ingredient in order to enhance efficacy and broad range of applicability [10]. According to [18], the additives, basically, have five functions (Table 5). Once again, it is important to emphasize that the use of additives will never correct or fix failures from poor management of the silage-making process.
### Functions

<table>
<thead>
<tr>
<th>Functions</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentation stimulators</td>
<td>Homofermentative lactic acid bacteria</td>
</tr>
<tr>
<td></td>
<td>Glucose, sucrose, molasses, cereals, wheat, citrus pulp, and enzymes</td>
</tr>
<tr>
<td>Fermentation inhibitors</td>
<td>Formic acid, acetic acid, lactic acid, benzoic acid, acryllic acid, citric acid, and sorbic acid</td>
</tr>
<tr>
<td></td>
<td>Formaldehyde, sodium nitrite, sodium metabisulfite, sodium chloride, antibiotics, and sodium hydroxide</td>
</tr>
<tr>
<td>Aerobic deterioration inhibitors</td>
<td>Heterofermentative lactic acid bacteria</td>
</tr>
<tr>
<td></td>
<td>Propionic acid, caproic acid, sorbic acid, and ammonia</td>
</tr>
<tr>
<td>Nutrients</td>
<td>Urea, ammonia, biuret, and limestone</td>
</tr>
<tr>
<td>Moisture absorbents</td>
<td>Citrus pulp, ground corn, cassava meal, straw, and coffee hulls</td>
</tr>
</tbody>
</table>

Table 5. Silage additives [18].

### 4.3.1. Fermentation stimulators

The additives that promote the desirable lactic acid fermentation are called fermentation stimulators, by either providing additional fermentable sugars or increasing the LAB population in the ensiled crop.

Additives containing water-soluble carbohydrates will improve the fermentation in crops containing low sugars such as some legumes and tropical grasses. The use of molasses in the ensiling process was a practice widely used in the past to accelerate and increase the lactic acid fermentation. However, it was recommended to be used in relatively high concentrations (40–50 g/kg of fresh matter) and crops containing low DM content showed increase in effluent losses. Due to the high cost and viscosity, which are difficult to apply the molasses, today it is not too used in the farms. Other products or by-products can also be used for the same purpose, but attention should be paid to the availability and cost [19].

Enzyme additives usually are active enzyme combination (cellulases, hemicellulases, and amylases) used to break down the crop fiber and starch to release water-soluble carbohydrates, which could be fermented by LAB. The best results are improvements in silage fermentation and decreases in fiber content. However, the enzymes require certain conditions for maximum activity such as the pH, temperature, surface area, dry matter content, and crop proteases may inhibit enzyme activity. In addition, their positive effects also depend on the LAB initial population, crop characteristics, and application rate. The most suitable role for enzymes may be in combination with microbial inoculants [17, 19].

Inoculants containing homofermentative LAB are used with the purpose of increasing the initial population of this bacteria ensuring efficient fermentation to produce lactic acid. In addition, the use of homofermentative inoculants may accelerate pH reduction because the lactic acid is a stronger acid (pKa 3.86) than acetic acid (pKa 4.76) [4]; improving the lactic acid:acetic acid ratio consequently reduces dry matter losses. Homofermentative inoculation would also limit degradation and deamination of crop proteins and reduce ammonia produc-
tion, which increases silage quality [20]. It was observed by [21], when evaluating the effects of homofermentative inoculants in alfalfa silage; they observed that some of the evaluated inoculants, with faster growing and ability to dominate the epiphytic microflora, decreased the pH since the first day of fermentation (Figure 3).

![Graph showing pH, ammonia nitrogen, and lactic acid of alfalfa silages over fermentation periods](image)

**Figure 3.** The pH (a), ammonia nitrogen (b) and lactic acid of alfalfa silages as a function of microbial inoculant within each fermentation period. **Means** followed by different letters in bars are different according to the predicted difference ($P < 0.05$). CTRL = control (without inoculant); CI = commercial inoculant, Sil-All® 4 × 4 W.S. (Alltech, Sao Paulo, Brazil); S1 = *Pediococcus acidilactici*, Strain 10.6; S2 = *P. pentosaceus*, Strain 6.16 [21].

Microbial inoculants include one or more of these bacteria: *Lactobacillus plantarum*, *L. acidophilus*, *L. salivarius*, *Pediococcus acidilactici*, *P. pentacaceus*, *Enterococcus faecium*, and *Streptococcus bovis*. Some combinations are used in accordance with the LAB capacity and potential of synergistic actions. For example, the use of *Streptococcus*, which exhibit faster growth and simultaneous drop in pH, combined with *Pediococcus*, which are more tolerant to conditions of temperature, pH, and high dry matter content. However, *Lactobacillus plantarum* is the most common species used [17]. According to [22], the inoculant should be added at a rate that is at least 10% of the epiphytic population to fermentation improvement. For commercial inoculants, recommendation ranges from $1 \times 10^5$ to $1 \times 10^6$ colony-forming units (cfu)/g of fresh forage.

4.3.2. Fermentation inhibitors

These are all chemical additives that affect the undesirable fermentation and microorganism growth. Based on the same principle of food conservation, several substances are used for this purpose. However, the choice of a suitable additive depends on cost-efficiency and historical occurrence of silage with poor-quality fermentation. Generally, they are used in wet crops with low WSC content and/or high buffer capacity. In addition, in crops containing high WSC, the acid-tolerant yeast can proliferate and decrease the silage quality. Salts of acids have become
the most popular fermentation inhibitors, because they are easier and safer to handle [10], and they are effective on controlling yeast growth [23].

4.3.3. Inhibitors of aerobic deterioration

During the feed-out phase, when opening the silo, the presence of oxygen allows the development of molds, yeasts, and aerobic bacteria that consume the silage nutrients. The length of time that silage remains cool and does not spoil after it is exposed to air is called aerobic stability. There are chemical and biological additives that are used to improve the aerobic stability by inhibit aerobic spoilage, mainly yeasts and acetic acid bacteria, because these microorganisms are responsible to initiate the aerobic deterioration. Generally, the chemical additives are more expensive and difficult to handle than are biological, and successful treatment depends on application rate. However, the variation in the effects when chemical additives are used is lower than the biological additives. Chemical additives with strong antimycotic activity are sorbic and benzoic acid [19, 23]. Besides the use of chemical additives, there is the possibility of using of biological additives based on heterofermentative LAB, such as Lactobacillus buchneri, which anaerobically degrade lactic acid to acetic acid and 1,2-propanediol causing a yeast inhibition [10, 23]. Yeast inhibition by organic acids is due to the undissociated form in acid pH. The inhibition effectiveness depends on the dissociation constant (pk) of organic acid; the acids with the highest pk are more effective in inhibiting. The ascending order of pk is formic acid, lactic acid, acetic acid, and propionic acid (3.75, 3.86, 4.76, and 4.87, respectively) [4].

4.3.4. Nutrients

The quality of crop can be improved by supplementation of dietary components that are essential for ruminants through of specific additives at the time of ensiling. In addition, despite of the buffering effect, the urea and ammonia can improve the aerobic stability of silage and increase crude protein content [6]. Grains can be added to increase levels of metabolizable energy in the silage. In other cases, some minerals can be added in order to meet a possible deficiency of the crop to better animal performance [19].

4.3.5. Moisture absorbents

Good results have been obtained in crops with a low DM content (<25%) at the ensiling to prevent excessive effluent losses and clostridial fermentations. Some additives can also improve the nutritive value and final silage quality [6]. Grains can be added to increase moisture absorbent to reduce silage effluent losses [19].

4.4. Using mixed crops

It can be used with several goals always taking advantage of a potential synergistic effect from improvement of soil tillage and fertilization and increased nutritive value, and/or supply the dry matter content and water-soluble carbohydrates to ensure a high-quality silage. Mixing legumes with cereal crops has been to increase grain yields and crude protein of crops while
improving soil fertility but can increase the buffering capacity, which can decrease the fermentation efficiency in drops in the pH [24].

5. How does the fermentation process affect silage quality?

All microorganisms present in the silo, crop epiphytic population, and possible contamination primarily consume energy of water-soluble carbohydrates and other compounds for their growth and proliferation. Theoretically, the homolactic fermentation recovers 99% of the energy from glucose. However, in the silage fermentation process, many pathways occur simultaneously with different extensions, beyond the initial cellular respiration and enzymes activity, which are decisive in the final silage quality. Reducing losses by effluent is also important because it contains cellular content with high nutritional value that can contaminate the environment [6, 25]. High-quality silage is the result of adoption of appropriate techniques, starting with soil preparation and fertilization. In addition, the crop must have high DM yield, adequate nutritional value, and good characteristics for fermentation at the ensiling. Actually, even if high-quality crops are harvested efficiently, significant losses in the quality can occur if the ensiling process is inadequate (Table 6).

<table>
<thead>
<tr>
<th>Source</th>
<th>Management</th>
<th>Good</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration</td>
<td></td>
<td>0–4%</td>
<td>10–15%</td>
</tr>
<tr>
<td>Fermentation</td>
<td></td>
<td>4–6%</td>
<td>10–15%</td>
</tr>
<tr>
<td>Seepage</td>
<td></td>
<td>0–2%</td>
<td>5–15%</td>
</tr>
<tr>
<td>Aerobic storage</td>
<td></td>
<td>5–7%</td>
<td>10–20%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>9–17%</td>
<td>20–40%</td>
</tr>
</tbody>
</table>

Table 6. Dry matter losses in silage under good or poor management [26].

Forages should be harvested for silage making when they have high nutritional value and the DM content is between 30 and 35%. Therefore, the monitoring of dry matter content at harvest period is essential, because some crops are required to be wilted or ensiled with additives to reach the recommended DM content. The crop must be chopped to about 0.5–1.5 cm length so that the work of packing and taking out is carried out easily. The chopped forage must be well packed in the silo, so less air will be trapped inside the stack, and the peripheral area should have packed more intensely. Filling the silo as quick as possible (within 3 days) limits the forage exposure to air, but each night until it is filled, the stack should be covered. The last step is complete seal with plastic as soon as filling and compaction is completed. In addition, the plastic should be covered, usually with tires or soil to eliminate gases and to prevent damage of the plastic. The packing density at of a good silage should be about 650 kg of fresh silage per cubic meter.
5.1. Chemical composition and nutritive value

Changes are inevitable in chemical composition during the ensiling process; it is due the conversion of soluble carbohydrates into organic acids, as well as degradation of fiber and protein of fresh crop. First, changes in the composition start immediately after cutting, still in an aerobic environment. Early in this phase, enzymes break down fructans, starch, and hemicellulose, releasing simple sugars, and also degrade protein to peptides, amino acids, amides, and ammonia. In addition, during the respiration, soluble carbohydrates are converted to CO$_2$ and water by releasing heat. If the respiration period is extended, it can increasing losses due the development of molds and yeasts. Also, the heat released by respiration may decrease the digestibility due to the Millard reaction. The heat binds amino acids to the hemicellulose increasing the indigestible fiber and undegradable protein [4].

During LAB fermentation, the soluble carbohydrates are converted to lactic acid, acetic acid, ethanol, CO$_2$, and water, which represents slight losses of DM and energy. However, if there is a clostridial fermentation, which causes major problems in the silage quality, it converts the soluble carbohydrates and amino acids to organic acids, glycine, biogenic amines, ammonia-nitrogen, H$_2$, and CO$_2$. The fermentation length is important in the crop preservation. When the fermentation length is extensive, the losses and changes in nutritional value are greater [4].

Another major problem about the silage chemical composition is at the silo opening. With air exposure, the microorganisms, which were inhibited, can proliferate and consume the silage energy. Heating and spoilage during feed-out is one of the greatest contributors to DM losses. In addition, it can produce some substances, like mycotoxins, that may pose risks to animals fed with this silage [26].

5.2. Animal performance

The feed intake is the key constraint limiting performance of ruminant animals fed diets containing forages. Regulation of feed intake in ruminants involves multiple mechanisms and complicated interactions between animal and feed characteristics. Evaluating factors that affect the silage intake of dairy cows, [27] concluded which silage intake can be predicted based on the silage digestibility, total acids, and DM content. Silage intake increased with increasing silage digestibility which was influenced by stage of maturity at harvest. The same authors showed that the total organic acids produced by silage fermentation process depress the silage intake, but it will depend on the proportion of the silage included in the diet. In addition, a positive association between DM content and silage intake, and DM content independently affects the silage fermentation and animal performance.

Feeding spoiled silage can be a big problem, because the deterioration decreases silage digestibility and intake in cattle. In addition, molds in spoiled silage can produce mycotoxins that cause serious health problems in the animals and farmers [26]. Silage additive is one of the ways to try to ensure efficient fermentation and thus obtain high-quality silage. When studies from North America evaluating the effects of silage additives on animal responses were summarized, [28] showed that although not replace good techniques of the ensiling process, the microbial inoculation can improve the silage quality and animal performance. This activity
in animal performance is not well understood and might inhibit detrimental microorganisms in both silage and rumen to enhance the animal health and performance [29].

5.3. Animal health

The microorganisms in the microbial epiphytic population are usually nonpathogenic. However, the contamination, especially with the soil, may increase the presence of enterobacteria and spores of clostridium and bacillus in the silage. Therefore, in some cases, the silage can be a contamination source of animal products, such as meat, milk, and cheese, besides affecting the animal health [4]. During the silage fermentation process, a succession of microorganisms and denaturation and production of several compounds occur. However, the main problem is the occurrence of undesirable fermentations, which reduces the nutritive value of silage. Furthermore, the presence of some microorganisms or compounds produced may be a risk to the animal health [26].

Enterobacteria present in the crop may have a small positive effect on the hygienic quality of silage because during the first stage of ensiling, they reduce the nitrate ($\text{NO}_3^-$) to intermediates as nitrite and nitric oxide which inhibit clostridial fermentations. However, enterobacteria are undesirable because they have an endotoxin, which can reduce the silage intake and increase cases of mastitis, besides the less effective fermentation than LAB [4].

The anaerobic environment into the silo is essential for high-quality silage and inhibition of molds that produce mycotoxins. Generally, the mycotoxins in silage are related to molds with high tolerance to CO$_2$ concentrations. Feeding spoiled silage results in reduced intake, increased abortions, hormonal imbalances, and suppressed immune function. In addition, good ensiling conditions reduce the most of the population of potential pathogens such as *Listeria monocytogenes*, *Escherichia coli*, and several *Salmonella* species, because they are strongly inhibited by acid pH (<4.5). Actually, the biggest problems are caused by clostridia and bacilli due the ability to form endospore and their presence later in food production systems, requiring special treatment for their elimination [4].

6. Future trends

With the knowledge of the silage process, some techniques are being developed to improve the efficiency of the preservation and production of high-quality silage. The development of monitors for the DM content of the crop at harvest will help farmers to know the crop quality and ensiling characteristics to choice of additives when needed at accurate rates. In addition, the development of specific additives for each culture that are used in the world with ample effect, since the silage fermentation until the animal performance. Furthermore, today many researches are aimed to developing plastic films more resistant and impermeable to oxygen. Through improvements in the plant, breeding is possible to obtain suitable crops for the most different environmental conditions with high quality and productivity, besides the suitable characteristics for silage production and animal performance.
7. Conclusions

Despite being a well-known technique, it is not easy to produce high-quality silage. Starting with the crop containing high nutritional value that usually is expensive and requires much care. In addition, the ensiling process needs specific machinery, physical structure (silos), and plastic sheets for the coverage. Moreover, the farmers cannot afford the risk of losing the entire crop with a poorly made silage.

Some crops at better nutritional value also have good ensiling characteristics such as corn and sorghum. However, to ensure a high-quality silage is often necessary to use techniques such as crop wilting and application of additives, which can become the process more expensive. A quick and efficient fermentation in reducing the pH is the most desired at ensiling. It depends on the anaerobic environment, water activity, and substrate for LAB fermentation. The homofermentative LAB are the most efficient in preserving the crop characteristics. However, some heterofermentative LAB are also desirable because of its effect on the aerobic stability of silage.

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