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“Software applications for sizing silos to maximize silage quality”

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Introduction

Producers considering a new silage storage frequently have three criteria that influence their decision about the storage size. They often want to minimize the space requirements because of space limitations on the farmstead. There must be sufficient volume to contain the stored crop. Finally, the initial investment should be as low as possible. These criteria influence the size of the storage resulting in high and wide bunker and pile silos. A square silo has the lowest initial investment compared to a long narrow rectangle when storing the same volume of feed. Since bunker silo walls are quite expensive, producers tend to use piles over bunker silos to keep the initial investment low. Wide and tall silos will have a lower feed removal rate for a given volume of feed removed compared to a feed out face which is narrower and shorter. Low feed out rates result in higher dry matter losses (Figure 1). Higher dry matter losses contribute to increased annual costs.

One method of reducing dry matter loss at the feed out face is to have a large enough feed out rate. Establishing the feed out rate is the first step in the design process. Selecting a feed out rate of 0.31 m/day assures a feed out dry matter loss rate of less than 3% for a wide range of bulk densities (Figure 1). Since feed out rates may have to change over the life of the storage structure, selecting a high design feed off rate allows a significant reduction of feed out rate down to about 0.15 m/day without incurring losses greater than the desired value of 3% for most bulk densities. The next design step is to establish the maximum height of the feed out face. The maximum height should be limited by the reach of equipment available to safely remove silage while maintaining a smooth feed out face. The feed out face should not be undermined causing an overhang. Overhangs can collapse on people causing injury and death. Collapsed faces leave rough and fissured faces prone to aerobic deterioration with consequent high dry matter loss and feed value loss. Front end loaders can reach 3.7-4.9 m from the floor of the silo while

telehandlers might reach as high as 9 m from the floor. The taller the feed out face, the narrower the silo will be but the farther any avalanching silage will fall away from the feed out face. The third step in design is to establish the volume of silage to be removed from the storage each day. This volume is determined by the weight of this particular silage fed to each animal per day, the number of animals fed from the silo and the bulk density of the silage in the storage. Once the daily volume stored is determined, the width of the silo can be calculated by Equation 1.

$$W = V/(H \times RR) \qquad 1$$

W = silo width, m
V = volume per day, m^3/day
H = height assumed, m
RR = removal rate, m/day

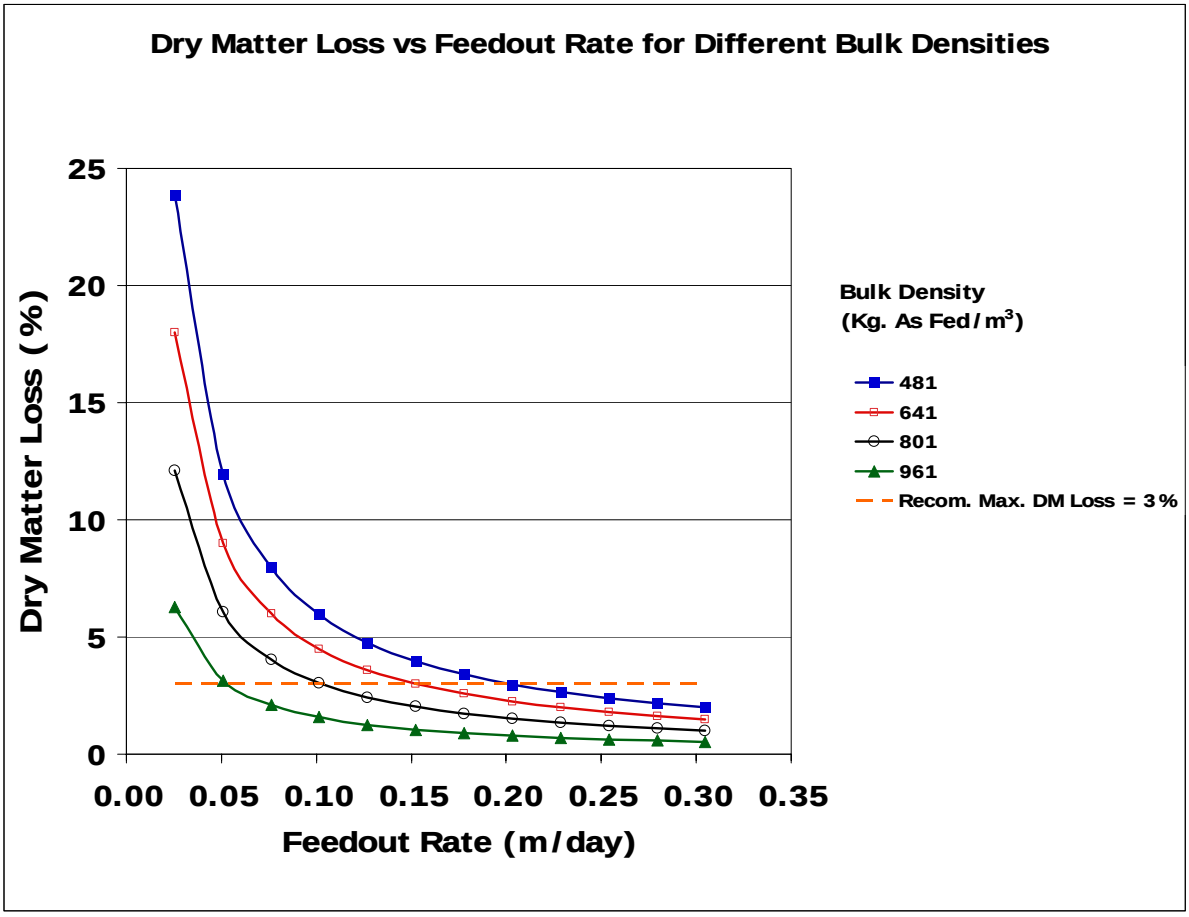

Figure 1. Dry matter loss as a function of feed out rate on the face of a silo. Derived from Pitt and Muck, 1993.

This procedure, examples and a manual worksheet are given in a fact sheet by Holmes and Muck, 2004. Consider the example of a 200 cow herd fed 19.5 Kg AF/cow – day of corn silage and the silage has a bulk density of 705 Kg AF/m^3 where AF means as fed. Assume the equipment on the farm can reach a maximum height of 3 m and the deign feed out rate is 0.31 m/day. The daily quantity of corn silage removed from the silo is 3,900 Kg AF/day (200 cows x 19.5 Kg AF/cow-day). The volume removed is 5.53 m^3 (3,900 Kg AF/day / 705 Kg AF/m^3). Using equation 1, the average width is 6 m (5.53 m^3 / (3 m x 0.31 m)). Since the width is greater than 5 m which is twice the width of a typical packing tractor, the whole surface can be packed and the width is acceptable for a bunker silo. For the silo to store enough feed, the total average length must be determined by equation 2.

$$AL = SP \times RR \qquad 2$$

AL = Average Length, m
SP = Storage Period, day
RR = removal rate, m/day

In our example, assume a 365 day storage period. The Average Length is calculated as 113.2 m (365 d x 0.31 m/d). Since the maximum recommended bunker silo length is 46 m, plan to use three bunker silos of 38 m average length each to satisfy the storage requirement. Thus in our example, we need three silos with average width of 6 m, depth of 3 m, average length of 38 m and a face removal rate of 0.31 m/day. A similar procedure would be used to size storage for hay silage for this herd. The overall footprint for the storage is about 18 m x 38 m. This is far from a square storage shape but will result in far less than 3 % dry matter loss at the feed out face because the removal rate is 0.31 m/day. Note: actual bunker silo floor length will be greater than 38 m. Each end may have a sloping silage surface of 3:1 slope ratio representing an additional 9 m of floor length. There will also be a filling apron of 13-15 m. Thus the total floor length might be 60-62 m (38m + 13m + 9m).

Steps to Successful Silage Making

Harvest At the Correct Moisture and Stage of Maturity

- Harvest hay at 60-65% moisture to assure good bulk density and good fermentation. Higher moisture runs the risk of clostridial fermentation (Butyric Acid). High moisture

also contributes to leachate losses. Low moisture results in low acid production resulting in a higher pH and reduced microbial inhibition. Lower moisture causes high porosity and rapid oxygen penetration at the feed out face. High levels of oxygen penetration support aerobic organisms that deteriorate silage dry matter. Low moisture reduces thermal mass which contributes to rapid heating when oxygen is present.

- Harvest whole plant corn at 65-70% moisture to assure good bulk density and good fermentation.
- Harvesting at the correct stage of maturity assures adequate sugars are available for fermentation to lactic acid. Harvest Alfalfa at early to mid bloom and whole plant corn at 1/3 – ½ kernel milk line.

Chop to Correct Particle Length

- Shorter particles pack better and release more soluble carbohydrates
- Set knives to obtain 0.95 cm theoretical length of cut (TLC) for hay and unprocessed whole plant corn and 1.27-1.91cm TLC for processed whole plant corn.

Size Storage for Large Face Removal Rate and Optimal Top Surface Area

- Design for 0.31 m/day removal rate to reduce the time silage is exposed to oxygen during feed out and to keep ahead of spoilage organisms. This generally means narrower bunkers and piles vs what is commonly seen.
- The forage at the top of a silo is typically of lower bulk density than that below it. Lower bulk density silage has higher porosity and high oxygen penetration rate when exposed to oxygen. Bolsen et. al. (1993) showed silage at the top of bunkers has a higher loss rate in the top 0.9 m than that below. The exclusion of oxygen by plastic films helps to control this loss compared to no cover. Deciding to use a silage pile over a bunker silo exposes more top area and consequently more silage in the top 0.9 m and can thus be expected to have higher dry matter loss in storage and feed out all other factors being equal.
- Use Bunker, Pile and Bag sizing software .

Use Additives to Improve Silage Quality and/or Remedy Problems

If quality has been a problem in the past, consider additives designed to remedy these problems. Plan to apply these materials at the chopper so good mixing can be accomplished before entering the silo.

- Adding Homofermentative organisms increases the likelihood of a rapid and complete fermentation and low acetic acid levels. They reduce dry matter loss on average 2-3% and increase animal performance 3-5% when effective.
- Use *Lactobacillus buchneri* to reduce heating at feed out and extend bunk life. Buchneri reduces dry matter loss on average 1-2%.
- Propionic acid reduces heating at feed out and extends bunk life.
- Sugar (molasses) reduces pH at a faster rate when inadequate sugar is available naturally.
- Urea reduces heating at feed out and extends bunk life and adds non-protein nitrogen
- Ammonia reduces heating at feed out and extends bunk life and adds non-protein nitrogen.

Fill Storage Quickly

- Harvest at a high enough rate to fill silo in fewer than three days. The quicker forage is placed in the silo, the quicker fermentation can begin to exclude oxygen. While silos are open, forage is exposed to oxygen thus supporting microbial deterioration. Exposed forage is also susceptible to precipitation which can leach soluble carbohydrates. Oxygen exclusion halts plant and microbial respiration which consume readily available dry matter. Size silos small and/or provide enough equipment and labor to harvest, transport forage and fill storage quickly.
- Cover surface of forage with plastic when filling must be stopped. This helps exclude oxygen and precipitation which can hasten dry matter loss.

Pack Forage to High Bulk Density

- Aim for greater than 705 Kg/m^3 bulk density and less than 0.4 porosity. Minimize porosity to limit oxygen penetration into the silage. Oxygen supports aerobic organisms which decompose silage quickly, causing heating and dry matter loss.
- Keep silo bag surface smooth to limit pockets of low density silage.
- Place forage or bags on all weather surface of gravel, asphalt or concrete.
- Pack bunkers and piles with heavy tractors in thin layers (<0.15m). Use a shallow slope on the filling surface to keep layer thickness thin. Multiple tractors may be needed with high forage delivery rates (more than 72.6 t AF/hr).
- While packing, make at least two passes over silage surface with both sets of wheels. This will require extra passes near the wall compared to interior of the storage.

- Use bunker/pile packing software to investigate “what if” scenarios before harvest.
- Slope the top surface to drain water from the silo without draining into the silage.

Cover Forage with Plastic to Exclude Oxygen and Water

- Cover silage immediately (<24 hr) upon completing filling. The longer forage is exposed to air, the higher will be the dry matter loss.
- Use 8 mil plastic or oxygen barrier plastic with tarp and weighting to hold plastic to forage surface. Joints in plastic should be sealed by overlapping sheets by 1.5 - 2 m, Berger and Bolsen (2006). Overlap in such a way as to shed water away from the silage. Use gravel filled bags or soil piles at perimeter to seal edges. Use tires or tire sidewalls touching to hold plastic against the forage uniformly.
- On bunker silos, use wall plastic to exclude runoff water and oxygen from penetrating the silage. On silage piles, extend the plastic past the silage and onto the ground a distance of 1.5 - 2 m to keep water away from the silage and to exclude oxygen, Berger and Bolsen (2006).
- Develop a vermin control program to limit damage to the plastic cover.
- Examine the integrity of plastic film on a weekly basis. Patch holes in plastic as they occur with tape designed for that purpose.

Feed Out So As to Minimize Deterioration

- Remove no more than three days of plastic cover. Consider leaving plastic to cover the top of silage near the feed out face. This may result in plastic overhanging the feed out face at times. This remaining plastic will shed precipitation.
- Weight the edge of the plastic at the feed out face to prevent billowing. Billowing of cover plastic draws air under the plastic.
- Remove visibly spoiled silage. Spoiled silage causes cattle to reduce their dry matter intake and can cause health problems due to mold spores and toxins.
- Ragged silage has larger surface area exposed to oxygen and fissures and cracks allow oxygen to penetrate deep into silage at the feed out face. With a front end loader or facer, remove at least 0.15 m per day from the silo face leaving a smooth surface. High face removal rates reduce the time silage is exposed to oxygen. A smooth face with no fissures reduces the surface area of silage exposed to oxygen. Techniques for front end loader operation that help to maintain a smooth feed out face include: downward force with the

edge of the bucket scraping the face of silage; use the side of the bucket to shave the face while driving parallel to the face; removing a cavity near the floor and then breaking the face into the cavity with the edge of the bucket. Practices that should not be used include: jamming the bucket edge into the feed out face and lifting the silage up; undermining the silage near the top creating an overhang; tunneling through the silage leaving a long exposed wall of silage.

- Remove from the face only that amount of silage to be fed in one feeding. Removed silage has low density which allows oxygen to penetrate deeply. Rapid heating can result in feed left at the base of the feed out face.

Software for sizing and managing bunker and pile silos

Bunker and pile silo sizing and management can benefit from many mathematical calculations to consider some of the "What if?" scenarios. Multiple calculations using the same set of equations can benefit from the use of computer software. Many software packages have been developed in spreadsheet format and are available for download from the Harvest and Storage page of the University of Wisconsin-Extension Team Forage web site located at URL:
www.uwex.edu/ces/crops/uwforage/storage.htm

Bunker Silo Sizing Calculator

The bunker silo sizing spreadsheet uses the principals described in the Introduction of this paper. It is designed with three major areas within the spreadsheet. The first area allows the operator to list the number of animals in various animal groups within the herd and the amount of dry matter for each animal in three hay silage categories and one corn silage category. The section then calculates the total quantity of each of the four forages required to be fed to the herd each day. These values are then entered by the user one at a time into the second (Input) section of the spreadsheet. The user also enters values for storage loss, feeding loss, bulk density, moisture content, face removal rate, storage period and maximum storage length. Finally, the results are tabulated in section three (Results) as a listing of bunker silo average dimensions that will satisfy the design criteria. To use the Results table, one selects a wall height that is the maximum his/her feed out equipment can reach. On the same line as the wall height, one selects the average bunker width, number of bunker silos, and bunker length. Also listed on this line are estimates

for the quantity of dry matter placed into storage, quantity of feed dry matter lost to spoilage, and the percent dry matter lost from storage and feeding losses.

Bunker Silo Volume and Weight Calculator

Often the question arises about the quantity of silage in a constructed bunker silo. This is usually needed as part of a feed inventory process. In this spreadsheet, the operator is asked to enter bunker silo dimensions, silage moisture content and dry matter density. The spreadsheet output includes the dry matter and as fed weight and the volume of silage in the bunker.

Silage Pile Dimension Calculator

This spreadsheet uses operator information to determine dimensions of a drive over pile. The number of animals and the quantity of silage fed per animal each day from the pile are used to calculate the quantity of this silage fed to the herd each day. The peak height, side wall slope and daily removal rate is used to determine the bottom and top width of the pile. The daily removal rate, length of feeding period and end slopes are used to calculate the top and bottom lengths of the pile. Other information reported by the spreadsheet include quantity of dry matter and as fed silage being placed into the storage and that removed assuming a 20% loss of mass during storage and feed out.

Silage Pile Capacity and Cost Calculator

Often the question arises about the quantity of silage in a constructed pile silo. This is usually needed as part of a feed inventory process. In this spreadsheet, the operator is asked to enter pile dimensions, silage moisture content and dry matter density. The spreadsheet output includes the silage dry matter and as fed quantity in each pile and total quantity of silage. By including dimensions of the buffer space of the storage pad around the pile and the cost per unit area of the pad, an estimate of the initial investment of the storage pad is calculated.

Average Density of Silage in Storage

The average density of silage in storage is very important for limiting oxygen penetration into silage and for determining the quantity of silage in the storage. This spreadsheet determines average bulk density and dry matter density in bunker, pile, bag and tower silos. It uses the

principle of weight of silage removed divided by the volume removed to calculate the density. The weight is determined by summing weights placed in the feed mixer wagon during feeding. The volume is calculated after the user enters the dimensions of the volume of feed removed from the storage. Accuracy is influenced by precision with which weight is recorded and how much feed volume is removed during the test period. Dimensions will be difficult to measure accurately if the test period is only a few days. However, measurement accuracy increases if the test period is greater than one week. This method has the possibility of giving more accurate values than the face probing method used by many researchers in that the point density can vary quite a bit over the face of a storage and the probing method can have inaccurate results if a bore hole is made into a non-representative site on the feed out face.

Silo Bag Sizing Calculator

This spreadsheet determines the number of silo bags and the pad needed to store them for three hay silage qualities, corn silage and high moisture corn. The user enters the following data: quantity of dry matter fed to the herd each day for each feed ingredient, dry matter density, bag diameter, bag length, storage period, distance between bags, pad buffer length on the ends of each bag and the dry matter loss. Output of the spreadsheet includes: number of bags for each forage type, quantity of feed dry matter placed into storage and that removed, storage pad length, storage pad width and pad area.

Silage Bag Capacity Calculator

Often the question arises about the quantity of silage in a silo bag. This is usually needed as part of a feed inventory process. The operator is asked to enter bag dimensions, silage moisture content and dry matter density. The spreadsheet output includes the silage dry matter and as fed weight stored in the bag.

Bunker Silo Density Calculator

Packing bunker silos to achieve high bulk density is important to limit the silage porosity and subsequently the penetration of oxygen into the top surface, under the plastic, and at the feed out face (Holmes and Muck, 2007b). Attaining a high silage density is important for two primary reasons. Most importantly, density and dry matter content determine the porosity of the silage.

Porosity, in turn, sets the rate at which air moves into the silage and subsequently the amount of spoilage which occurs during storage and feed out. The higher the density, the greater is the capacity of the silo. Thus, higher densities generally reduce the annual cost of storage per tonne of crop by both increasing the amount of crop entering the silo and reducing crop losses during storage. General recommendations have been to spread the crop in 0.15 m layers and pack continuously with heavy, single-wheeled tractors. Holmes (2006a) summarizes some of the research and field trials related to density achieved in bunker/pile silos. Many field trials are finding:

Dry matter density is greater near the bottom of the silage than toward the top. (Muck and Holmes, 2000; Visser, 2005; Craig and Roth, 2005; D'Amours and Savoie, 2004; Oelberg et al, 2005) This may be due to self-compaction and more time spent packing the lower layers.

Dry matter density is lower next to the wall than in the center of the bunker/pile silo. (Visser, 2005; Craig and Roth, 2005; D'Amours and Savoie, 2004; Oelberg et al, 2005) This may be due to reduced packing time next to the wall and the lower depth on the sides of piles.

Average dry matter density is higher for hay than for corn silage. (Visser, 2005; Oelberg et al, 2005) This may be the result of faster harvest rate for whole plant corn than hay with resultant lower packing time for whole plant corn. Hay is often harvested at a higher dry matter than corn silage. Research has shown dry matter density to be directly related to dry matter content.

Increasing packing tractor weight, number of packing tractors and reducing layer thickness result in increased dry matter density. (Muck and Holmes, 2000; Visser, 2005; Craig and Roth, 2005; D'Amours and Savoie, 2004; Oelberg et al, 2005).

The recommended procedure for filling a bunker silo or silage pile is to spread the forage in thin layers on the sloped filling face and drive over it several times with one or more heavy tractors. The progressive wedge technique of filling continually covers previous layers of forage, thus reducing exposure to air. Filling the storage quickly (within 3 days) limits the forage exposure to air throughout filling. Consequently, equipment to harvest, transport and fill the storage as well as labor should be capable of filling the storage rapidly. Each of two smaller bunkers/piles can be filled more quickly than one large one, minimizing the exposure to oxygen during filling.

One practical issue is packing time relative to crop delivery rate. Assuming one packs continuously with one tractor throughout filling, packing time per tonne (1 to 4 min/t As Fed) is high under low delivery rates (<30 t As Fed/h) and generally declines with increasing delivery rate. This result suggests farmers using high capacity harvesters need to pay particular attention to spreading the crop in a thin layer and would benefit from using several heavy packing tractors simultaneously. If a satisfactory dry matter density is not being achieved, a producer can select one or more of the following options a-g to increase density;

a. Reduce delivery rate of silage to the storage.
b. Increase dry matter content by allowing longer crop field drying time.
c. Increase depth of silage in the bunker/pile silo.
d. Increase average tractor weight by adding more weight to each tractor, or replace existing tractors with heavier tractors.
e. Add more packing tractors. Use heavier rather than lighter tractors so the average weight is not reduced when adding a tractor.
f. Reduce packing layer thickness.
g. Pack for additional time.

Items a. to c. are somewhat difficult to accomplish if the harvest rate and bunker silo are currently being pushed to the limit. Few will be willing to slow the harvest rate so packing can be accomplished. Fermentation occurs best in the range of 30-40% dry matter. Increasing dry matter content beyond 40% to improve density is counterproductive for good preservation because of incomplete fermentation, heating, and increased porosity. If the bunker is full, adding silage depth above the full mark can be dangerous.

Items d. to g. are more often within the control of the producer. Producers achieving high packing density have adopted the use of very heavy tractors and are using a shallow (< 0.15 m) packing layer thickness. When the delivery rate to the silo is quite high (as with self-propelled harvesters operating in corn silage), one or more additional packing tractors will be needed. In a well-packed silo, all tractor tires will pass over the entire packing layer surface at least once. More passes are beneficial. Because density near the wall of a bunker silo is frequently lower than that toward the interior, packers should make additional passes near the walls.

The “Spreadsheet to Calculate the Average Silage Density in a Bunker Silo” (Holmes and Muck, 2007a**)** was developed to guide producers as they consider how they can increase bulk density in their bunker silos. User provided inputs to the spreadsheet include: bunker wall height and peak height, harvest rate, dry matter content, tractor weight and percent of filling time each of up to four tractors spend packing. Outputs of the spreadsheet include: an estimate of bulk and dry matter density following packing and fermentation, maximum achievable bulk and dry matter density and porosity. The operator can use this spreadsheet to try some “what if” scenarios by changing the input variables over which he/she has control to try to reach a desired density and porosity.

The “Spreadsheet to Calculate the Average Silage Density in a Silage Pile” (Holmes and Muck, 2008**)** was developed to guide producers as they consider how they can increase bulk density in pile silos. User provided inputs to the spreadsheet include: pile height, pile top and bottom width, harvest rate, dry matter content, tractor weight and percent of filling time each of up to four tractors spend packing. Outputs of the spreadsheet include: an estimate of bulk and dry matter density following packing and fermentation, maximum achievable bulk and dry matter density and porosity. The operator can use this spreadsheet to try some “what if” scenarios by changing the input variables over which he/she has control to try to reach a desired density and porosity.

Porosity

Porosity is a measure of the voids between the solid particles of a material. Pore space can be filled with fluids including gas and/or water in silage. The “air filled” porosity allows gases to move within the material. For gases to move throughout the material, the pores must be continuous. Closed pores do not contribute to gas flow.

Figure 2 shows porosity as calculated using the equations of Richard et al (2004). From Figure 2, porosity is most influenced by bulk density (as fed density) over the range of dry matter contents recommended (0.30-0.40) for ensiling in bunkers, piles, and bags. Bulk density in silage is affected by the same packing practices as dry matter density: tractor weight, packing time and spreading layer thickness as well as depth of silage, however, the same packing practices result

in a lower bulk density as dry matter content increases. This trend is the opposite of what occurs with dry matter density. Figure 3 was developed using a modified version of the spreadsheet for calculating average density in a bunker silo by Holmes and Muck (2007a). From Figure 3, it is apparent porosity increases with harvest rate and increasing dry matter content. To keep porosity below 0.4, multiple heavy tractors and lower dry matter content are needed when the harvest rate is high.

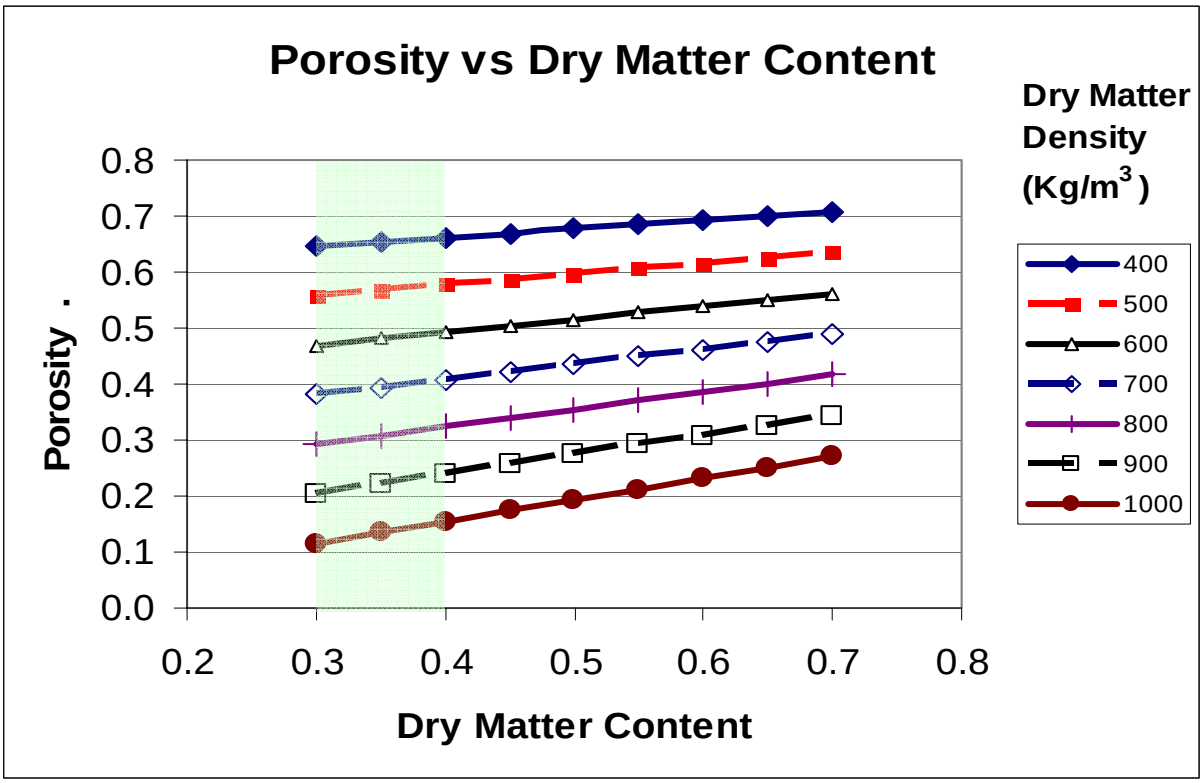

Figure 2. Graph of porosity (decimal) vs. dry matter content (decimal) for various bulk densities

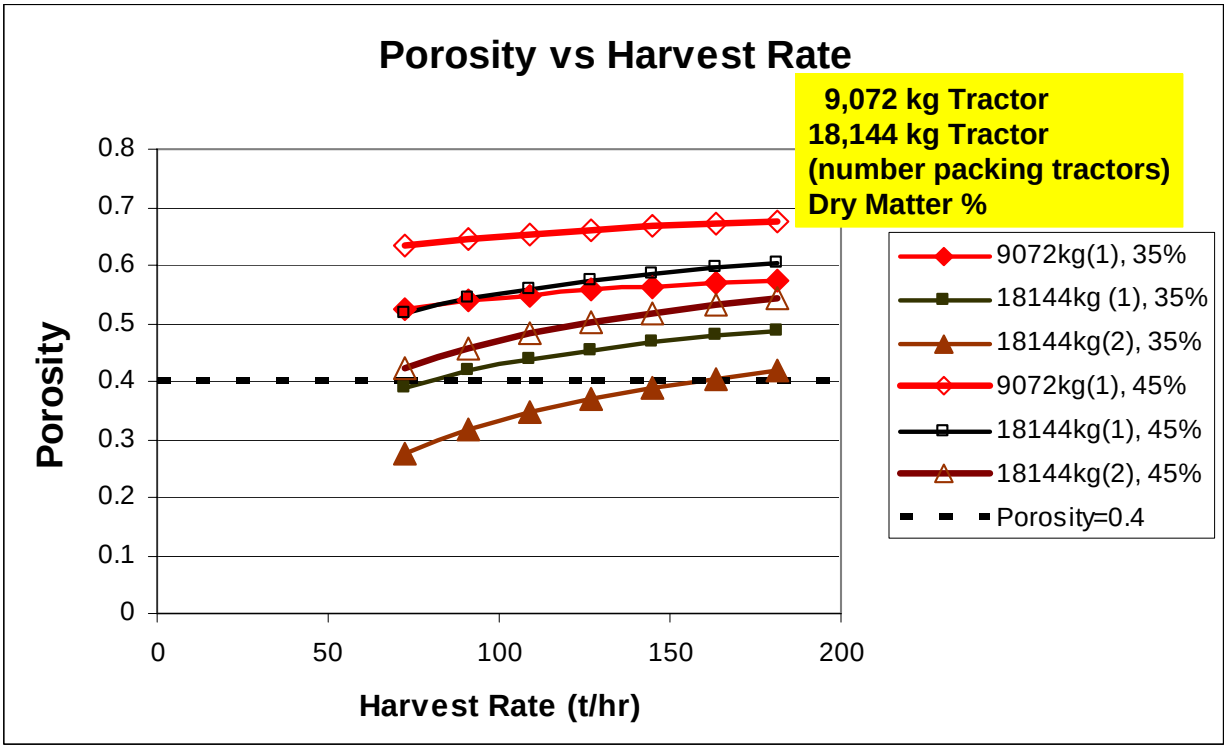

Figure 3. Porosity vs Harvest Rate

Calculator to Determine Length on Bunker/Pile Silo Floor to Achieve a Given Forage Layer Thickness

Recommendations for many years have included distributing forage in thin layers before packing. Preliminary research by Muck and Holmes (2007) has not confirmed the value of thin layers when packing time per tonne is kept constant. However, when a given weight of forage is distributed in thin layers, each pass of the packing tractor results in more packing time per tonne when the layer is thin than when the layer is thicker. Consider this example for determining the length of the filling slope when one load is pushed onto the filling slope at a layer thickness of 0.15 m:

Example 1.

Assume:

Weight per load = 6804 Kg DM/ Load = 6.8 tonne/Load
Forage density on filling slope = 80.1 Kg DM/m^3
Forage Dry Matter = 32% DM
Bunker height = 3.7 m
Packing Speed = 4.8 Km/hr
Tractor width = 3 m
Bunker width = 12.1 m
Packing layer thickness = 0.15 m
Tire width per trip = 0.5 m/trip

Packing Area = (6804 Kg DM/ 80.1 Kg DM/m^3) / (0.15 m) = 566 m^2
Length of Packing Surface = 566 m^2 / 12.1 m = 46.8 m, (Figure 4)
Packing Trips = (12.1 m -3 m)/0.5 m/trip = 18 trips per pass across the packing surface
Total Packing Length = 46.8 m/trip x 18 trips = 842 m
Time/Pass = 842 m/4800 m/hr = 0.18 hr = 10.5 min = 10.5 min/load
Packing Time per tonne = 10.5 min/laod/6.8 t DM/load = 1.55 min/t DM= 0.5 min/t AF

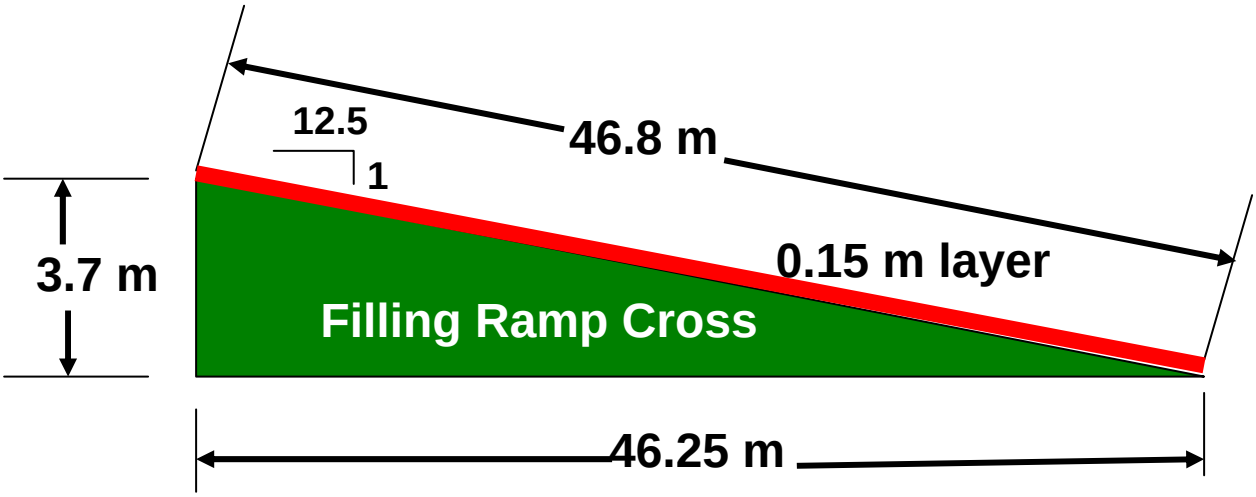

Figure 4. Cross section of progressive filling wedge for Example 1.

The more conventional recommendation is to use a 3:1 progressive wedge filling slope. In Example 2, a filling slope ratio of 3:1 is used and the remaining assumptions of Example 1 are used. The resulting layer thickness and the time spent packing that layer for one packing pass is developed in Example 2.

Example 2

Assume:

Length of Packing Surface = 11.6 m
Layer Thickness = (6804 Kg DM/ 80.1 Kg DM/m^3)/(12.1 m x 11.6 m) = 0.61 m
Total Packing Length = 11.6 m/trip x 18 trips = 208.8 m
Time/Pass = 208.8 m/4800 m/hr = 0.044 hr = 2.6 min = 2.6 min/load
Packing Time per tonne = 2.6 min/laod/6.8 t DM/load = 0.38min/t DM= 0.12 min/t AF

By selecting the longer filling slope of Example 1, the layer thickness becomes about one fourth that of Example 2 and the packing time per tonne becomes four times larger.

A spreadsheet is available to calculate the length of floor needed to achieve a given layer thickness while filling bunker and pile silos (Holmes 2006b). The user enters; average

bunker/pile fill height and width, desired unpacked forage layer thickness, unpacked dry matter density, and truck/wagon load dimensions.

How much value can be saved by implementing good silage management practices?

The answer to this question depends on current management practices. If a producer can be viewed as doing a moderate job of management, some savings can be obtained. If on the other hand, large improvement in practices are needed, much greater savings are possible. To help address this issue, a spreadsheet is available (Holmes, 2006c). This spreadsheet was used with the following assumptions and those in Table 1 to estimate the benefit of moving from "not so good management" to "good management":

100 cow herd with replacements

Hay Silage Value = $138/t DM, 40% of cow ration, 50% of heifer ration

Corn Silage Value = $110/t DM, 60% of cow ration, 50% of heifer ration

Table 1. Dry matter loss percentage assumed for analysis of management economics

	Dry Matter Losses with Good Management		Dry Matter Losses with Not So Good Management	
Forage Type	Hay	Corn	Hay	Corn
Loss Category	(%)	(%)	(%)	(%)
Feeding Loss	5	5	7	7
Feed Out Loss	3	3	5	5
Storage Loss	10	10	15	15
Filling Loss	1	1	3	3
Harvest Loss	6	1	8	2

Results of the economic analysis using the spreadsheet are found in Table 2. With these assumptions and those not presented about the ration formulation, the value of moving from not so good management to good management is $13,795/year ($33,571-$19,766). You can use this spreadsheet to enter your own assumptions about herd size, rations and estimated losses to find a savings for your situation for each management change you attempt and for the total savings.

Table 2. Economic loss for good and not so good management

	Economic Loss with Good Management		Economic Loss with Not So Good Management	
Forage Type	Hay	Corn	Hay	Corn
Loss Category	($)	($)	($)	($)
Value Lost	11,204	8,572	18,649	14,9927
Total Value Lost	19,776		33,571	

Conclusions

There are many opportunities to improve silage management and by doing so improve economic profitability for the producer. There are many spreadsheets available to help make decisions about proper silage storage management. Use of these spreadsheets to help guide silage storage management should help improve silage management and consequently profitability.

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Mycotoxin: impacts and control strategies

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1. Factors that interfere in micotoxin production

Mycotoxins are toxic substances that result from secondary metabolism of several strains of filamentous fungi. They are organic compounds with low molecular weight and no immunogeneticity. In tropical and subtropical climates the fungi development is enhanced by factors such as excellent humidity and temperature conditions. Fungi grow and spread well in cereals, especially in peanut, corn, wheat, barley, sorghum and rice, in which they generally find a highly nutritious substrate for their development.

Fungi growth and mycotoxin production in cereals can occur in several development phases, maturation, harvest, transportation, processing or storage of grains and forages. That is why a reduction of cereal humidity through a drying process and a correct compacting and sealing in silage, are of fundamental importance to reduce contamination levels.

More than 400 mycotoxins, presently known, are produced by approximately one hundred fungi. The main mycotoxins can be divided into three groups: Aflatoxins, produced by fungi *Aspergillus* such as *A.* fla*vus* and *parasiticus*; Ochratoxins, produced by *Aspergillus ochraceus* and several species of *Penicillium*; and *Fusarium* toxins, representing the main types the trichothecenes, zearalenone and fumonisins,

Mycotoxin production depends on a series of factors such as humidity, temperature, oxygen availability, fungi growth time, substrate composition, damage to the grains integrity caused by insects or mechanical/thermal factors, quantity of fungi inoculum, as well as the interaction/competition among fungi strains. Genetic characteristics represent a more decisive factor to the solution of the problem. This complex of factors shows that their control, as preventive measures, is more than often very difficult. As a result of wet weather conditions, Brazilian climate at harvest, do not favor grains drying, especially corn.

Other species, such as *Aspergillus fumigatus*, *Byssochlamys nivea*, *Monascus* spp., *Penicillium roqueforti* and *Trichoderma* spp. are adapted to the silage conditions and are generally identified in preserved feed. In this case, contamination by fungi can be present before or after the ensiling process.

Although they are exposed also when grazing, the feedlot feeding system submitted animals to a more vulnerable condition, especially bovine cattle, because practically all their feed is based on concentrate and conservated forages (silage, hay, haylage, etc). Concentrate have grains in their composition which are commonly contaminated with fungi and mycotoxins. Corn grains are a classic example, often found with high humidity, badly packed and more than often used even after fungi deterioration, presenting high levels of contamination.

Ensiling should be the ideal method to conserve forage or the grains contaminated by mycotoxins, because after the harvest the forage is stored in low anaerobic and pH conditions, an incompatible environment for the development of filamentous fungi. However, silage is potentially subject to aerobic deterioration, preferentially in peripheral areas of the silo. Filamentous fungi develop in high quantities when the silage remains in contact with the air for a long period of time. The protection against air infiltration during storage and the correct handling of the silo during the feed out process avoid the aerobic deterioration process, also inhibiting the spreading of microorganisms that produce mycotoxins.

The rupture of the plastic film that covers the silo, is usually due to animals stepping on it, or to excessive sun exposure that dry and crack it, exposing part of the ensiled mass.

For these reasons it is necessary to put in practice all the strategies to reduce oxygen penetration in the silo, avoiding its undesirable effects both in nutritional and hygienic issues, during storage or silage consumption.

On the other hand, some fungi are capable of producing mycotoxins in plants, even before ensiling. The plant in senescent stage allows this growth resulting in contamination of silage by mycotoxins, even when raised properly as agronomic management.

Therefore, before ensiling it is necessary to properly size the silo regarding daily demand, because if this step is neglected, silage is no longer an efficient option in food conservation because of problems related to losses and development of undesirable microorganisms.

2. General effects of mycotoxins in animal health

Besides direct losses of ensiled forage, the consequence of ingestion of spoiled silage reflects directly in the animal's performance. Cases of reproduction disorders, low weight gain, immunity depression, heterogeneous groups, are usually related to the presence of mycotoxins in silage. These intoxications become more and more frequent due to better observation and lower tolerance of animals with high potential yield.

When mycotoxins are ingested, the several effects are due to their different chemical structures, influenced by the fact of being ingested by different superior animal organisms and also by the variety of species, breed, gender, age, environmental factors, management, nutritional conditions and other chemical substances. Mycotoxicosis brings huge economical, sanitary and commercial losses, especially because of its anabolic, estrogenic, carcinogenic, mutagenic and teratogenic properties.

The major problem of mycotoxicosis are losses related to several organs and systems of animals, implying on the decrease of their productive performances.

Acute manifestations occur when individuals consume moderate to high doses of mycotoxins. Clinical signs can appear associated with a specific pathological state, depending on the ingested mycotoxin, the species susceptibility, individual organism conditions and the interaction or not with other factors. Injuries depend on each

mycotoxin. However the most common are hepatitis, bleeding, nephritis, necrosis of digestive mucous membrane and death.

Chronic mycotoxicosis is the most frequent, occurring when there is an intake of moderate to low dosage. In these cases, the animal exhibit symptoms characterized by the reduction of reproduction efficiency, lower feed conversion, decrease of growth rate and weight gain. This state is only detected with special care or by an regular analysis program of mycotoxins present in feed. Clinical signs can also be confused with poor nutritional management, other diseases including those that come from this mycotoxicosis or with nutritional deficiencies.

3. Prevalence of mycotoxins in grains and feeds

Results of mycotoxicologic analysis from the Laboratory of Mycotoxicologic Analysis (LAMIC) of the Federal University of Santa Maria, from 1986 to 2009, show the mean contamination by mycotoxins and the positivity of corn samples, where the aflatoxin, considered the most important group of mycotoxins, were detected in roughly half of the analyzed samples. On the other hand, toxins produced by *Fusarium*, fumonisins with 65% of prevalence, zearalenone with 22% and deoxynivalenol with 23%, are mycotoxins of high incidence in corn (Table 1). In Table 2 an average contamination by mycotoxins and the positivity of silage samples from all over Brazil is shown, illustrating that one fifth of the samples were contaminated by aflatoxins, over half were contaminated by fumonisins and more than one fourth by zearalenone and deoxynivalenol. When the silage samples in Table 2 are divided according to their origin from different Brazilian states, 4 states demonstrated a larger number of analyzes, Paraná, Rio Grande do Sul, Santa Catarina and São Paulo (Table 3). The major prevalence of fumonisins was detected in the samples from the states of Rio Grande do Sul and São Paulo, with almost 65% of positive samples. The contamination by zearalenone was higher in the state of Rio Grande do Sul, with almost 40% positivity. In Paraná, the contamination by deoxynivalenol was larger than in the other states, with 40% of presence in the samples.

Table 1 – Mean concentration of mycotoxins and percentage of prevalence in samples of maize analyzed in LAMIC/UFSM from 1986 until June 2009.

MYCOTOXIN	# of ANALYZED SAMPLES	MEAN (µg/kg)	CONTAMINATED SAMPLES (%)
Aflatoxin[1]	55,318	11.2	49.0
Fumonisin[2]	15,615	1,531.8	65.0
Zearalenone	45,343	36.7	22.0
T-2 Toxin	7,981	12.8	1.0
Deoxynivalenol	6,167	68.7	23.0
TOTAL (analysis)	311,993		32.0

[1] B1, B2, G1 and G2 Aflatoxin summation; [2] B1 and B2 Fumonisins summation.

Table 2 – Mean concentration of mycotoxins and percentage of prevalence in samples of silage analyzed in LAMIC/UFSM from 1986 until June 2009.

MYCOTOXIN	# of ANALYZED SAMPLES	MEAN (µg/kg)	CONTAMINATED SAMPLES (%)
Aflatoxin[1]	941	2.1	20.0
Fumonisin[2]	617	1,078.5	55.0
Zearalenone	914	63.4	28.0
T-2 Toxin	261	3.3	2.0
Deoxynivalenol	577	128.6	26.0
TOTAL (analysis)	6,750		26.2

[1] B1, B2, G1 and G2 Aflatoxin summation; [2] B1 and B2 Fumonisins summation.

Table 3 – Mean concentration of mycotoxins and percentage of prevalence in samples of silage from different states in Brazil analyzed in LAMIC/UFSM from 1986 until June 2009.

STATE	MYCOTOXIN	# of SAMPLES	MEAN (µg/kg)	POSITIVITY (%)
SC	Aflatoxin[1]	387	1.7	22.22
	Fumonisin[2]	350	1,020.1	50.57
	Zearalenone	393	40.5	22.13
	T-2 Toxin	199	1.0	2.51
	Deoxynivalenol	337	96.1	25.51
	Total (analysis)	3,177		24.58
RS	Aflatoxin[1]	305	2.0	17.70
	Fumonisin[2]	138	959.7	65.94
	Zearalenone	267	78.8	39.70
	T-2 Toxin	33	0	0.00
	Deoxynivalenol	118	144.0	24.57
	Total (analysis)	1,914		29.58
SP	Aflatoxin[1]	131	3.2	19.08
	Fumonisin[2]	67	1,865.8	67.16
	Zearalenone	137	118.5	25.55
	T-2 Toxin	16	41.6	6.25
	Deoxynivalenol	69	160.2	21.74
	Total (analysis)	880		27.95
PR	Aflatoxin[1]	99	2.2	20.20
	Fumonisin[2]	57	855.8	50.87
	Zearalenone	105	42.8	23.80
	T-2 Toxin	13	0	0.00
	Deoxynivalenol	50	275.9	40.00
	Total (analysis)	678		26.97
Other States	Aflatoxin[1]	19	2.3	21.05
	Fumonisin[2]	5	436.0	60.00
	Zearalenone	12	35.0	30.00
	T-2 Toxin	0	0	0.00
	Deoxynivalenol	3	0	0.00
	Total (analysis)	101		22.21
TOTAL (analysis)		6,750		26.20

[1] B1, B2, G1 and G2 Aflatoxin summation; [2] B1 and B2 Fumonisins summation.

4. Control and management of mycotoxins

Control and management of mycotoxins involve a process which presents a series of critical activities, starting with the definition of a monitoring program. This implies on the definition of the sampling process, goes through several analyses and controlling points and ends in the decision-making. This should consider the reliability of the diet in which the risk of intoxication by mycotoxins can be minimized and the cost-benefit is precisely quantified, allowing the maximization of herd productivity.

4.1 Mycotoxin analysis program planning

A mycotoxin analysis program planning requires knowing the characteristics of mycotoxin distribution. Knowing the dynamics of mycotoxin distribution and how to interfere in decreasing the sampling risk are fundamental in a program.

The most practical way that was found to decrease the risk and offer a suitable balance between cost-benefit is showed in Figure 1.

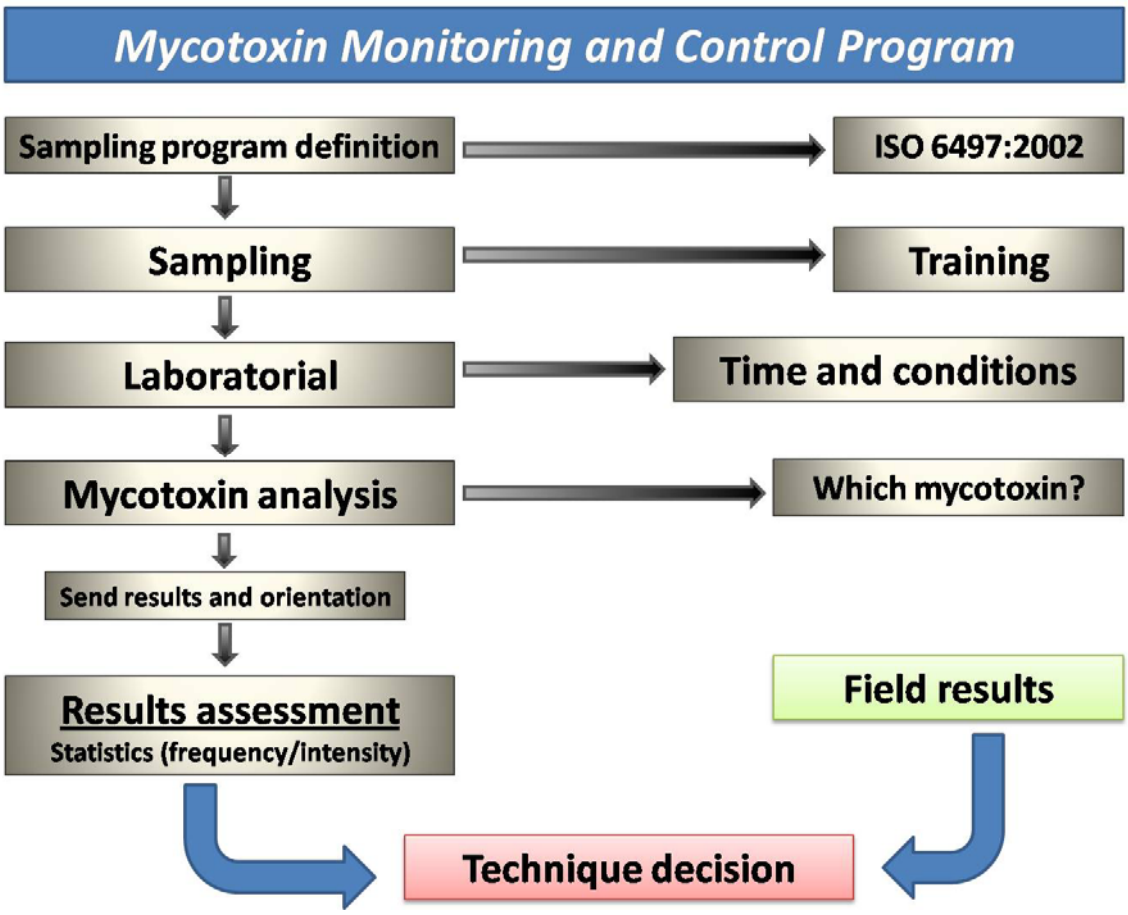

Figura 1 – Mycotoxin monitoring and control program

4.1.1 Cereals sampling

The sampling program used in most industries assisted by LAMIC is recommended by the UE standard committee (Amtsblatt der Europäische Gemeinschaften N L 102/1, TEIL II, 1976 e Futtermittelrecht mit Typenliste fur Einzel - und Mischfuttermitteln, 1994), and later standardized by ISO 6497:2002. Technical adaptations to attend the dimensions of food processing industries, were made to always sample raw material after being grounded, allowing better feasibility and representativeness. Sample collection should be done when the material is moving and grounded, in pre established time intervals, depending on the amount processed daily. Therefore an animal feed factory that produces 100 tons per day, will do a minimum composite sample of 44.7 kg of grounded material, according to the equation $\sqrt{20xT}$, T being the number of tons produced. This material will be better collected if dynamically sampled through the “hole in the screw” system, which allows obtaining a constant sample of the previously grounded material flow during processing. A sample of at least 1 kg must be destined to laboratory analysis.

The sample should be sent to the laboratory as fast as possible, accommodated in a resistant package. For distant areas, the use of air transportation is recommended, in order to reach the lab no longer than 48 hours.

The analysis and mainly the definition of which mycotoxins should enter in a control program can only be defined knowing their prevalence in specific regions where grains are harvested. Because of the need in making fast decisions in agribusiness, it is recommended for the analysis to be made as soon as possible, obligating the analytical process to be also fast and precise.

In the result evaluation it is necessary first to establish confidence interval for the sampling system. The usual procedure starts with the collection of at least 2 samples per batch. The results are evaluated in their amplitude so that the number of samples to be analyzed is adjusted. After an initial period, it is possible to reduce the number of samples to one per day, depending on the total production and the contamination variability of the original substrate.

4.1.2 Silage sampling

Doing the total sampling of a silage silo can seem of great worth, however, to do so it would be necessary to rip the plastic film that covers it favoring the entrance of

oxygen and consequent development of undesirable microorganisms. It is not so important to know the contamination by mycotoxins in the whole ensiled product, but to know the fractions that are being used and that are in contact with oxygen, which could be favoring the production of mycotoxins at a time. The accumulation of daily samples during a period represents the best way to trace the feed offered to the herd.

Another way to justify the sampling being made only in the open portion of the silo is that, generally in the ensiling process, the forage mass is disposed in layers, allowing the existance of slices of product, in which, the surface sampling is enough for the total silo representativity.

The method of sampling and shipping samples consists in:

- ✓ Taking out 200 to 500 g in 2 points per m² of the cut surface;
- ✓ Homogenize the silage in a clean surface;
- ✓ Reduce using the “cross system” with the support of adequate equipment;
- ✓ Remove a 2 kg portion of the fresh material;
- ✓ Drying with a heat source (except in microwave, in the sun or in temperatures higher than 70°C);
- ✓ Ground in 1 mm particles;
- ✓ Sending to laboratory in a thermal box with enough ice to keep the sample cool;
- ✓ Identifying correctly.

In case the sample is not dried before sending to the laboratory, it should be stored in a plastic bag, in a way that it is well compacted inside the package, which should be hermetically sealed. Sending the sample in a thermal refrigerated or vacuum sealed box, suppresses the fungi growth and mycotoxin production that may occur during the sample shipping and receiving period.

4.2 Mycotoxin Diagnosis

Nowadays the most specific, precise and reliable methodologies are obtained with the use of chemical processes. These procedures can be directed to Thin Layer Chromatography (TLC) as well as to High Performance Liquid Chromatography (HPLC). With the emergence of HPLC linked to the detection of Mass spectrometry (L/CMS and LC/MSMS) besides Gas chromatography-mass spectrometry (GC/MS), the diagnosis systems tend to be faster and more precise. The immunoassay tests can be used for selection and, in exceptional cases, for semi quantification. The use of chemical evaluations are accepted in international methodologies and recommended for the

diagnosis of mycotoxins. The use of extraction in the solid phase brings progress, especially in standardizing and automation of mycotoxic analysis.

The analysis system used in “on line” monitoring uses the HPLC joined with automated extraction, purification and derivation methodologies, as well as LC/MSMS systems, decreasing the use of laboratory technicians labor force**,** besides minimizing analytic errors and providing a fast response in diagnosis.

4.3 Decrease of mycotoxin toxic effect using Anti-Mycotoxin Additive (AMA)

Once mycotoxins are formed, the inhibition of the fungi growth will not imply on their concentration decrease. A frequently used method to control mycotoxicosis is the use of material nutritionally inherent to the animal’s diet, in order to decrease the absorption of mycotoxis in the gastrointestinal tract. These substances were called mycotoxin adsorbent and are now generically called Anti-Mycotoxin Additives (AMA).

In the Brazilian market, where approximately 100 AAMs for poultry and swine were evaluated, only about 30% presented effectiveness. Due to the fact that most of the product did not attend the demands for the use as AAM, the uncertainty of how much and how to use a truly effective product still remains. The answer comes with constant monitoring of the feed factory.

In a way, all diets for a pre initial phase should have inserted in their formulation an AAM. Diets for the other phases, must consider the Mycotoxin Risk (MR) before inserting AMA. This index considers the interaction between mean contamination of analyzed samples and mean prevalence of each mycotoxin in these samples, which is used as reference for decision making regarding the use or not of AAM.

5. Conclusion and Recommendations

The presence of mycotoxins in an animal’s diet can determine significant losses in the production system. The considerable presence of mycotoxins in the main components of the diet determines the need to adopt a continuous control program which can be based on the use of AMA (adsorbents). To adopt control measures it is necessary to know the prevailing contamination precisely, making it indispensable the implementation of a monitoring program of silages and feed destined to animal intake.

The future control of the mycotoxin problem, in livestock economy, depends on the implementation of adequate policies in agricultural management extent, as well as

production and storage systems. Only policies in these areas will bring solid and long lasting economical results for livestock industry.

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Challenges in the utilization of high moisture grains silage for ruminants

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ABSTRACT. The advantages of the high moisture grains regarding the dry grains are widely emphasized in the literature. In Brazil, the usage of high moisture corn started in the eighties and since then it is a constant expansion technology. Besides the economic and loss reduction aspects it is highlighted the better animal performance. Considering the research results in Brazil and abroad, in ruminants fed with high moisture corn, it is evident that the application of this technology may bring advantageous results to the producers. In Brazil, the increase of the finish beef cattle in feedlot has been one of the factors which demand the increase of grain silages use. Besides corn, the use of sorghum grain in the ruminants feed has also increased, which stipulates investments to explain some issues which have not been consolidated by the research.

I – Introduction

The use of high moisture grain silage, especially from corn, is a technology which has become more and more important in the animal production systems where the grain use is an important compound in the diet. In Brazil, the increase of finish beef cattle in feedlot system receiving diets with high grain has made an increase in the use of high moisture corn. Emphasis is given to the lower cost of production and to better silage feed conversion in relation to the dry grains. The lower production cost (estimated between 7 and 15%) is determined by elimination of stages such as transport to the grain storage silo, cleanliness,

drying, elimination and break due to high moisture.

The animal feeding in feedlot or semi-feedlot system deserves special attention, not only regarding the nutritive demands, but also specially because of the feedstuff costs, mainly concentrate. Due to it, it is necessary to find technological use which allows efficiency and economy in the exploration. In this context, the grains silages use may constitute an important alternative for the grains in the concentrate formulation. In Brazil, corn is the principal grain used in the ruminant feeding. However, other grains can present great possibilities of usage because of quality and regional availability. As an example, it is possible to mention sorghum grain, millet, white oat and triticale. The use of these grains is still small when compared to the corn use, but there are already some studies of these grains use in silage form to the feeding of different animal species (Jobim et al., 2003; Oliveira et al., 2007; Catelan et al., 2009).

Nevertheless, other progresses must be searched to add quality to these silages. Today the main issues are regarded to the microbial or nutritive additive use, chemical composition of the grains, with inference to its quality (nutritive value), and to the ensiling processing.

II- Ensiling effect on the grains starch

According to Mello Jr. (1991), the carbohydrates of the ruminant diets can be enzimatically digested in the rumen and large gut by microbial enzymes and, in the small gut, by the intestine and pancreatic enzymes. In the rumen, the starch is easily and quickly fermented by the amilolytic microorganisms, although the level which this occurs depends mainly on the physical and chemical properties of the starch granules. The effect on the matrix protein solubility which encapsulates the endosperm starch granules has been considered the most important factor that affects the usage efficiency. Studies made in the 70s and 80s (McNeill et al., 1975, Theurer, 1986) reveal that the ensiling process may improve the availability and/or the use of grain starch, depending on the ensiling technology, animal specie and grain source. The corn grain endosperm is formed by vitreous and floury zones and both present differences in chemical and physical composition. The corn grain presents texture differences, being classified as flint corn, dent corn or semi flint corn. An important way to evaluate grain texture is the Kernel vitreousness, due to the relation with the quantity of vitreous and floury endosperm. The bigger quantity of vitreous endosperm, harder the grain is, on the other hand, the more dent the grain is, the bigger the quantity of floury endosperm.

In the ensiling, the higher content of moisture, regarding the dry grain, favors the fermentation inside the silo, resulting in higher nutrients solubility and in an increase of the

starch susceptibility to enzymatic hydrolysis, causing improvement in the animal feeding efficiency (Gill et al., 1982; Simas, 1997) and in the microbial protein synthesis. Also, the starch gelatinization by heating can favor its digestibility, but this will hardly happen in ensiling normal conditions of corn grains, once the necessary temperatures (62 a 72ºC) for this gelatinization to occurs are not reached. However, according to Rooney and Pflugfelder (1986), starch can be gelatinized by the action of chemical agents. This way, silage acids can also contribute for a better starch digestibility. The pH reduction, due to acids production in grain ensiling process, results in acid hydrolysis, both the starch and the protein fraction, which favors the increase in gastric retention and pepsin activation time determining an increase in silage (Jones et al., 1974).

The corn starch preserved in silage form, both from the whole plant and moisture grains, is also digested mostly and more quickly in the rumen and only a small fraction goes to small gut (Owens et al*.,* 1986). In ruminants the low starch degradation in the rumen may reduce the total digestibility in tract and harm the rumen microbial protein production. Nevertheless, the rumen is the main local of starch digestion, with volatile fatty acids and microbial protein production. (Theurer, 1986).

III- Grain Processing and Starch Use

It is known that grains which suffer intense physical processing (triturated or compressed) and/or chemical processing (gelatinization) present higher ruminal digestion. The processing purpose is digestibility improvement by breaking the barriers that make it impossible the access of ruminal microorganisms and enzymes in the nutritive components of feeding (McAllister et al., 1990).

The grains processing increases excessively the starch ruminal digestion, because it acts in the increase of grains surface area or in the increase of starch granules solubility in water (Antunes and Rodrigues, 2006). Thus, grain starch and protein availability in the rumen and small gut also increases changing ruminal fermentation and passage rate characteristics and the digestion site (Theurer, 1986; Owens et al. 1986), making energy available for the microbial development and consequently in a higher volatile fatty acids production (Owens et al.,1997). The rate and extension of starch digestion in the rumen differ among the starch sources (Rooney and Pfugfelder, 1986) and from processing method and intensity (Theurer, 1999).

IV- Maturation stage and genotype effect in grains use

In situ incubation studies revealed differences among and inside starch sources in ruminal degradation due to the differences in the content of amylose and amylopectin, crystalinity, particles size and the technical process used (Tamminga et al. 1990, Tamminga, 1997). Chemically, starch is constituted by amylose and amylopectin polysaccharides, interlinked and wrapped up by a protein matrix or layer (Rooney and Pflegfelder, 1986).

The starch digestibility is inversely proportional to the amylose content, due to interactions with this protein matrix of starch granule (Rooney and Pflugfelder, 1986, Zeoula and Caldas Neto, 2001). This way, starch sources with bigger amylopectin contents, such as unripe corn grain, can present higher digestibility (Jobim et al. 2003). McAllister et al. (1993), consider that in practice starch digestion extension in the rumen seems to be more determinate by the material type which surrounds and protects the starch granule than by its physical and chemical proprieties. The protein matrix of corn endosperm is extremely resistant to digestion by ruminal microorganism (McAllister et al., 1990). Also for the sorghum, a potential limitation for the use of grain silage is the low digestibility due to the dense protein matrix of the peripheral endosperm (Gutierrez et al., 1982), which makes the starch little accessible to ruminal digestibility.

In this context, McAllister et al. (1991), using scanning probe microscopy, observed that the corn protein matrix limits the ruminal bacteria access to the starch granules. After the pericarp breaking by chewing or processing, the fermentation rate of starch granules is determined by the protein matrix rigidity and by the presence of cellular wall of the endosperm cells (Antunes and Rodriguez, 2006).

The starch degradation in the rumen varies with the corn maturation stage decreasing with the maturity advance (Jobim et al., 2003). Before grain maturation completion, the protein matrix which covers the starch granules, in flint corn it is already being formed and it will limit starch ruminal digestion (Philippeau et al., 1996). Because of this, the corn harvest for silage with higher moisture content, comparing to dry grain, has beneficial effect on the digestibility in the rumen (Jobim et al., 2003).

A way to manipulate the starch degradation rate is by hybrids selection (Philippeau et al., 1999). Studies performed regarding the corn maturation stage show a strong variability in starch ruminal degradation considering the genotype (Philippeau et al., 1996). Corn hybrids differ by endosperm texture (dent, flint) (Majee et al., 2003). There are evidences that the endosperm texture is related with rate and extension of protein and *in vitro* starch and *in situ* ruminal in cattle digestibility (Phillipeau et al., 1999). Kotarski et al. (1992), comparing

ruminal disappearance of *in vitro* starch between sorghum cultivars observed a faster disappearing rate for floury endosperm cultivars regarding cultivars with vitreous endosperm. Philippeau et al. (1999), studying relations between starch ruminal degradation and physical characteristics of corn grain in 14 corn hybrids, observed a effective average degradability of 50%, varying from 39.7% for the grains with flint texture to 71.5% to dent grain (Table 1).

Table 1. Influence of corn grain texture on ruminal DM and starch degradation

Item	Dent			Flint			SE	P
	Average	Min	Max	Average	Min	Max		
Effective DM degradability, %	55.8	51.9	71.5	42.3	39.7	45.3	1.3	.0001
Effective starch degradability, %	61.9	55.1	77.6	46.2	40.6	50.5	1.5	.0001
Vitreousness, %	51.4	38.5	57.3	71.8	66.8	79.1	1.4	.0001

Source: Adapted from Philippeau et al. (1999)

The effective degradability of starch was higher to dent corn grain than to flint corn grain, on average of 61.9 e 46.2%, respectively. These two types corn differed in Kernel vitreousness, with averages of 51.4% and 71.8%, respectively. The authors evidenced that the ruminal degradability and the starch physical characteristics varied between the materials, where 88.5% of starch degradability variation in the rumen was associated with the grain endosperm Kernel vitreousness. Therefore, the grain texture seems to make an important part in the starch ruminal degradation (Philippeau e Michalet-Doreau, 1997).

Evaluating the influence of the endosperm Kernel vitreousness and the grain moisture extension in corn digestion of high moisture in feedlot cattle, Szasz et al. (2007), observed that the portion readily degradable in the rumen, both for DM and starch, increased linearly with the grain harvesting moisture. This decreased proportionally to insoluble fraction potentially degradable of DM and starch. The authors also highlighted that corn with high moisture and higher Kernel vitreousness presented smaller sized particles and bigger surface area when compared to hybrids with floury endosperm. This smaller particles size was associated with a faster *in situ* digestion and an intestinal and total tract digestibility of starch slightly higher. Thus, these authors concluded that the negative effects on the starch digestion associated with vitreous endosperm can be avoided by ensiling and processing of high moisture corn.

V- Microbial additives use

The use of microbial inoculants in grain silages has shown inconsistent results, the same way that the application of these inoculants in plants silages. A relevant aspect is to consider that the grains can present a bacteria population, specialized in lactic acid production, lower regarding the forage volume of the same plant. This because epiphytic bacteria is usually found on leaves surface and in basal region of the plant. Thus, it is possible that the bacteria population added in silage present relevant effect in the fermentative process, with higher preserving efficiency. Then, Schaefer et al. (1989), studied the silages inoculation of the air part and of the high moisture corn and verified higher effects of inoculants addition on the microbiological counting in moisture grains silages. Yet, they did not detect effects on the nutritional quality.

There are evidences that the success in microbial inoculants use in silages depends, among other factors, on the presence of adequate substratum and the bacteria population added via inoculants in relation to the epiphytic population (Muck, 1988). On the other hand, it is possible that the lactic bacteria population (CFU/g of silage) necessary for a good fermentation pattern in grain silages is much smaller than in plant silages. In high moisture silages, this is because of the lower buffer capacity (3.17 m.eq NaOH/100 g DM – Calixto Júnior et al., 2009), demanding lower acids production for the ensiled volume stability. This thesis would be proven by lower acids concentration observed in grain silages, with lactic acid values of 0.80 and 0.78% and acetic acid of 0.40 and 0.12% (DeBrabander et al., 1992, Jobim et al.,1997).

Working with high moisture corn and high moisture sorghum silage, Ítavo et al. (2006) verified that the pH values obtained from the sixth day after the ensiling were 4.22 and 4.14, respectively, for control and inoculated silages. As for the silages of high moisture corn, the regression equations for the variable pH, in sorghum silages, inoculated or not, indicated pH stabilization after the third day of ensiling (pH<4.2). The regression equations for the variable pH, of inoculated or not silages, indicate a pH stabilization on the first days of fermentation. This way, the use of microbial inoculants in grain silages must follow bacteria specificity and product cost criteria.

VI- Nutritional additives use

The impossibility of previously concentrate formulation is a disadvantage for use high moisture grain silage, for being not able to be stored with a mixture ready for a later use. This

fact makes it necessary to mix it, daily, to the other diet ingredients, before supplying it to the animals. Thus, the corn grains ensiling with additives which raise the nutritive value, specially referring to crude protein and energy content, is interesting, because it can make a silage with nutritive value available similar to the ones of the commercial concentrate. In this context, the Conservation Forage Group, of Animal Science Department of University of Maringá (UEM) has been performing studies which have proved the feasibility of soybean, sunflower or urea addition in high moisture corn.

The use of nutritive additives in high moisture corn is still rare and the studies are recent (Jobim et al. 2002, Jobim et al., 2008, Andrade et al., 2009, Jobim et al., 2009). The aim of other grains addition, of urea or other product is to improve the chemical composition of silage, resulting in a better quality feed. It is stated that this can be an easy application technology, with an improvement in silage quality. This proceeding can be a viable alternative for producers who intend to reduce the concentrate formulation in feedlot system or even for dairy cows feeding. It is possible to use nutritive additives to obtain silage with protein and energy levels similar to the ones seem in commercial concentrate or formulated on the farm.

The soybean can be an alternative, since the raw soybean is a rich protein, besides being considered good energy source due to its high oil content. At the same time, in specific time of the year, the soybean is available in more accessible prices than defatted meal. The grain of soybean can become a low cost protein source, when produced on the farm or in cases of market prices for sales are very low.

The data presented in Table 2 are results of a study which evaluated quality and chemical composition of corn grain silages with levels of soybean. After one year of storage the silages presented excellent preserving quality, with reduced losses. It was verified that the corn grain ensiling added with raw soybean allows improving the silage feed chemical composition, mainly regarding the protein and energy contents. This way, the soybean use added to corn grain silage can determine the reduction in the commercial concentrate use and, consequently, reduce the production costs, being able to contribute to solve the serious grains storage problems on farms.

Table 2 – Chemical composition and gross energy (GE) content of corn silage added with soybean

Treatment	DM (%)	Ash (%)	CP (%)	NDF (%)	NNE (%)	GE (Kcal/g)
CG *	65.9	1.8	9.5	6.1	5.9	4020
CG + 10% SG	66.9	2.3	13.7	8.0	7.8	4309
CG + 20% SG	68.6	2.8	17.9	9.9	9.7	4471
CG + 30% SG	70.9	3.2	22.1	11.8	11.6	4538
CG + 40% SG	73.8	3.5	26.3	13.7	13.5	4730

*CG = corn grain SG = soybean
Source: Jobim et al. (2002)

Still emphasizing the increase of grain silage protein content, Jobim et al. (2008) studied the soybean, sunflower grains and urea addition to high moisture corn, evaluating the animal performance in sheep. Nowadays the sunflower crop has been growing, but its production is addressed for the oil extraction, where the sub product has been used in animal feed in the form of meal. Thus, urea was added to corn grains aiming the increasing of crude protein content in silage. This way, Santos (2009), points outs that the use of urea in ration for finishing cattle can be costs reduction source, without reducing the animal performance. Studies performed in the USA and Brazil, comparing the use of urea *vs* true protein in cattle diet, evidence that there is not reduction in the animal performance when urea was used. Santos exemplifies that in a feedlot with 20 thousand animals for a period of 90 days, the substitution of 0.5 kg of soybean meal (R$ 800/t) for 0.5% of the mixture with 87% of corn and 13% of urea (corn = R$ 370/t and urea R$ 1,000.00/t), the savings can reach R$ 313,200.00 (Brazilian Real).

In this line the high moisture corn with urea addition can reduce even more the feeding cost and result in higher profit to the producer. Information obtained in farms allows estimating that the corn grain silage ton cost is R$ 200 to 232.00 (Brazilian Real), while the dry corn cost is between R$ 250.00 a 260.00 (Brazilian Real). The urea addition can result in economical benefits, because it is considered the ensiling cost of corn grain around R$ 200.00 (Brazilian Real). Adding 1% of urea (R$ 870.00/t) the cost would become R$ 208.70/t (Brazilian Real).

Considering the expense with commercial concentrate used in cattle feedlot, with an estimate cost of R$ 0.38 to 0.42/kg Brazilian Real, it can be verified that the soybean or urea addition can result in economical benefits.

In Table 3 some values of chemical composition of high moisture corn with soybean, sunflower or urea addition. These silages presented good preserving quality and, in the animal performance evaluation (feedlot sheep), showed great results. In this study, it was evaluated the chemical composition, the ruminal degradability of dry matter and crude protein and the

starch disappearance in the rumen. It was also evaluated the silages aerobic stability. It was verified that the there was an effect of soybean, sunflower and urea addition in the silages chemical composition, especially on the crude protein and ether extract contents. There was also an effect on the effective DM degradability and crude protein. The silage which presented higher aerobic stability was the high moisture corn added with urea. It can be concluded that the addition of soybean or sunflower or urea in the high moisture corn results in an improvement in the feed chemical composition.

Table 3 – Chemical composition of high moisture corn and silages with 20% of soybean, 20% of sunflower grain or 1% of urea.

Treatment	DM (%)	OM (%)	Ash (%)	ENN (%)	CP (%)	Starch
HMC	62.1	94.5	5.5	5.2	10.1	64.2
HMC+S	65.6	93.0	7.0	10.3	17.7	51.2
HMC+SF	65.0	95.1	4.9	11.9	10.9	56.8
HMC+U	65.7	94.4	5.6	4.9	20.2	59.3

HMC = high moisture corn; HMC+S = high moisture corn + soybean; HMC+SF= high moisture corn + sunflower grain and HMC+U= high moisture corn + urea. *CV = Coefficient of variation.
Source: Jobim et al.(2008)

VII- Grain Silage for Ruminant

The use of grain silage, especially from corn, has increased in all the regions of Brazil, principally in properties with technologies. The increase in grain silage usage is promoted by an increase in finish beef cattle. However, the books review show that, in Brazil, there are few studies evaluating the grain silages quality and animal performance. The biggest numbers of studies have been published related to the corn and sorghum use in swine feeding and in lower number relating to cattle, sheep and equine.

VII.1. Sheep

When studying the performance and characteristics of carcass in sheep in *Creep Feeding* receiving high moisture corn compared to the dry grain, Almeida Jr. et al. (2004), verified that the average daily gain and slaughter age at 28 kg of live weight were not affected by treatments. For carcass traits and characteristics there was not effect from grain type either. Nevertheless, the animals which received grain silage presented a tendency of lower slaughter age, what can be seen on Table 4.

Table 4 – Birth weight average, daily feed intake, average daily gain and slaughter age of feed with (HMC) in lambs on creep feeding

Variable	0% HMC	50% HMC	100% HMC
Birth weight (kg)	4.90	4.87	5.05
Daily feed intake (kg MS)	0.387	0.308	0.365
Average daily gain (kg)	0.368	0.396	0.385
Slaughter age (days)	64.88	61.13	61.43

Source: Almeida Jr. et al. (2004)

The feedlot sheep performance, fed with dry corn (DCG), high moisture grains (HMC) or hydrated corn grains before ensiling (HHMC) was evaluated Reis et al. (2001). The authors verified beneficial effect in the high moisture grain usage regarding the dry grain (Table 5). Regarding the average daily gain (ADG) and feed conversion ratio (FC), it was evidenced that the animals which received DCG+HHMC or DCG + HMC presented better results and that the best answer of animals fed with high moisture corn can be attributed, among other factors, to the starch composition granting higher digestibility.

Table 5 – Average initial weight and average daily gain, gramas per day.

Item	DCG	HMC	HHMC	DCG + HMC 50:50	DCG+HHMC 50:50
Initial weigh	8.98	9.94	9.90	9.34	9.99
ADG 28 days	108.50a	154.10a	150.30a	114.70b	93.60b
ADG 56 days	87.30b	147.40a	121.20a	170.40a	101.10b
ADG 73 days	123.70a	160.90a	153.70a	145.00	126.00b

Within a row, means without a common superscript letter differ ($P < 0.05$), Tukey test.
DCG = dry corn grain; HMC = high moisture corn; HHMC = hydrated corn grains before ensiling
Source: Reis et al. (2001)

Regarding the moisture corn grain silage with nutritive additive, Lombardi (2007) verified that the addition of sunflower grains or urea in the ensiling does not interfere in the sheep performance. In table 6, data is shown referring to the animal performance in relation to weight gain and carcass variation. It is concluded that corn grain silage associated with 20% of sunflower grains or 1% of urea, in feedlot sheep feeding, do not affect the carcass quantitative variable, recommending its use for feedlot sheep supplying.

Table 6 – Performance and carcass characteristics from lambs fed with high moisture corn (HMC), HMC added with sunflower grain (HMC+S), HMC added with urea (HMC+U)

Characteristic	HMC	HMC+S	HMC + U	Average	F	CV (%)
Initial BW, kg	22.71	23.22	23.00	22.98	0.09	10.22
Final BW, kg	31.43	30.85	30.88	31.05	0.60	3.85
ADG, kg	0.17	0.15	0.15	0.16	0.32	19.51
Hot carcass, kg	13.55	13.18	13.43	13.39	0.27	7.57
Chilled carcass, kg	12.95	12.54	12.92	12.80	0.44	7.61
Carcass yield, %	50.51	50.51	50.49	50.51	0.30	5.69
Commercial carcass yield, %	48.27	48.10	49.54	48.63	0.28	4.32

F = F test
CV = coefficient of variation

VII.1.Cattle

In studies performed by researchers from FMV/UNESP-Botucatu, aiming to get information about the nutritive aspects in feedlot systems, in Brazil, and published by Tonin (2009) reveal that the main grains applied in feedlot cattle diet are corn with 79% and sorghum with 21%. Some studies have compared sorghum grains with corn grain in cattle diet, with satisfactory results. When evaluating dry corn, HMC, tannin - sorghum grain, tannin-sorghum silage, dry sorghum and high moisture sorghum Almeida Jr. et al., (2008), did not verify effect on the weight and slaughter age of calves. Also they did not observed difference for daily gain and live weight total, being then real daily gain of 0.96 Kg. The data evidence that sorghum, with or without tannin, in grounded dry grains forms or moisture grain silage, and the moisture corn grains silages, can be used for post weaning Holstein calves, with satisfactory performance.

When evaluating HMC or sorghum moisture grains (HMS) silage in a performance experiment of calves (F1 Red Angus x Nelore), Igarasi et al., (2008), did not observed trait effect on the final live weight, daily weight gain, subcutaneous fat thickness and back fat thickness, indicating that sorghum moisture grain can substitute corn moisture grains in feedlot, in high concentrate diets (Table 7).

Table 7 – Carcass characteristic and animal performance

Item	HMC	HMS	CV (%)	P value
Initial BW, kg	299.69	293.69	-	-
Daily gain, kg/day	1.41	1.43	13.20	0.64
Final BW, kg	482.54	486.81	4.86	0.40
Initial fat thickness, mm	1.92	2.02	-	-
Final fat thickness, mm	4.28	4.16	21.69	0.54
Back fat thickness, mm	5.87	5.38	21.38	0.06

Source: Igarasi (2008)

The performance and characteristics of yearling steers carcass fed with corn and sorghum moisture grains silage with different protein levels was evaluated by Passini et al. (2002). The authors do not find significant difference for the daily weight gain in the traits during the total period of feedlot. The average daily gain was satisfactory, that is around 1.219 kg/day. It was not found effect for ribeye area, subcutaneous fat thickness and tenderness which evidences the sorghum grains potential for finish beef cattle.

Berndt et al. (2002), compared the effect of harvested dry corn grain or moisture ensiled, associated to the silage of corn plant or sugar cane bagasse, on the corporal composition and muscle tissue deposition in young bulls and observed that animal fed with moisture corn presented higher rates of lipids and energy deposition, without changing the empty body weight gain. Also Henrique et al., (2007) did not observed difference in carcass characteristics for finishing bulls regarding the type of corn grain, but they verified an improvement of 9.7% in feed efficiency when it was use moisture corn grains silage comparing to the dry grain.

VIII- Conclusion

The high moisture grains ensiling is still an expending technology in Brazil. The ensiled sorghum or corn grains usage in ruminant feeding in motivated by the increase in the feedlot finish beef cattle. Emphasis is given to the lower production cost and better feed conversion of silage comparing to dry grains. However, in Brazil, there are still some issues which must be better evaluated by research for increase in the storage efficiency and animal performance. Also, it is needed studies evaluating other grains (sorghum, millet, oat, triticale), which in certain situations can result in economical benefit. In the authors' opinion, the use of nutritive additives in corn grains ensiling, such as urea and the soybean grain, is an alternative fully justified in most of the production system, given to costs and results observed regarding preserving quality.

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Temperature measurements of large scale silo face to asses aerobic deterioration of corn silage on farm

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1. Introduction

Silage is an efficient technique for the conservation of the energy of the harvested forage since microorganism fermentation transforms sugars into organic reduced compounds (lactate, acetate, mannitol and ethanol), transferring to ATP a very small amount of energy compared with that obtained from a system in an atmosphere of oxygen (McDonald et al., 1991). It has long been recognized that the presence of air produce deleterious effect on silage (Beck and Gross, 1964; Woolford, 1990). Oxygen enables various aerobic spoilage microorganisms, which survive in the anaerobic phase of ensiling, to become active and to multiply, causing aerobic deterioration (Woolford, 1990). Aerobic deterioration usually results in high dry matter (DM) loss (Bolsen et al., 1993) and the loss of important nutritional components (Kung et al., 1998) and may lead to potentially toxic substances or undesirable microorganisms which can negatively affect food quality and safety (Wilkinson et al., 1999; Ivanek et al., 2006; Vissers et al., 2007; Tabacco et al., 2009). A large part of the silage stored in horizontal silos is exposed to air penetration, especially in the upper part near the walls which are difficult to seal properly (Ashbell and Lisker, 1988). Much greater losses can occur, however, when the silos is opened for feeding, because air penetrates the silages and promotes the growth of aerobic, acid-tolerant microorganisms and the oxidation of fermentation products present in the silages (Danner et al., 2003). On the farm this deterioration is usually manifested by a rise in temperature and by the appearance of molds in the peripheral areas of the silo (Ashbell and Weinberg, 1992). Silages that spoil and heat in the silo or in the feedbunk may dramatically affect daily DM intakes, because cows are reluctant to consume unstable silages especially during the warm season when DM intake is already compromised (Mahanna and Chase, 2003).
Since the evaluation of microbiological and chemical quality of the working face of a silage during the feed-out phase would require many samples, expensive labor and equipment, qualified personnel, and time-consuming laboratory analyses, a simple method to assess silage quality, with good accuracy and quickness is necessary.
The aim of this paper is to show the correlation between microbial and chemical composition of the silage during the feed-out phase and some easily measurable parameters by technicians and farmers, that are useful to quantify the extent of aerobic deterioration at a farm level.

2. The role of yeast in the aerobic deterioration of silages

Yeasts that metabolize lactic acid are the primary spoilage microorganisms in silage, although acetic acid bacteria can also initiate the aerobic deterioration process in corn silage (Pahlow et al., 2003). It has been suggested that silage with yeast populations in excess of 10^5 g^{-1} DM are regarded as highly susceptible to aerobic deterioration (Beck and Gross, 1964; Daniel et al., 1970).
Immediately after ensiling yeasts compete with other microorganism for fermentable substrates and, during the first week of ensiling, yeast populations can reach 10^7 cfu g^{-1} (Jonsson and Pahlow, 1984). A gradual decrease of yeast counts usually takes place during subsequent storage, depending on factors such as degree of anaerobiosis, pH, and concentrations of undissociated organic acids. The pH levels usually reached in silage are not inhibiting for silage yeast, which usually can grow

within a pH range of 3-8. Under aerobic conditions, yeast tolerate organic acids better than most other microorganisms and consume organic compounds such as lactic, acetic, succinic, malic, citric, propionic acids and ethanol (Moon and Ely, 1979; Middelhowen and Franzen, 1986). These characteristics together with the effect of oxygen and sugars explain why yeasts are notorious spoilers of fermented products and why anaerobic conditions are absolutely necessary to provide an adverse environment for their development (Jonsson, 1989).
Over air exposure, the growth of lactate-utilizing yeasts causes a rise in pH and, as this process proceeds, other aerobic organisms, such as moulds and bacilli, start to proliferate. Lactic acid, acetic acid, and water soluble carbohydrates (WSC) are the main sources of energy for the microorganisms involved in aerobic deterioration. The oxidation of these nutrients results in the production of carbon dioxide and water, with the evolution of heat (McDonald et al., 1991). With the complete oxidation of glucose the temperature rise in the silage mass, assuming a specific heat capacity of 1.89 kJ kg^{-1} $°C^{-1}$ for the silage DM and no loss of heat to the atmosphere is given by the expression:

$$T = (DM * GLUCOSE) / (267.5 – 0.147 * DM)$$

Where DM is DM content in g kg^{-1} and GLUCOSE is the amount of glucose (g kg^{-1} DM) oxidized (McDonald and Whittenbury, 1973). For example, the temperature rise through the complete oxidation of sugars in an insulated silage of DM content 320 g kg^{-1} and sugar content (as glucose) of 50 g kg^{-1} DM, would be 73°C.
When well fermented silages with low values of residual sugars are concerned (as corn silage), the main product oxidized is likely to be lactic acid. The heat produced from the complete oxidation of lactic acid is marginally less (15.16 kJ g^{-1}) than from glucose (15.64 kJ g^{-1}) and therefore the temperature increase from lactic acid oxidation in the above example would be slightly lower, about 70°C. In most practical situations a part of the heat produced through oxidation will be dissipated into the atmosphere, although temperatures as high as 50°C have been recorded in aerobic deteriorated silages (Henderson et al, 1982). Therefore, temperature can be useful as an index of heating associated with aerobic deterioration, since rises in temperature are clearly linked with yeast activity and DM loss and could have application in alerting farmers to the onset of aerobic deterioration (Williams et al., 1994; Tabacco and Borreani, 2002a, b).

3. Temperature measurements at the silo face

Several systems to measure temperature of the working face of a silo could be applied to describe the areas involved in aerobic microbial activity. One method consists in burying temperature loggers inside the silo at the time of silo filling and retrieving them at the feed-out (Kung, 2009), but this option is very expensive and permits to measure only some points of the silage mass. Another options is to use temperature probe or “spike” thermometer (Tabacco and Borreani, 2002a; Schoonmaker, 2009) to monitor the working face of a silo. Tabacco and Borreani (2002a) proposed the use of a probe thermometer to monitor the silage face and to quantify the extent of aerobic deterioration at the farm scale. Briefly, the temperatures are measured by probe thermometer within the stored silages during feed-out at 20-cm depths into the working face. Measurements were taken at 11 locations across the working face (0.25, 0.5, 1.0, 2.0, 3.0 m from both the right and left side walls and in middle of the silo face) and at seven elevations (0.1, 0.2, 0.3, 0.6, 1.0, 2.0 below the top surface and 0.2 m above the base) (Figure 1). Ambient temperature is recorded at the same time. Area of the working face with visible moulds is also determined and measured. The difference between the temperature of the silage sample and the temperature measured in the central zone of the silo (h/2 and x/2, where h = height of the silo; x = width of the silo) were used as an index of heating associated with aerobic deterioration (dT). A silo is considered aerobically deteriorated

when at least 10% of the area of the working face was interested by temperatures 10°C above those recorded in the central zone of the silo and/or visible moulds were present in top layers near side walls.

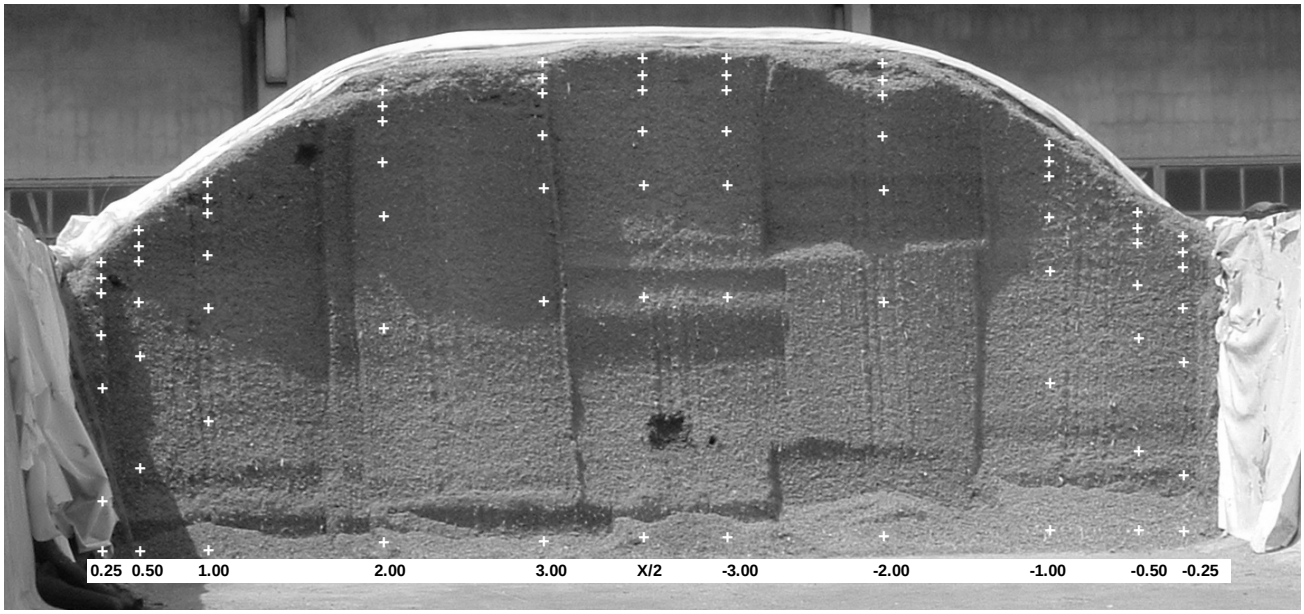

Figure 1. Scheme of temperature measurement on the working face of the silo, with a probe thermometer.

The more recent method proposed to measure temperature at the working face is infrared thermography by heat-sensing digital cameras (Seglar, 2008). Infrared thermography is a noninvasive technique capable of detecting thermal radiation from the surface of any object. Infrared thermography has been widely used in animals to diagnose inflammatory conditions such as sole abscesses and laminitis in horses (Eddy et al., 2001), to predict changes in udder temperature in dairy cows (Berry et al., 2003), and to detect other subclinical inflammation in cattle and dairy cows (Nikkhah et al., 2005). Heat-sensing digital cameras could capture in a single picture the whole temperatures of the working face of a silage. The first attempt to assess the heat production at the silage face was made in 1993 by Cassinis et al., working on bunkers of corn, Italian ryegrass and alfalfa silages. These authors concluded that the temperatures measured on the silo face could be correlated to the "heat status" of the silage, even if the values were always lower than those measured into the silage mass to a dept of 12 cm. The same results were reported by Collombier et al. (2001), who utilized infrared thermography for visualizing deteriorations of corn silage and for testing the efficacy of the *Lactobacillus buchneri* inoculant on silage aerobic stability. These authors found that the surface temperatures were lower than those measured by probe thermometer to a dept of 15 cm in the silage mass, especially in the peripheral areas, where the heat generated by aerobic deterioration is higher than in the silo core. Furthermore it was pointed out that the temperature measurement by infrared thermography is highly influenced by weather (sunny, cloudy, rainy ...), the time of the day, the exposure of the silo face, and the homogeneity of the feed-out face (Cassinis et al., 1993; Collombier et al., 2001).

4. Linking temperature to microbial status of the silage

The heat produced during silo filling is the result of respiratory process that is enabled by the high amount of air trapped in the silage mass, especially when the filling operation are protracted for long time (Woolford, 1990). Under anaerobiosis there is less opportunity for energy to escape from the system in the form of heat. The ATP produced during fermentation is used for microbial

biosynthesis and although it would result in some loss of energy in the form of heat, because of limited ATP production, this loss will be very small. The ATP formed from anaerobic metabolism is only 1 to 2 mol compared with 38 mol obtained for complete oxidation in the respiration process (McDonald et al, 1973a). In temperate environment during the winter season, steam could be released from the core of the silo because of the difference between retained heat (produced by respiration during silo filling and to a lesser extent by fermentation processes) and the ambient temperature. Therefore the presence of steam from the core of the silo does not always mean that silage is spoiling (Borreani et al., 2002; Kung, 2009).

After silo opening under aerobic conditions, the metabolism of lactic and acetic acids by aerobic microorganisms (mainly yeasts) results in an increase in pH, which is well correlated with temperature rise and also with DM loss.

Tabacco and Borreani (2009) presented results from a survey carried out in western Po Plain (Italy) on 54 dairy farms performing well, moderately well and badly to ensure that not only high quality silages were surveyed, encompassing different farm enterprises and representative of a range of ensiling practices. The mean chemical and microbial composition from core, peripheral areas, and molded spots of silages are reported in Table 1. The cores were characterized by a DM content ranging from 26.2 to 41.4% with a mean value of 34.3%, an a_w of 0.981, and a pH of 3.64. The lactic acid ranged from 1.44 to 8.98% of DM, the acetic acid from 0.17 to 5.48% of DM, whereas the butyric acid was always below the detection limit. The microbial characteristics of the core resulted in a mean yeast count of 2.93 log cfu/g, mold count of 1.76 log cfu/g and clostridial spore content of 1.36 log MPN/g. The samples from the peripheral areas with no visible molds presented chemical values that ranged from values similar to those of the core to values that are characteristic of deeply altered silage (pH up to 8.71, absence of lactic and acetic acids and presence of butyric acid with values up to 0.64% of DM). As expected, samples from the molded spots presented a deeply altered chemical and microbial profiles, with mean pH of 6.84, a_w of 0.993, yeast higher than 6 log cfu/g, mold around 8 log cfu/g, and clostridial spore from 1.48 to 7.04 log MPN/g. Furthermore the microbial activity of the deteriorated silage altered the acid contents with absence of lactic and acetic acids and butyric acid content that ranged from <0.001 to 0.24% of DM. The silage temperature in the middle of the silo was in average 18.6°C with values ranging from 12.0 to 22.9°C. Higher values were observed in the peripheral areas and in molded spots of the silo with temperatures up to 54.5°C. As a consequence the calculated dT were on average 9.9 and 13.3°C for the silage from the peripheral areas and from the molded spots, respectively, whereas it was close to 0°C in the silage core. The core samples always showed a pH below 4.0 and a dT below 2°C, whereas silages from the peripheral areas were split into two groups, 53% of them had a pH lower than 4 and a dT lower than 3.5°C and 47% had a pH higher than 4 and a dT higher than 5°C. A positive dT higher than 5°C correspond to pH higher than 4.5 in most of the silages from the peripheral areas (48 out of 51 samples) and in all silage from moldy spots.

Table 1. Mean values of chemical and microbiological characteristic from core, peripheral areas, and molded spots of corn silages observed in the farm survey (Tabacco and Borreani, 2009).

Items		Silage core	Peripheral areas	Molded spots
	n.	108	108	153
DM content (%)	Mean	34.3	34.1	33.4
	SEM	0.27	0.54	0.57
	Range	26.2 – 41.4	16.3 – 47.4	11.5 – 48.4
Water activity (a_w)	Mean	0.981	0.988	0.993
	SEM	0.0010	0.0013	0.0008
	Range	0.960 – 0.990	0.963 – 1.00	0.970 – 1.00
pH	Mean	3.64	4.97	6.84
	SEM	0.013	0.14	0.051
	Range	3.45 – 4.03	3.53 – 8.71	4.70 – 8.33
Yeast (log cfu/g)	Mean	2.93	5.48	6.33
	SEM	0.18	0.22	0.10
	Range	<1.00 – 5.70	<1.00 – 8.57	3.00 – 8.40
Mold (log cfu/g)	Mean	1.76	3.71	8.00
	SEM	0.10	0.18	0.07
	Range	<1.00 – 4.04	<1.00 – 6.65	5.70 – 9.40
Clostridial spore (log MPN/g)	Mean	1.36	2.75	5.08
	SEM	0.057	0.48	0.27
	Range	<1.18 – 2.36	<1.18 – 6.46	1.48 – 7.04
Sample temperature (°C)	Mean	18.6	30.6	35.4
	SEM	0.26	1.10	0.90
	Range	12.0 – 22.9	7.6 – 51.8	12.4 – 54.5
dT (°C)[1]	Mean	-1.5	9.9	13.3
	SEM	0.19	1.06	0.90
	Range	-5.9 – 1.9	-5.7 – 33.5	-6.0 – 33.5
Nitrate (mg/kg fresh matter)	Mean	349	298	48
	SEM	48	79	48
	Range	<100 - 3367	<100 - 4791	<100 – 1523
Lactic acid (% DM)	Mean	5.45	2.91	0.02
	SEM	0.17	0.30	0.0081
	Range	1.44 – 8.98	<0.001 – 7.09	<0.001 – 0.85
Acetic acid (% DM)	Mean	1.67	1.63	0.02
	SEM	0.10	0.20	0.0066
	Range	0.17 – 5.68	<0.001 – 6.16	<0.001 – 0.72
Butyric acid (% DM)	Mean	<0.001	0.03	0.02
	SEM	-	0.020	0.020
	Range	<0.001	<0.001 – 0.64	<0.001 – 0.24

5. Practical examples

In a farm survey carried out over 2 year in western Po Plain (Italy) on 19 dairy farms, Tabacco e Borreani (2002b) identified three groups of farms on the basis of the status of the corn silage during feed-out: A) aerobic deterioration both in winter and in summer; B) aerobic deterioration only in summer; C) no aerobic deterioration both in winter and in summer. Main farm characteristics are listed in Table 2. Farms of group C were characterized by a higher number of lactating cows, higher milk production per cow, and lower area of silo face per cow that result in a higher daily feed-out

rate (Table 3). Over winter top temperatures at the working face exceeded 30°C in farms of group A, while in farms of groups B and C were lower than those recorded in the silage core. During summer the top temperatures were higher than 30°C also in silos of group B. As an example, in Figure 2 are reported the contour map of temperatures of the silo face of two representative farms of group A and C in winter and summer periods. Group A and B had similar feed–out rate both in summer and in winter, but differed for care used to seal and weight down the silo surface (1 *vs*. 2 plastic sheet used; absence *vs.* presence of plastic sheet on side walls; tires only (20-30 kg m^{-2}) vs. tires plus soil (70-100 kg m^{-2}) to weight down the silo surface; absence vs. presence of sandbags to weight down the plastic sheet near side wall). These management practices avoid heating in silages of group B during winter but were not sufficient in summer, if not coupled with feed-out rate higher than 0.25 m, as for farms in group C.
The results underscore the importance of coupling high feed-out rates with careful silo management in order to control aerobic deterioration.

Table 2. Characteristics of the farms involved in the survey (Tabacco and Borreani, 2002).

Group	Farm	Lactating cows	Milk production	Forage crop	Silo	Silo size (m)			Silo face
	(no.)	(no.)	(kg yr^{-1} cow^{-1})	(ha)	(no.)	width	height	length	(m^2 cow^{-1})
A	10	48±9	8352±1536	22±6	1-3	7.4±1.4	2.0±0.5	22±6	0.32±0.10
B	6	50±11	8958±1315	26±5	2-3	7.3±1.1	2.1±0.3	21±3	0.31±0.09
C	3	75±7	11030±150	36±4	3-4	6.4±0.9	2.4±0.4	21±4	0.19±0.01

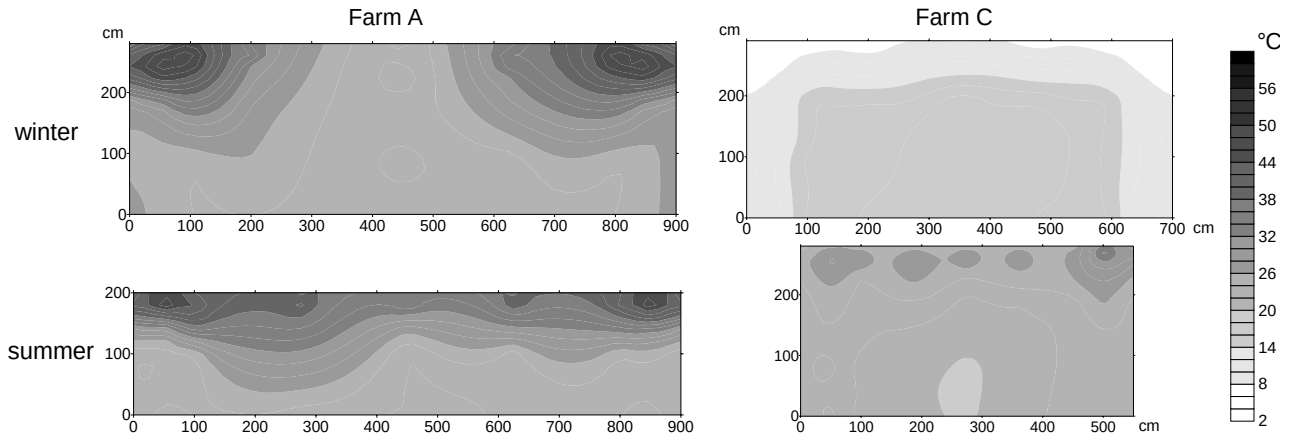

Figure 2. Contour map of temperatures recorded at 20-cm depths of the silo face in two farms of group A and C during winter and summer (Tabacco and Borreani, 2002).

Table 3. Main characteristics of maize silos [‡] involved in the survey (Tabacco and Borreani, 2002).

Group	Daily consumption	Density (kg FM m^{-3})	Winter				Summer			
			Temperature[†]		pH	Feed-out	Temperature		pH	Feed-out
	kg FM cow^{-1}	top/centre	top	centre	top	(m/d)	top	centre	top	(m/d)
A	21	221/621	35	19	4.9	0.11	36	24	5.1	0.14
B	22	312/634	16	17	3.9	0.13	35	22	4.7	0.16
C	25	321/576	11	16	3.7	0.24	28	23	3.9	0.31

[†] temperature (°C) was calculated as an average of the 20-cm depth measurements.
[‡] Main fermentation characteristics of central zone of maize silages (n = 76) were: DM content 345±27 g kg^{-1}; pH 3.61±0.14; lactic acid 41±12 g kg^{-1} DM; acetic a. 15±9 g kg^{-1} DM; NH_3-N 62±13 g kg^{-1} TN; no butyric acid.

6. Conclusions

Since the prevention of aerobic deterioration at farm level is crucial to animal health, feed and dairy food safety, temperature measurements at the working face during feed-out is a good and simple way to identify the microbial status of the silage and to quantify the extent of spoilage at a farm level.

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Effects of Microbial Additives in Silages: Facts and Perspectives

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Abstract

Silage inoculants have been used to maintain and (or) improve the nutritive value of forages stored as silage. Inoculants based on homolactic acid bacteria have been used to ensure a quick fermentation and rapid drop in pH based on the production of lactic acid. In turn, there is usually less production of acetic and butyric acids and lower concentrations of ammonia-N. Newer inoculants based on *Lactobacillus buchneri* have been developed to improve the aerobic stability of silages by increasing acetic acid and decreasing yeasts. Some inoculants have also resulted in improvements in fiber digestion via moderations of rumen pH and production of ferulic acid esterase. Silage inoculants work best when applied at correct application rates and when they are evenly distributed through the forage mass. They are also more effective when the management of the silage has been good.

Introduction

The primary goal of making silage is to maximize the preservation of original nutrients in the forage crop for feeding at a later date. However, fermentation in the silo is a very uncontrolled process can easily lead to less than optimal preservation of nutrients. In order to assist in the fermentation process, various silage additives have been used to improve the recovery of nutrients and energy in silage, often with subsequent improvements in animal performance. This review will focus on some practical aspects of the fermentation process and the uses of microbial inoculants. For a more in-depth review on all silage additives see the review by Kung et al., 2003.

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. If air is not removed quickly, high temperatures and prolonged heating are commonly observed. 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.

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 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.

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, yeasts metabolizes 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. Various silage additives (which will be discussed later in this paper) can also improve aerobic stability.

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 results in excessive amounts of air trapped in the forage mass that can result in a decrease of fermentable water-soluble carbohydrates. 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.

Silage Inoculants

As shown in Table 1 many end products are commonly produced during the fermentation process but many of these end products are associated with less than desirable fermentations. Of the several types of acids produced during, lactic acid is the strongest acid (stronger than the other acids) and preferred end product of silage fermentation. In fact, homolactic acid fermentation that produces only lactic acid is a desirable fermentation because of the high energy and dry matter recoveries (Table 2). Note that in the undesirable fermentations, large amounts of carbon dioxide (CO_2) are produced. Because CO_2 is a gas, the carbon (or dry matter) is lost to the environment. This explains why these fermentations have low DM recoveries.

Homolactic Acid Bacteria. Because forage often naturally contains many detrimental types of bacteria, the concept of adding a microbial inoculant to silage was to add fast growing homofermentative lactic acid bacteria (^{ho}LAB) in order to dominate the fermentation resulting in a higher quality silage. Some of the more common homolactic acid bacteria (^{ho}LAB) used in silage inoculants include *Lactobacillus plantarum* (note: this organism is now officially classified as a heterolactic acid bacteria), *L. acidophilus, Pediococcus acidilactici, P. pentacaceus, and Enterococcus faecium.* Microbial inoculants contain one or more of these bacteria, which have been selected for their ability to dominate the fermentation. The rationale for multiple organisms comes from potential synergistic actions. For example, growth rate is faster in *enterococci* > *pediococci* > *lactobacilli*. Some *pediococci* strains are more tolerant of high DM conditions than are *lactobacilli* and have a wider range of optimal temperature and pH for growth (they grow better in cool conditions found in late Fall and early Spring). Table 3 lists several common microbes that have been studied as silage inoculants.

Fermentation responses to homolactic acid bacteria. Alfalfa, grass, and small cereal grain crops have responded well to microbial inoculation with ^{ho}LAB. The fermentation of high moisture corn has also been improved with ^{ho}LAB. However, ^{ho}LAB microbial inoculation of corn silage has resulted in less consistent results. For example, I found 14 published (peer reviewed) studies in North America where corn silage was treated with a ^{ho}LAB microbial inoculant. Improvements in animal performance where found in only 3 instances and changes in

fermentation end products were small. However, Bolsen et al. (1992) reported that in 19 studies conducted at Kansas State University, with corn silage, silages inoculated with ^{ho}LAB had 1.3 percentage units higher DM recovery, supported 1.8% more efficient gains, and produced 1.64 kg more gain per ton of crop ensiled with beef cattle. Similar results were found with treated sorghum silages. In certain instances, significant animal responses have been observed with inoculation although there was little effect on traditional end products of fermentation (Gordon, 1989; Kung et al., 1993). These data suggest that lack of detectable changes in classically measured fermentation end products is not a good indicator of the effectiveness of an inoculant.

When compared to untreated silages, silages treated with adequate numbers of a viable ^{ho}LAB should be lower in pH, acetic acid, butyric acid and ammonia-N but higher in lactic acid content (Table 4). In a review of the literature between 1990-95, Muck and Kung (1997) reported that microbial inoculation lowered pH, improved the lactic: acetic ratio, and lowered ammonia nitrogen content in more than 60% of studies. Dry matter recovery was improved in 35% of the studies. Dry matter digestibility was also improved in about one third of the cases. Microbial inoculation usually has little or no effect on the fiber content of silages because most lactic acid bacteria contain little or no ability to degrade plant cell walls. Decreases in fiber content may be due to partial acid hydrolysis of hemicellulose. Some data suggests that certain microbial inoculants can increase fiber digestion (Rice et al., 1990; Weinberg et al., 2007).

Effects of homolactic acid bacteria on animal performance. Relative to animal responses, Kung and Muck (1997) reported positive responses to microbial inoculants on intake, gain, and milk production (Table 5). The average response in milk production was a +1.36 kg per day in studies where milk production was statistically improved. Although literature summaries are encouraging, caution should be used when interpreting such data because all inoculants are not equal and the conditions (e.g. rate of application, inoculant viability, species of bacteria, crop, and moisture levels) varied markedly among the studies. As many have pointed out in the past, products with organisms with the same name are not necessarily the same organism and may not have the same effectiveness (Dennis, 1992). For example, Rooke and Kafilzadeh (1994) reported that various strains of ^{ho}LAB improved silage fermentation but animal performance was improved by only 1 strain of organism. Probably the most impressive data set for a single

inoculant is that of animal experiments conducted using *Lactobacillus plantarum* MTD1. A summary of 14 lactation studies conducted in University and government research institutes in North America and Europe using resulted in an average increase of 4.6% (Moran and Owen, 1994). Improvements in milk yield were obtained with a variety of crops (grass, corn, and alfalfa) across a wide spectrum of DM contents (15 to 46% DM). Similarly, 19 comparisons among untreated silages and Moran and Owen (1995) summarized silages treated with MTD1 for beef cattle. Across all studies and types of forage, cattle fed inoculated silage inoculated with MTD1 ate 7.5% more DM and gained 11.1% more weight.

Unfortunately, there is no good way to predict the effectiveness of microbial inoculants. A model developed by Pitt (1990) suggested that inoculants would be most effective on alfalfa during cool conditions of first, third and fourth cuttings. However, there are numerous products that have little or no research to support claims of improved fermentation or animal performance. Another factor, which complicates the evaluating process, is that the majority of bacterial inoculants are repackaged for distribution under private label and numbers of bacteria may be low and/or other additives (e.g., enzymes, fermentation extracts, minerals) are included in the formulations.

Effects of homolactic acid bacteria on bunk life. Bunk life or aerobic stability improved in only 33% of the studies and in fact inoculation with ^{ho}LAB has, in many instances, made aerobic stability worse (Muck and Kung, 1997). This is probably due to a lower content of acetic acid and other potential antifungal end products. This finding is extremely ironic because, many producers buy microbial inoculants because they perceive an improvement in aerobic stability. The most recent thought from silage researchers suggest that there needs to be a compromise in silage fermentation end products such that recovery of nutrients in maximized that results in silages that are stable when exposed to air.

Propionibacteria. Several microorganisms that are not ^{ho}LAB have been used as silage inoculants specifically for the purpose of improving aerobic stability. For example, the *Propionibacteria* are able to convert lactic acid and glucose to acetic and propionic acids that are more antifungal than lactic acid. Florez-Galaraza et al. (1985) reported that addition of *P.*

shermanii prevented the growth of molds and markedly reduced the initial population of yeast in high moisture corn where the final pH was greater than 4.5. Weinberg et al. (1995) saw little benefit from adding *Propionibacteria* to pearl millet and corn silage (final pH < 4.0) but reported improvements in the aerobic stability of wheat silage when the decline in pH was slow. Similarly, in 3 studies using laboratory silos, we (Kung et al., unpublished data) did not observed beneficial effects of *Propionibacteria* in corn silage (final pH 3.6 to 3.8). However, Bolsen et al. (1996) reported more propionic acid, lower yeasts and molds, and greater aerobic stability in corn silage (pH of 3.6) treated with *Propionibacteria*. Some concerns relative to the use of *Propionibacteria* that have not been adequately addressed are the loss of DM (from CO_2 production) and the fact that *Propionibacteria* have proteolytic activity. The primary reasons for the ineffectiveness of these organisms include the facts that they are strict anaerobes, they are slow growing, and they are relatively acid intolerant.

Heterolactic lactobacilli. Contrary to past thinking, new research suggests that heterolactic acid bacteria may also be useful as silage inoculants when aerobic stability is a problem. Muck (1996) first suggested that *Lactobacillus buchneri* could improve the aerobic stability of silage. Driehuis et al. (1996) reported that corn silage treated with *L. buchneri* was more stable than untreated silage. They suggest that improved aerobic stability was due to the ability of *L. buchneri* to ferment lactic acid to acetic acid and 1,2 propanediol (Oude-Elferink et al., 1999). In a meta analysis, Kleinschmit and Kung (2006) reported significant improvements in aerobic stability when silages were treated with *L. buchneri.* Mari et al. (2009) collected samples from farm silos and showed that corn silages treated with L. buchneri had greater populations of this organism, fewer yeasts and greater aerobic stability over corn silages that had not been treated. Recently, Nsereko et al. (2008) developed a strain of *L. buchneri* capable of producing ferulic acid esterase which when used as a silage inoculant has the potential to improve fiber digestion in silages. However, digestion of NDF was not been consistently improved in several studies (Kang et al., 2009; Hofherr et al., 2008).

Inoculation Rate, Use, and Storage of Inoculants. The organism(s) from microbial inoculants must be present in sufficient numbers to effectively dominate the fermentation. The most commonly recommended inoculation rate for homolactic acid based-inoculant results in a

final concentration of 100,000 (or 1×10^5) colony-forming units of this organism per gm of wet forage. There is limited evidence to support the suggestion of some that doubling or tripling this amount (e.g. 200,000-300,000 cfu) is more beneficial.

Most microbial inoculants are available in powder or granular form. Inoculants applied in the dry form are often mixed with calcium carbonate (limestone), dried skim milk, sucrose or other carriers. These products can be applied by hand or by solid metering devices as per manufacturer's recommendations. Inoculants to be applied in the liquid form come as dried powders and are mixed with water just prior to use. (Use of chlorinated water may be detrimental to the inoculant if levels exceed more than 1.5 to 2 ppm.) Application can be with a simple watering can by weighing the incoming forage load and adjusting application based on the average unloading time. A better method is to use a metered liquid sprayer to evenly disperse the inoculant on the forage. Unused liquids should be discarded after a period of 24 to 48 h because bacterial numbers begin to decline. Water in inoculant tanks should not be allowed to increase over about 37°C because high heat will kill the inoculants (Mulrooney and Kung, 2008).

Kung (2009) reviewed a wide variety of causes that might cause a microbial inoculant to fail. Correct application rate and adequate distribution throughout the forage mass was highlighted as major factors that could cause failure of a microbial inoculant. However, application to forage at the chopper is highly recommended in order to maximize the time that microorganisms have in contact with fermentable substrates. Application at the chopper is more important if silage is being stored in a bunk or pile because it is difficult to achieve good distribution onto silage from a forage wagon. Distribution of the inoculant is less of a problem if it is applied at the blower of an upright silo or at the bagger. Throwing a can of dry inoculant onto a load of forage and hoping for even distribution is not an acceptable practice! Inoculants can be applied in a liquid or solid form. Data from our lab (Whiter and Kung, 2001) suggests that on higher DM silages (greater than about 45% DM), using a liquid based inoculant is preferable because the low moisture in these silages limits fermentation. Inoculants applied in a liquid form may be more advantageous because the bacteria are added with their own moisture to help speed up fermentation.

Storage is an important aspect of a high quality inoculant that contains live microorganisms. Some inoculants require refrigeration or freezing for optimum storage. Those that do not require cold temperatures for storage should still be kept in cool, dry areas away from direct sunlight. Moisture, oxygen and sunlight can decrease the stability of inoculants resulting in lower viable counts and a product that does not meet label guarantees. Opened bags of inoculants should be used as soon as possible and, if not completely used, probably not carried over into the next season.

Conclusions

Silage additives can be useful tools to improve silage quality and animal performance; however, they are not replacements for good management practices. The question of which additive to use can sometimes be a difficult one. Table 6 shows some suggestions for use of silage additives. Cost of the product should not be the most important factor when choosing an additive! Proof of efficacy and cost should be considered together. Why buy a cheap additive that is ineffective? In contrast, the most expensive additive might not be the best either. How should one evaluate a silage additive? In my opinion, the three major issues that are relevant in North America for choosing an additive include a broad and extensive data base (proving efficacy under a broad range of conditions, crops, moistures, etc.) that 1) supports improvements in animal production, 2) supports improvements in DM or nutrient recovery, or 3) supports improved aerobic stability. Finally, choose an additive from a reputable company that stands behinds their products and offers excellent technical service support.

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

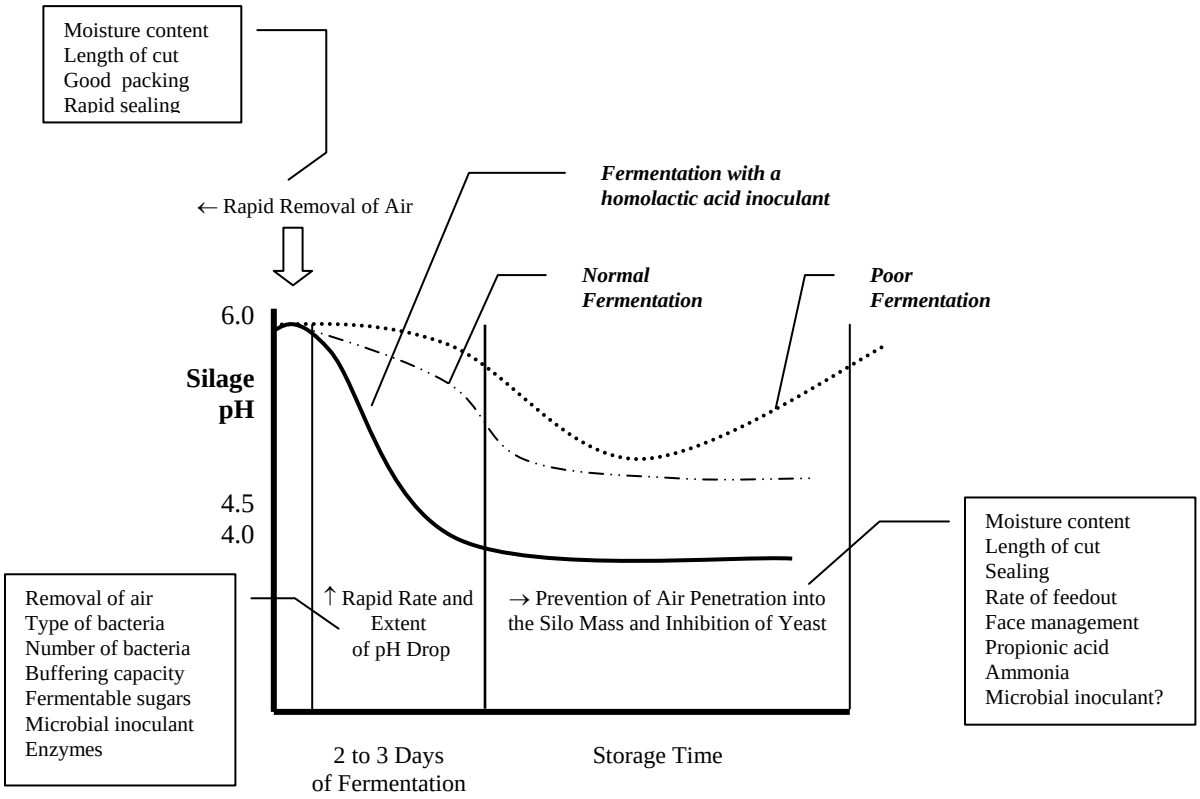

Table 1. Common end products of silage fermentation.

Item	Positive or Negative	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.

Table 2. Predominant fermentation pathways in silage.

Type of fermentation	End-products	Theoretical DM recovery, %	Theoretical Energy Recovery, %
Homolactic (glucose)	lactic acid	100	99
Heterolactic (glucose)	lactic acid, ethanol, CO_2	76	98
Heterolactic (fructose)	lactic acid, acetate, mannitol, CO_2	95	99
Yeast (glucose)	ethanol, CO_2	51	99
Clostridia (glucose and lactate)	butyric acid, CO_2	49	82

Table 3. Some of the more common bacteria used as silage inoculants and some reasons for their use.

Organism	Type of Organism	General Reasons for Addition	Primary End Products
Lactobacillus plantarum	Lactic acid bacteria, traditionally considered homolactic but now classified as heterolactic	-rapid production of lactic acid -relatively acid tolerant	Lactic acid
Pediococcus sp.	Lactic acid bacteria, homolactic	-rapid production of lactic acid -faster growing than lactobacilli -some strains show good growth at cooler temperatures -some strains have good osmotolerance	Lactic acid
Enterococcus faecium	Lactic acid bacteria, homolactic	-rapid production of lactic acid -faster growing than lactobacilli	Lactic acid
Propionibacterium sp.	Propionibacteria	-production of antifungal compounds	Propionic and acetic acids, CO_2
Lactobacillus buchneri	Lactic acid bacteria, heterolactic	-production of antifungal compounds -ferulic acid esterase for improved fiber digestion	Lactic and acetic acids, propanediol, CO_2

Table 4. Theoretical effect of adding a microbial inoculant containing homolactic lactic acid bacteria on the end products of silage fermentation.

Item	Theoretical Effect
DM recovery	Greater recovery
Rate of pH decline and final pH	Faster decline and lower final pH
Ammonia nitrogen	Lower content
Lactic acid	Greater content
Acetic acid	Lower content
Butyric acid	Lower Content
Ethanol	Lower content
Fiber (NDF/ADF)	Increased
DM digestibility	Increased
Animal performance	Increased

Table 5. A summary of animal responses to microbial inoculants between 1990 and 1995.

Type of Study	Intake	Gain	Milk Production
Number of Studies	67	15	36
Studies with Positive Responses	28%	53%	47%

(Kung and Muck, 1997)

Table 6. Some suggestions for use of silage additives.

Item	Additive of choice
1) Consistently make good quality silage. 2) No significant heating problems.	1) Homolactic acid based inoculant.
1) Consistently make good quality silage. 2) Some heating problems during warm weather.	1) Homolactic acid based inoculant and 2) Buffered propionic acid preservative added to TMR at feeding.
1) Usually make good quality silage. 2) Spoiled or hot silage usually only at silo opening and when feeding out the last silage from silo.	1) Homolactic acid based inoculant and 2) Buffered propionic acid preservative or new microbial inoculants designed to improve aerobic stability on several first and last loads into silo.
1) Consistently have problems with heating silage. 2) Inadequate daily removal of silage leading to hot feed.	1) Buffered propionic acid preservative at ensiling or 2) New microbial inoculants designed to improve aerobic stability.
1) For bunk, pit, or drive-over silos, significant spoilage on top layer even after covering.	1) Buffered propionic acid preservative or 2) New microbial inoculants designed to improve aerobic stability only on last loads into silo.
1) Extremely dry forage or forage chopped too long	1) Buffered propionic acid preservative or 2) New microbial inoculants designed to improve aerobic stability.
1) Silage that is moved, silage for selling, silage fed from intermediate feeding piles	1) Buffered propionic acid preservative or 2) New microbial inoculants designed to improve aerobic stability.

Optimization of the animal production system based on the selection of corn cultivars for silage

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Introduction

Originally from the tropics, specifically Mexico and Guatemala, corn belongs to the C4 plants group, and is traditionally the most used plant for silage production. There are several aspects that can contribute for this, such as, high dry matter production, optimum dry matter content at harvesting, at least 3% of soluble carbohydrates on an as-is basis, lower buffering capacity, and besides that, adequate microbial fermentation.

The use of modern corn cultivars which are more productive, environmentally adapted, structural and physiologically more efficient, are considered the characteristics responsible for the effective gains in production of these plants. However, despite corn silage is extensively known, still is common miss concepts related to cultivars selection, cultural managements, harvesting time, and ensilaging steps, where the quality of final product usually is impaired.

The structural composition has direct influence in the quality of corn plant. Its fraction variations, due to genotypic and phenotypic factors, have direct consequences on production and plant composition. Thus, to produce good corn silage, beside the amount of grains on the ensiled mass and the high productivity, the proportion of other plant fractions and their fiber digestibility are also very important.

To target elevated dry matter production and adequate quality, it is necessary to know, through the maturation period, the improvements in nutritive value due to the higher grain participation in the plant, and the losses accounted for the reduction in stem digestibility,

pondering up to get positive balance on the digestible nutrients production. The main idea of these aspects is to achieve high quality final products, which could be responsible for improvements on animal performance on several production systems, independent if they are based on milk or beef production, and its economic feasibility, as well.

Thomison (2008) consider five steps in the selection of corn hybrids:

1) Select hybrids with maturity ratings appropriate for your geographic area or circumstances;
2) Choose hybrids that have produced consistently high yields across a number of locations. Hybrids will perform differently based on region, soils, and environmental conditions;
3) Minimize stalk lodging, choosing plant hybrids with good standability;
4) Select hybrids with resistance and/or tolerance to stalk rots, foliar diseases and ear rots;
5) Never purchase a hybrid without consulting performance data;

1. Corn hybrids

The current market shows great diversity of materials, with specific characteristics to attempt regional requirements, taking in to account the environmental conditions, soil type, expected productivity, and resistance to pests and diseases.

The corn plant shows different vegetative cycles, with genotypes extremely early, where the pollination can occur 30 days after emergence, or genotypes which cycle can target up to 300 days. In Brazilian conditions, the cycle varies between 130 and 150 days for normal materials, 120 days on early materials, and between 105 and 110 for those that are the earliest (Fancelli & Dourado Neto, 2000).

Normal cycle materials usually show higher vegetative development with higher number of leaves below the ear (10 to 12). However, on early materials, this number is between 6 to 8 leaves, which gives to this group higher photosynthetic efficiency, due to the higher light incidence on the lower leaves (Nussio, 1991).

Besides that, they are also more efficient to convert energy into grains, showing higher grain proportion on the mass (50% vs. 38%) compared with normal genotypes (Nussio, 1991;

Penati, 1995). According to some studies (Cruz & Pereira Filho, 2001), the maturity stage where the grains are on the hard farinaceous point, considered the ideal moment for the ensilage process, early materials show higher dry matter content than normal cycle genotypes.

Ettle & Schwarz (2003), comparing normal cycle material with stay green characteristic and an early cycle hybrid, observed that the normal cycle hybrid showed lower stem dry matter content (26 vs. 31%), and higher ear dry matter content (61.9 vs. 58.4%) for plants harvested at the same age, comprising 38 to 42% plant DM.

The type of cross is also important on corn hybrids selection. Single hybrids are uniform in plant and ear height, ear size, and disease tolerance, while the double crosses are the most variable for all traits. Single crosses have the maximum hybrid vigor, and thus the greatest yield potential, followed by modified single crosses, three-way crosses, and double crosses. Although some double crosses may yield better than some single crosses under certain circumstances, most modern single crosses can outperform double crosses even under unfavorable growing conditions (Carter, 1992). Pixley & Bjarnason (2002) did not observe differences in grain production of single-cross, three-way and double cross corn hybrids.

Considering the endosperm type, there are two mainly genetic groups: Flint (*Zea mays* ssp. Indentura) and Dent (*Zea mays* ssp. Indentata). On the Flint corn, the external endosperm layers are completely connected by the zein protein forming vitreous and extremely hard layers, while the more internal layers have a small proportion of farinaceous endosperm, showing non dented and rounded core.

The Dent group is characterized by the presence of certain amounts of zein, however, the corneous layers do not cover the whole endosperm surface, that gives vitreous aspect only on the lateral of the grain, and at the inner grain, shows farinaceous texture. When dehydrated, the central part of the grain hardens to get dented conformation (Brieger & Blumenschein, 1966). The Dent group represents the highest part of USA corn cultivars, while in Brazil, the Flint materials are predominant.

Pereira et al. (2004), evaluating the proportion of vitreous endosperm on Flint and Dent materials in three maturity stages (doughy, ½ milk line, and physiologic maturity), observed values around 59.9; 67, and 72.4%, for the Flint materials, and 38.2; 46.9, and 47.9%, for the Dent hybrids, respectively. \

2. Maturity

Corn silage quality is dependent of several production system steps, which can be divided in 3 mainly stages: pre culture establishment; plant establishment and growth; and ensilage. During the plant establishment and development, special attention should be given to the culture tracts, such as: fertilization, pest, diseases, and weeds control. Besides that, the harvest point determination is essential for appropriate forage fermentation in the silo.

The harvest point of corn plants for silage production directly affects forage production per area, quality, and silage intake. So it is responsible to determine the production levels to be reached and consequently economic results in each animal production system.

As the corn plant matures and reaches the blooming stage, nutrients are stocked in the grain, primary as sugars and later as starch. The plant maturation process is associated with dry matter content increase, starting with less than 20% at blooming to more than 40% when plant reaches physiologic maturity (Wilkinson et al., 1998). Although, corn plants show maturation gradient with variable rates and it is dependent of cultivars and edafoclimatic conditions (Oliveira et al., 2002 cited by Possenti et al., 2004).

The results have suggested the hard-farinaceous point as a reference to harvest plants, because it conciliates an adequate dry matter content to ensile the plants (32-35%), and permits a potential grain filling (6,000 kg/ha) and total plant dry matter accumulation (12,000 - 14,000 kg/ha). Moreover, the animal intake also is improved (Nussio et al., 2001)

According to Wilkinson et al. (1998), the intake of silages harvested with dry matter content higher than 35%, is reduced in 25% when compared to plants harvested with 30% DM.

The advantages of harvesting corn plants with dry matter content between 32 and 35% are: considerable increases in dry matter production per area; storage losses reduction, mainly by runoff production; and animal dry matter intake improvements (Cruz & Pereira Filho, 2001).

Actually, the scientific community has shown variable results, due to the large number of hybrids offered in the market. On this wise, the decision by the harvesting moment, should consider that the plant need to be harvested in a physiological stage where the NDF content is diluted by the progressive starch increase due to the grain filling. Grain filling and stem digestible losses are concomitants events, so historically, minimum dry matter digestibility

variation had been observed with the plant dry matter content increase, since the milky up to the hard grain stage.

To sum up, the ideal harvest point recommendation always suggested advanced plant physiological stage, where was possible to conciliate higher liquid biomass accumulation both in grain and in the whole plant; higher percentage of grain/ear, that suggests higher NDF dilution by starch, maintaining the TDN unaltered; higher dry matter content, favoring the fermentative process and potential intake by the animals (Nussio et al., 2001). Planting several hybrid maturities each year spreads the harvest season and reduces the weather risks and is optimum for timely harvest management (Carter, 1992). The author recommends a hybrid maturity mix of 25% short-season, 50% medium season, and 25% full-season hybrids.

3. Productivity

Corn shows maximum dry matter production before the highest grain dry matter accumulation. The greatest dry matter production occurs when grains are at the semi-hard stage, while maximum grain dry matter accumulation occurs when the black layer is formed, which often occurs with grain hardening (Ferreira, 2001).

Corn hybrids, with early cycle, show lower dry matter production (12,270 vs. 14,945 kg/ha) and grain production (5,978 vs. 6,721 kg DM/ha) than normal cycle hybrids (Russel et al., 1992). Furthermore, according to Penati (1995), usually, in adversely environmental conditions, production damages in early hybrids cycle are higher than those in normal cycle materials.

Corn hybrids production varies between 26,000 and 75,300 kg/ha fresh basis (Costa et al., 2000; Beleze et al., 2003; Neumann et al., 2006), while dry matter production reaches values between 8,470 and 35,900 kg/ha, depending on the cultivar and harvesting time (Silva et al., 1999; Filya, 2004; Jaremtchuk et al., 2005; Baron et al., 2006).

Fiber fractions production (stem, leaves and bracts) and grains are also important for the evaluation of corn hybrids for silage production, and varies between 4,700 to 11,750; and 4,000 to 12,700 kg DM/ha, respectively (Tolera et al., 1999; Farinelli et al., 2003; Jaremtchuk et al., 2005; Ferreira et al., 2006). The stem fraction reached productions between 2,380 to 3,620 kg DM/ha in a hybrid evaluation study conducted by Beleze et al. (2003) for plants harvested between 20.74 and 48.27% DM.

Nussio (1997) evaluated the plant fiber fraction influence of different corn cultivars on the dry matter production per hectare, and on the *in situ* digestibility of plant fractions. The author observed that the selection of corn hybrids for silage production, based mainly on the dry matter production, should be revised due to the variable production potential of the available materials, and also due to the wide dispersion between agronomic and qualitative characteristics. Consequently, the importance of information about the genotypic origin of corn hybrids, and the quality of the material to be ensiled as well, is gaining space in this scientific field.

Some hybrids perform at consistently high levels over many sites while others may not perform equally well under variable conditions. Superior hybrid performance over several locations and years is the best prediction of superior future performance (Carter, 1992).

Different participations of plant components can result in similar plant dry matter production (Penati, 1995). Therefore, it is clear the relevance of the analysis of corn plant production and the quality of its fractions on the evaluation programs of corn silage hybrids, aiming to join good production and digestibility of the whole plant.

4. Morphological composition

In despite of being used as a forage source, the factor that is highlighted on the evaluation of corn silage quality is the grain percentage in a dry matter basis (Silva et al., 1999). Grain proportion has been recognized as an adequate criterion on silage material selection because there is a relationship between potential grain and whole plant dry matter production (Nussio & Manzano, 1999).

On the other hand, the importance of grain participation, as the main factor responsible for corn silage quality, was questioned by Hunter (1978), who observed genotypic variation on plant fiber fraction quality, expressed by the dry matter intake and forage digestibility. These values were independent of grain proportion (DM basis) what suggests important contribution of fiber on forage quality.

A study conducted by Lauer et al. (2001) showed that, in the last 70 years, the improvements in corn silage production and quality is due to grain production increase, with small changes in cultivars fiber fraction, moreover, they reinforce the importance of improvement programs based on productivity and quality of this fraction.

The different plant fractions contribute distinctly to the dry matter production, and its variation can be attributed to the genotype as well as plant maturity stage. Santos et al. (2002) observed lower correlation, positive and significant ($P<0.05$) between grain productivity and plant height ($r^2=0.51$), and ear insertion height ($r^2=0.52$). Actually, corn plant has shown height variation between 1.6 to 2.65 m, with ear insertion around 0.78 to 1.43 m (Santos et al., 2002; Farinelli et al., 2003; Jaremtchuk et al., 2005; Neumann et al., 2006).

Additionally to the corn plant fractions participation on the silage production, the quality of these fractions is also a determinant factor on the corn hybrid selection.

5. Nutritive value

The great nutritive value of corn plant, characterized by high digestibility or energy density, determines the excellence of this plant, and generally, this is the attribute that qualifies it as an option for the animal production systems. Whole plant composition can be observed on the summary data showed in Tables 1 and 2.

Actually, modern corn hybrids had reached harvest point early and with higher starch content, one of the main breeders objectives. However, this occurred at the expense of lower cell wall digestibility, and with little effect on the organic matter digestibility (Givens & Deaville, 2001).

Table 1 – Dry matter content (DM), ether extract (EE), crude protein (CP), starch, and *in vitro* true digestible dry matter (IVTDDM) of corn plants for silage production

Author	DM	EE	CP	Starch	IVTDDM
			%		
Russell et al. (1992)	34.8 - 51.4	-	6.9 - 7.6	-	71.9 - 74.8
Coors et al. (1997)	30.0	-	7.5	-	77.4
Thomas et al. (2001)	36.9 - 41.3	3.5 - 3.6	7.7 - 9.6	15.2 - 18.3	77.8 - 79.9
Ballard et al. (2001)	27.3 - 33.7	2.2 - 2.6	6.6 - 6.8	12.6 - 14	73.7 - 79.2
Filya (2004)	21.1 - 42.0	-	-	-	-
Lewis et al. (2004)	28.0 - 42.0	-	7.1 - 8.4	11 - 22	82.5 - 90.7
Kung et al. (2008)	33.7	-	7.1	29.74	-

Table 2 – Cellulose (CEL); Hemicellulose (HEMI); Neutral detergent fiber (NDF); Acid detergent fiber (ADF), and NDF digestibility (NDF-dig) of corn plants for silage production

Author	CEL	HEMI	NDF	ADF	NDF-dig
			%		
Russell et al. (1992)	-	-	43 – 50	24 - 27	-
Coors et al. (1997)	-	-	48.6	25.6	-
Thomas et al. (2001)	-	-	40 – 42	21 - 25	-
Ballard et al. (2001)	21 - 23	18 - 21	41 – 45	23 - 26	44 – 49
Filya (2004)	20 - 33	19 - 20	44 – 56	24 - 35	-
Lewis et al. (2004)	-	-	39 – 46	-	56 – 79
Kung et al. (2008)	-	-	42.91	25.84	51.7

In despite of the dry matter production increases when plants are sowed with high density, the same behavior is not observed for the *in vitro* true digestible dry matter (IVTDDM). Cusicanqui & Lauer (1999) observed dry matter digestibility decreases of 0.35 g/kg for each increase of 1,000 plants/ha, explained by the improvement of plant NDF and ADF content when the population increases.

Table 3 shows some nutritive value results from plant fractions found by some authors.

Table 3 – Fraction compositions of corn plants for silage production

Plant fraction[1]	DM	CP	NDF	ADF	IVTDDM	NDF-dig
			%			
Stem	22 - 26	3.0 - 6.3	57 - 81	44 - 60	26 - 63	20 – 39
Leaves	25 - 43	8.6 - 14.6	56 - 74	33 - 44	55 - 77	55 - 65
Bract	25 - 40	3.6 - 6.4	72 - 82	-	58 - 71	51 - 62
Cob	32 - 43	2.5 - 3.4	78 - 88	-	42- 63	37 - 56
Grain	41 - 59	10.7 - 12.0	11 - 14	3.3 - 3.5	98 - 99	77 - 90

[1]Russell (1986); Philippeau et al. (1999a); Thomas et al. (2001); Zeoula et al. (2002); Zeoula et al. (2003a); Zeoula et al. (2003b); Rosa et al. (2004); Neumann et al. (2006) and Borstmann et al. (2006)

In the last years, some corn hybrids had been developed specifically for silage production. The focus has been on the fiber digestibility improvement, assuming that this can result in dry matter intake increases, and consequently gains in milk or meat production (Thomas et al., 2001). Weiss (2001) observed that cows consumed more DM when fed diets that contained forages with high *in vitro* NDF digestibility.

Philippeau & Michalet-Doreau (1998), in a corn hybrids study with Dent and Flint endosperm, observed that, for non-ensiled material, effective dry matter degradability did not differ between treatments. On the other hand, the genotypic differences were significant based on starch degradability. For non-ensiled material, the effective starch degradability was 72.3 and 61.6% for Dent and Flint genotypes, respectively. For the ensiled material, effective starch degradability was 78.6 and 67%, respectively.

The lower starch degradability for Flint genotypes on *in situ* studies was probably due to the lower rapidly degradable fraction proportion, lower degradation rate, or from both effects (Philippeau et al., 1999a). The difference on starch ruminal degradability could be related to the vitreous endosperm content that showed little farinaceous endosperm proportion.

With the lower starch fraction digestibility, the hypothesis of "dilution" may not always be occurring, leading to the lower digestibility of plants harvested on advanced maturity stage.

It is also important to highlight that, in despite of evident starch degradation superiority for the Dent genotype, usually, the same slope is not applied for the vegetative plant portion. Some current studies suggest that whole plant nutritive value should not follow the reduction in starch digestibility, independent of the analyzed material (Dent or Flint), because there is a compensation on the vegetative portion (Nussio et al., 2001).

The variability observed for ruminal starch digestion in corn cultivars with different endosperm, suggests that the genetic selection can manipulate the local and extension of starch digestion in ruminants (Philippeau et al., 1999b). Ruminal and post ruminal starch digestibility can vary widely among feedstuffs. However, intestinal starch digestion is always compensated by reductions in its ruminal digestion, so that total tract digestion variation could be relatively small (Huntington, 1997; Taylor & Allen, 2005).

The site of starch digestion alters the final products nature (volatile fatty acids in the rumen, and glucose in the small intestine), and finally, its metabolic utilization efficiency by the ruminant (Philippeau et al., 1999a). These authors observed effective dry matter and starch degradability, closed to 42.3 and 55.8; 46.2 and 61.9% for Flint and Dent genotypes, respectively. The highest Dent genotype degradability is explained by the higher degradable rate, and higher starch fraction rapidly degradable than those observed in Flint genotypes. As a result, forage and energy intake should be optimized based on the dry matter content and starch concentration as well, without compromising nutrient digestibility.

A study evaluating the influence of corn endosperm type on nutrient digestibility in lactating dairy cows showed that total-tract starch digestibility was greater (+6.3%) for diets that contained floury and opaque corns, compared with vitreous corn diets. In contrast, apparent total-tract NDF digestibility was lower for floury (-8.4%) and opaque (-0.4%) diets (Lopes et al., 2009).

Wolf et al. (1993) observed that ear proportion and plant fiber fraction production have stronger associations on whole plant digestibility and cell wall composition than on grain production. However, they suggested that the improvements on fiber fractions should be targeted without sacrificing grain production.

Penati (1995) observed that cell wall components (NDF, ADF, cellulose, hemicelluloses and lignin) are responsible for negative effects on plant digestibility, highlighting the importance of stem and leaves fractions analysis, when high quality silages are expected.

In this context, Nussio & Manzano (1999) suggested that quality prediction models, for corn cultivars selection programs that aim silage production, should be established based on two mainly factors: grain percentage on the ensiled mass (% DM), and nutritive value of stem-leaves portion (% of the *in vitro* true digestible dry matter) as well.

According to Tovar-Gomez et al. (1997), a considerable share of the animal responses associated with distinct corn genotypes had occurred due to differences in ruminal plant cell wall degradability, highlighting so, the importance of knowing this component quality. As a result, corn silage hybrids improvement programs that aim gains on fiber fraction quality should consider the NDF digestibility as an important criteria for hybrids selection (Lauer, 2003).

6. Cutting height

Increasing the cutting height of corn silage decreases silage yield. Corn plant parts contain different amounts of fiber and digestible energy. Raising the cutter bar on a silage chopper will leave more of the lower corn stalk in the field, which is typically higher in fiber and lower for digestible energy. According to Lauer (2001), corn silage yield decreased 15% as the cutter bar was raised from 15.2 to 45.7 cm above the soil surface. Neylon and Kung (2003) observed reductions of 5-10% in the dry matter production in plants harvested at 45.7 cm height, when compared to plants harvested at 12.7 cm height.

In a study evaluating high (42 cm) and normal height cut (20 cm), Restle et al. (2002) observed reductions in the dry matter (-6,7%), and NDF (13,24%) content, and increases in the IVTDOM (+13,77%), and in the digestible energy (+11,8% - Mcal/kg DM) in the high cutting treatment. However, the dry matter production was reduced in 6,7% (10,945 vs. 10,207), and the dry matter intake, daily weight gain and feed conversion were not affected.

According to Bernard et al. (2004), increasing the cutting height from 10.2 to 30.5 cm, did not improve silage quality or milk yields of cows. Corn silage harvested at 30.5 cm had lower (-5.3%) concentration of ADF, but no differences were observed in concentrations of NDF or digestible NDF.

Wu and Roth (2005) summarized 11 studies that evaluated low (17.8 cm) versus high (48.2 cm) cutting height and observed reductions in ADF (-10.2%), NDF (-7.4%), forage yield (-7.4%), and increases of DM (+6%), CP (+2%), starch (+5.9%), digestible NDF (+4.7%), and DM digestibility (+2.5%).

Kung et al. (2008) observed that high cutting of a normal corn hybrid increased concentrations of DM (+4%), CP (+5%), NE_L (+3%), and starch (+7%), but decreased the concentrations of ADF (-9%), NDF (-8%) and ADL (-13%). The NDF digestibility was not affected by the high harvest height.

7. Cultivars evaluation models

Besides the agronomic characteristics evaluation from current market corn cultivars, animal performance studies are extremely important, and to do it, some productivity indices are created aiming to evaluate these factors together.

Corn hybrids selection based on plant IVTDDM and total dry matter production as well, sounds a reasonable way on the high production and quality hybrids selection (Hunter, 1978). Recent models of corn cultivars for silage consider factors, such as dry matter production per hectare associated with plant dry matter digestibility, or with digestible dry matter production per hectare (DDMP/ha) as a criteria for material selection.

Ballard et al. (2001) found digestible dry matter productions between 11,000 and 11,700 kg/ha for early hybrids (94 to 95-d relative maturity), while in higher spacing (0.9 vs. 0.76 m), Costa et al., (2000) observed DDMP/ha between 5,540 and 8,560 kg/ha.

Plant dry matter content affects total dry matter production (Russell et al., 1992; Ballard et al., 2001; Wilkinson & Hill, 2003; Filya, 2004) and forage digestibility as well (Bal et al., 1997; Zeoula et al., 2003b; Lewis et al., 2004), and therefore, also has influence on plant DDMP. In a study comparing corn hybrids for silage, Thomas et al. (2001) got digestible dry matter productions closed to 11,400 kg/ha for materials harvested with 49.2% DM against 11,000 kg/ha for plants harvested with 53.9% DM. Paziani et al. (2009) observed that the digestible dry matter production (kg/ha) was affected by dry matter and grain production, and plant and stem digestibility as well

Nutrient composition data and *in vitro* NDF digestibility should be used to estimate economic value of the production system. Corn silage is a major source of both NE_L (net energy for lactation) and eNDF (effective NDF) and these components should be part of the hybrid selection process (Undersander et al., 1993; Weiss, 2001)

Therefore, the possibility of knowing silage corn hybrids behavior throughout its development, based on production evaluation and plant nutritive value as well, allows the ideal harvest moment decision for each material. Thus, it guarantees adequate fermentation, which enables a quality feedstuff, and consequently, results in better animal responses allowing technique-economic feasibility on this important production system phase.

8. Conclusions

The optimization of the animal production system based on the selection of corn cultivars for silage is achieved when producers consider the performance of hybrids over multiple sites or multiple years within a given region.

Information of both silage yield and digestibility of the whole plant and fiber, are needed when selecting hybrids. The digestible dry matter production is an effective measure that reaches most of the quality aspects of corn cultivars, because variables such as digestibility of several fractions and productivity of the whole plant are in accordance of this estimation. As a result, it is a versatile tool on the corn cultivars evaluation. However, sometimes companies do not have this information, so the formula below can help in this situation. It also can be used as a tool to evaluate the efficiency and get predictable values for the production system.

- ✓ Digestible DM production (kg/ha) = Plant digestibility (%) x DM production (kg/ha)

The cultivar evaluation based on productive aspects should be utilized after supplied some basic recommendations, such as the cultivar requirements on determined region, soil type, rainfall, etc. So, the digestible dry matter production should be used as a fine adjustment to figure out or help on the final decision.

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Strategies to enable the use of legume silage in ruminant production

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1. Introduction

The Brazilian livestock is based on pastures use, the ones which represent the more practical and economical way of ruminant feed. Despite having the biggest commercial herd in the world, approximately 200 millions of cattle, the productivity indexes obtained in Brazil in most of animal production systems in pasture are still low. This low productivity is related to the season pattern in feed supply which comes from pastures, responding to climatic changes, resulting in crop period and intercrop period of animal products.

Regarding to this, it shall proceed to the adjust between forage demand and supply, by an adequate feed planning, aiming to diminish the forage shortage in the dry period, making it possible this way, the uniform animal production all year long. The use of ensiled forage is a feasible alternative to guarantee the high quality forage supply, during the food shortage period. In this context, the sorghum and corn crops has been stood out as the most used species in ensiling process, by its ease of cultivation, high productivity and especially by the produced silage quality. However, recently, the interest for the use of legume silage in animal feeding system has been studied in several countries, being that, in Brazil, the soybean silage *(Glycine max L.* Merrill*)* has been of bigger interest.

It is aimed with the present text to discuss some aspects referring to the legume use as silage, to the fermentative pattern in legume silages, and to cattle feed supplementation with legume silages, as well as to show data related to the bioeconomic feasibility of legume silage use in ruminant feeding, pointing out the soybean silage.

2. Legume in the ensiling process

The legume use in pasture areas promotes increments in animal production, by the increase of nutritive value and forage supply resulting not only from the participation of legume in the animal diet, but also by indirect effects related to nitrogen biologic fixation. However, although these benefits, its participation in animal production system in pasture in our country is still insignificant, being the low persistence of legumes in pastures considered as the main cause of little use by the producers.

As for the use for silages production, historically, the legumes have always been considered inadequate to ensiling, due to high buffering capacity, low soluble carbohydrates and dry matter contents. Besides these restrictive characteristics to the fermentative process, it is important to stand out that the legumes in general present lower productivity of dry matter when compared to tropical grass. These factors together explain the reason of the low use of legumes as silage in tropical countries. However, these characteristics undesirable to fermentative process can be overcome by the additive use and forage wilting before ensiling.

The first studies about legume silage in Brazil are from the seventies (Faria et al., 1971; Tosi et al., 1973), being then approached in the lecture about Forage Preserving, approached by Dr. Tosi, in the 1º Symposium about Pasture Management, in 1973, in the city of Piracicaba, São Paulo. Farias et al. (1971) evaluated silages of perennial soybean (*Neonotonia wightii*), centrosema (*Centrosema pubescens*, Benth) and *Macroptilium atropurpureum* cv. Siratro and concluded that the silages of the three legumes were low quality, based on the high pH value and in the low lactic acid content. In the 80s or 90s the main goal of the studies was to increase the protein content of corn silages by consorting this grass with legumes of annual cycle, cultivated under different cultural arrangement (Evangelista et al., 1983; Obeid et al., 1985, 1992a,b). Actually, the consorted silages resulted in higher food intake and weight gain

of animals compared to exclusive corn silage, due to higher protein content in corn x soybean consorted silages (Zago et al., 1985; Obeid et al., 1992b).

However, the corn x legume consort presents some limitations, highlighting the competition for light among species, the difficulty of these species to present the adequate growth stage to cut at the same time, besides the limits for fertilizations and weed control, since these ones have anatomic and physiologic differences, and also in the type and quality of fertilizer necessary for its adequate nutrition.

Knowing this, the exclusive legume planting, especially soybean, has been an interesting option by presenting high dry matter production per area, besides a higher facility in cultural practices and in harvest. In fact, more recently, it has been seen a growing interest in soybean cultivation for silage production in many countries, such as United States (Griffin, 2000; Blount et al. 2003; Seiter et al., 2004), Canada (Johnston & Bowman, 2000, Bello-Pérez et al., 2008), United Kigdom (Koivisto et al., 2003), Costa Rica (Tobia & Villalobos, 2004), Argentina (Castro & Andreo, 2008) and Brazil (Keplin, 2004; Melo Filho et al., 2005; Pereira et al. 2007 a,b; Rigueira, 2008). The interest for the soybean cultivation as forage is partly due to an improvement of ensiling process technology as well as the appearance of new varieties, with distinct length cycles, developed especially for forage. Nevertheless, the available information about silage production and use of this legume in ruminant feed are still scarce.

The new varieties are the results of old varieties crossing developed for haymaking with modern varieties developed for grain production, resistant to diseases such as root rot, caused by *Phytophtora*. From these crossings, some characteristics like plant height, shoot formations, lodging resistance, pods number, canopy, were selected, producing lineage of almost two meters high (Devine & Hattley, 1998; Devine et al., 1998 a,b). Pioneer work in soybean improvement for forage in Brazil was made by Melo Filho (2006), who evaluated 20 soybean variety, two lineage and 11 F2 segregate population originated from varieties crossing with the referred lineage, aiming to select progenitors and segregate lineage, for the use in improvement programs directed to silage production. In Brazil, technologies were developed which make the soybean cultivation possible all over the national territory, appearing new varieties and cultivars adapted to different soil, temperature and humidity conditions.

3. Fermentative pattern in legume silages

The ensiling process principle consists in producing enough lactic acid quantity to inhibit the growth of undesirable microorganisms, maximizing the forage nutrients preservation. During the stages of ensiling process there are biochemical changes of the carbohydrate and nitrogen compounds of fresh forage, under the enzymatic activities of the plant and microorganisms. The soluble carbohydrates are converted in organic acids and gases, while part of the protein is degraded to non-protein components. The magnitude of these changes can affect the food intake potential of nutritive attributes in the ensiled material (Van Soest, 1994).

Although they present high nutritive value, in general, legumes have undesirable characteristics for the proper fermentation process such as high moisture content at the harvest moment, high buffering capacity, low soluble carbohydrates contents and tabulate and hollow stem, which prevent the complete air removal at the ensiling moment (McAllister et al., 1998). Besides that, because of higher crude protein (CP) of legumes compared to the grass, it normally presents high proteolysis extension in the ensiling process.

Between 7 and 87% of nitrogen compounds of legume silages is converted in non-protein nitrogen (NPN) in the silo (Papadopoulos & McKersie, 1983; Muck, 1987). The high proteolysis extension is presented as a potential depression factor of dry matter ingestion, efficiency of nitrogen compounds utilization and animal productive performance. Among the factors which affect the proteolysis extension, it is emphasized:

a) Specie: Remarkable differences are observed in the ensiled mass proteolysis among legume species. The proteolysis is high in alfalfa silages, intermediate in red clover silages (*Trifolium pratense* L.) and trefoil (*Lotus corniccuneata* L.) and low in *Onobrychis viciifolia* Scop. (Albrecht & Beauchemin, 2003). Between 44 to 87 % of CP in alfalfa silage was degraded in NPN in the silo, while the value for red clover silage varied from 7 to 40% (Papadopoulos and McKersie, 1983; Muck, 1987). The lowest value for clover silage was due to the action of polyphenol oxidase enzyme which converts o-diphenol, present in high concentration in clover, into reactive o-quinones (Jones et al., 1995). These compounds react rapidly with proteases and protein substrates, reducing the proteolysis extension in the ensiled mass (Hatfield & Muck, 1999).

The low proteolysis extension in the silo in legumes such as trefoil and *Onobrychis viciifolia* Scop is due to higher tannin content (Albrecht & Beauchemin, 2003). Tannins are secondary metabolic compounds of plants, composed by hydroxylated phenyl rings which might have esterification with simple sugar (hydrolysable tannins) or polymerization through carbon-carbon bounds (condensed tannins). These polyphenolic molecules have the capacity of making complexes with many nutritionally important substances, pointing out among them the proteins. Besides acting directly, making the nutrients not available, they can complex the enzyme, reducing or inhibiting its catalytic power, or bind to glycoproteins present in bacteria cellular envelop, making the nutrient transport for the interior of the cells difficult and, consequently, decreasing the microbial growth (Mcsweeney et al., 2001). This way, the protease action is reduced both in the silo and rumen.

b) Dry matter content (DM) of ensiled mass: Legumes in less advanced maturity stages, although having higher potential nutritive value, present higher proteolysis extension due to lower dry matter content. Merchen & Satter (1983) evaluated the composition of fresh alfalfa (20.8% DM) and dehydrate alfalfa containing 29, 40 or 66% DM, before and after the ensiling, and the effect on the digestion local, in dairy cows with mean milk production of 23.7 kg d^{-1}, fed with diets containing 65% alfalfa. A little effect was observed in the total nitrogen compounds content, but its composition was affected by DM content. After ensiling, the soluble N fraction increased 77% (407 to 721 g kg^{-1} of N), 64% (390 to 641 g kg^{-1} of N) and 13% (373 to 423 g kg^{-1} of N) in alfalfa with 29, 40 and 66% DM. Due to a reduction in the proteolysis and deamination in the silo with the increase of DM content, the non degradable protein intake in the rumen and the duodenal flow of non ammoniacal N were higher using alfalfa with 66% DM (357 and 920 g kg^{-1} of total ingested N) regarding to alfalfa with 29% DM (148 and 688 g kg^{-1} of total N ingested) and 40% DM (148 and 726 g kg^{-1} of total N ingested). However, the higher temperature in the ensiled mass with 66% DM, originated from the plant respiration, resulted in higher increase in ADIN (acid detergent insoluble nitrogen) fraction after ensiling, which implicated in lower total protein digestibility of alfalfa diet containing 66% DM (676 g kg^{-1}), compared to the alfalfa diets containing 29% DM (723 g kg^{-1}) and 40% DM (724 g kg^{-1}). There was no effect on the total digestibility of fiber in neutral detergent.

Tabacco et al. (2006) estimated reduction of 12.29 percentage units of NPN fraction (% of total N) for each increase of 10 percentage units in dry matter content in alfalfa silages without additive submitted to wilting. (Figure 1).

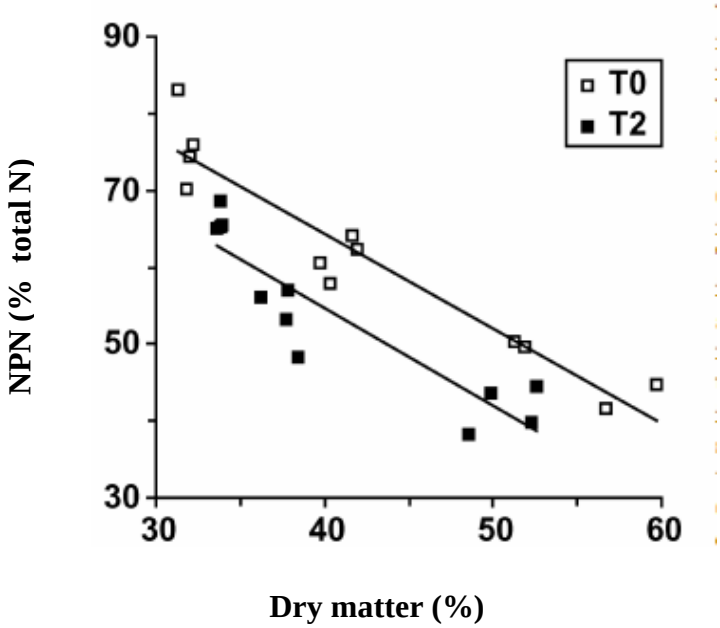

Figure 1 – Non-protein nitrogen content (NPN) of alfalfa silages without (T0) and with addition of 4% (dry matter basis) commercial hydrolysate tannin (T2), according to the dry matter (DM) of ensiled mass submitted to wilting. NPN-T0 (% total N) = 113 −1.229MS (r^2 = 0.90; MSEP = 4.1). NPN-T2 (% total N) = 105 – 1.269MS (r^2 = 0.81; MSEP = 4.8). PMSE = prediction mean square error.
Source: Tabacco et al. (2006).

c) Additive use: Some additives which inhibit protease activity of plant or bacteria have been effective in preserving protein fractions in legume silages with higher proteolysis potential, which can be highlighted the formic acid, formaldehyde, ammonium tetraformate (ATF) and commercial tannin (Nagel & Broderck, 1992; Tabacco et al., 2006; Broderick et al., 2007).

Nagel & Broderick (1992) observed that treatments with 2.8 g of formic acid/100g of DM or 0.31 g of formaldehyde/100g DM of alfalfa with 37% DM, decreased 32.5% and 17.6% the NPN fraction, regarding the alfalfa silage without additive (NPN of 430 g kg^{-1} from total N). According to the authors, the lower formaldehyde effectiveness to inhibit the proteolysis is because of lower application rate

or the inability of it to reduce the forage pH quickly. However, because of the high cost and corrosive power of formic acid, other additives have been preferred.

The ATF is a buffer containing 1 mol of ammonia and 4 moles of formate. This compound is less corrosive and easier to handle than formic acid and presents potential to reduce proteolysis in legume silages. Broderick et al. (2007) verified that the addition of 7 liters of ATF/ton of alfalfa silage (fresh basis) reduced in 9.2% the NPN fraction (453 vs. 499 g kg^{-1} of total N) and in 23.4% the N fraction of free amino acids (301 vs. 393 g kg^{-1} of total N), compared to alfalfa silage without treatment. Besides, when supplying isonitrogen diets (180 g kg^{-1} DM) and isoenergetic (1.58 Mcal of liquid energy for lactation kg^{-1} of DM) for Holstein cows containing 40% of alfalfa silage (DM basis) without or with ATF addition, the authors verified that the ATF addition increased the dry matter intake in 4.2%, milk production in 8.6%, 3.5% fat-corrected milk production in 6.9%, milk protein production in 11.1% and non fat solids production in 10.0%, without affecting the urinary N excretion, indicating the efficiency increase in the N usage.

The commercial tannin use in low levels (lower than 5%, DM basis) has been adopted as silages additives because of its potential effect of proteolysis reduction during the ensiling process (Tabacco et al., 2006). The available commercial tannins can be divided in two main groups: condensed and hydrolisate, with several industrial usages. The tannin extracted from chestnuts (*Castanea sativa* L.) is the most hydrolyzed tannin, while quebracho and acacia tree extracts are the main commercial condensed tannins.

Tabacco et al. (2006), when evaluating the addition effects of four levels (0; 2; 4 and 6%, DM basis) of commercial hydrolyzed tannin (with 92% DM and 77% pure tannin, DM basis) originated from chestnut, in the fermentation quality in laboratory silos and ruminal degradation, concluded that the 4% tannin level (DM basis) was the most effective in reducing the proteolysis in the silo (13.9% reduction) and the ruminal protein degradation rate, without affecting the intestinal digestibility of rumen undegradable protein, indicating a possibility to increase the efficiency of using dietetic protein.

3.1.Ensiling and fermentative pattern in soybean silage

The soybean, besides restrictive characteristics to the fermentative process inherent to legumes, already pointed out, present high ether extract content which can inhibit the bacteria in the ensiled mass, affecting the fermentation process, resulting in silages with high pH. It is added to this a low autochthon population of bacteria which produce lactic acid (Pereira et al., 2007b).

According to Undersander et al. (2007), the soybean harvest for ensiling can be made from stage R_3 (start of pod formation) to R_7 (start of maturity). However, Muñoz et al. (1983) recommend the R_6 stage (full seed) as the most appropriate for the soybean harvest, due to nutritive aspects (high protein value and high dry matter digestibility) associated to the high dry matter production. Besides it provides higher silage acceptability by the animal regarding the ones harvested in a younger stage (Coffey et al., 1995a). Nevertheless, even in this development stage, the soluble carbohydrates content required for an adequate fermentation is low (Blount et al., 2003). This way, addition of a sugar source readily fermentable (molasses e.g) and/or, microbial additive use, can help for an adequate fermentation, when soybean is ensiled alone (Pereira et al., 2007 a, b; Pereira et al., 2008).

Opposing other forages, the nutrient contents and the forage quality of whole soybean plants do not change drastically with the maturity advance because the seed is rich in protein and energy (Willms, 2003).

Coffey et al. (1995b) evaluated the nutrient composition of soybean silage, obtained from the average of two varieties of maturation group IV and V, for two years, at the R_2, R_4 and R_6 growth stages, and found values varying from 16.0 to 20.6%; 38.3 to 48.3% and 27.3 to 37.3% for crude protein, neutral detergent fiber and acid detergent fiber, respectively.

Decrease in the pH and increase in the acid lactic/acetic acid ratio with the advance of growth stage were observed by Muck et al. (1996), cited by Panciera et al. (2003), for a grain cultivar and two forage lineage, according to Table 1. The authors also report that the fermentative characteristics of the soybean silages resemble to the ones from alfalfa silage.

Table 1 – Characteristics of soybean silage thirty days after ensiling[1]

Types	Harvest stage	DM (%)	pH	Lactic acid (%)	Acetic acid (%)
PA (Forage)	R_1	32.1	5.60	2.87	3.45
OR (Forage)	R_1	35.9	5.17	5.16	3.12
FS (Grain)	R_2	37.8	5.88	2.40	3.13
PA (Forage)	$R_{2.7}$	31.1	5.29	4.37	3.42
OR (Forage)	$R_{3.3}$	31.6	5.16	5.02	3.14
FS (Grain)	$R_{5.7}$	32.7	5.22	4.85	2.56
PA (Forage)	R_3	30.3	4.93	6.21	2.99
OR (Forage)	R_4	34.3	4.86	5.97	2.56
FS (Grain)	R_6	38.6	4.96	3.00	1.21

[1] Muck et al. (1996) cited by Panciera et al. (2003)

Pereira et al. (2007a) evaluated gases and effluent losses, dry matter recovery and fermentative profile in soybean silages (SS) submitted to the following treatments: exclusive SS, SS with inoculants (SSI), SSI with 2.5 % powder molasses (SSIM) and SS with 25% powder molasses (SSM), using 20 liter buckets. The authors registered lower value ($P< 0.05$) of pH in silage treated with inoculants and molasses (Table 2), probably by soluble sugar supply which stimulates lactic fermentation, associated with inoculants presence, suggesting that there was higher growth of lactic acid bacteria. In fact, when evaluating microbial population of soybean silage in different fermentation periods, submitted to the same treatments above (non published data) it was observed population with higher number of lactic acid bacteria on the day-7 (8.94 log CFU/g) and day-14 (62 log CFU/g) of fermentation, for the SSIM and SSM silages, respectively, while in SS and SSI silages the maximum population of these microorganisms was only registered on the day-28 of fermentation.

Table 2- Mean values of pH, effluent (EL) and gases losses (GL), dry matter recovery (DMR), ammonia nitrogen:total nitrogen ratio ($N\text{-}NH_3$), and lactic acid (LA), acetic acid (AA) and butyric (AB) content of soybean silage (SS) treated with different additives.

Item	Silages				VC (%)
	SS	SSI	SSIM	SSM	
pH	4.69^{a}	4.66^{a}	4.43^{c}	4.55^{b}	1.19
$N\text{-}NH_3$ (% DM)	21.64^{a}	15.75^{b}	12.38^{c}	12.58^{c}	8.51
LA (% DM)	4.69	4.79	5.12	6.51	18.9
AA (% DM)	3.70^{a}	3.30^{a}	2.32^{b}	2.84^{ab}	15.15
AB (% DM)	0.1768	0.2666	0.0287	0.0703	90.43
GL (% DM)	12.97^{a}	10.16^{b}	9.05^{b}	9.56^{b}	12.8
Effluent (kg/t OM)	18.5^{ab}	19.29^{a}	14.73^{bc}	11.31^{c}	13.1
DMR (%)	86.2^{b}	88.8^{ab}	9.6^{a}	91.9^{a}	2.39

Exclusive SS, SS with inoculant (SSI), SSI with 2.5% molasses (SSIM) and SS with 2.5% molasses (SSM).
Means in the same row followed by different letters differ ($P < 0.05$) by Tukey test

It is recognized that the soybean and other legumes silages stabilize in higher pH, as mentioned before. Melo Filho (2006) evaluated the direct and indirect effects of DM, carbohydrate soluble in water, CP and $N\text{-}NH_3$, inherent to soybean plants silage, on the pH of the referred silage. The factor which has most influenced the pH variation was the $N\text{-}NH_3$ concentration (% of total N), presenting positive and significant correlation (0.730) and positive direct effect (0.652); higher therefore than the residual effect (Table 3). Indeed, silages that present high values of $N\text{-}NH_3$, usually, present high pH, indicating low quality fermentation of the ensiled material.

Table 3- Direct (underline) and indirect effects of inherent parameters of whole plants soybean silage on its pH*

	Correlation effect*						Total effect
Parameters**	DM	WSC	CP	$N\text{-}NH_3$	ADF	EE	gc
DM	<u>0.175</u>	0.109	-0.033	0.009	-0.184	-0.003	0.073
WSC	-0.07	<u>-0.273</u>	0026	-0.239	0.028	-0.058	-0.586^{+}
CP	0.042	0.051	<u>-0.136</u>	-0.023	-0.103	0.027	-0.142
$N\text{-}NH_3$	0.002	0.1	0.005	<u>0.652</u>	-0.023	-0.006	0.730^{+}
ADF	-0.089	-0.021	0.039	-0.042	<u>0.364</u>	-0.05	0.200
EE	-0.003	0.086	-0.02	-0.02	-0.099	<u>0.183</u>	0.127

* $R^2 = 0.76$, $P\varepsilon = 0.49$; ** DM, WSC, CP, N-NH3, ADF, EE, = contents of dry matter, water soluble carbohydrates, crude protein, ammonia nitrogen, acid detergent fiber and ether extract, respectively; gc = genotype correlation; +,Significant at 5%, through the bootstrap method, with 1000 simulations.
Source: Mello Filho (2006)

An interesting fact is related to the good aerobic stability of legume silages. Griffin (2001), evaluating research data from LEGSIL project (legume silage),

conducted in the period of 1997-2001, in four countries of the European Community, relates that from 264 legume silages evaluated, none have presented heating or fungal deterioration when exposed to air for four days, and that 90% of silages were stable for seven days. The mixture of legumes and grass (50:50) also produced silages with high aerobic stability, while 90% of exclusively grass silages deteriorated in four days. The author also points that these results regarding preservation and aerobic stability were confirmed in field experiments, using both big bales and bunker silo.

4. Optimization of legume silage based diets for cattle

4.1 Nutrition characteristics of legume silages

Legume forages normally present high nutritive value for ruminants due to high protein content, lower neutral detergent fiber content (NDF), higher ruminal degradation rate of potentially digestible NDF and higher physical fragility of NDF particles when compared to the grass ones, which promotes higher animal dry matter intake, in spite of the lower digestion extension of NDF (Allen, 1996; Oba & Allen, 1999). However, the high proteolysis extension during the ensiling process, associated to the low starch content in legume silages challenges the nutritionists to minimize potential losses of nitrogen compounds in legume silages based diets for ruminants.

Several nutrition strategies have been researched aiming to maximize the efficiency of nitrogen utilization in legume silage based diets, mainly alfalfa silages, such as the combination of legume silages with higher starch content silages (corn silage) (Dhiman & Satter 1997; Brito & Broderick, 2007), use of legume silages with lower proteolysis potential (Hoffman et al., 1997; Broderick et al., 2007), and the use of additives that inhibit proteolysis at the ensiling moment (Messmann et al., 1997; Nagel & Broderck, 1992; Broderick et al., 2007).

4.1.2 Alfalfa silages vs. corn silages

In Table 4 it is presented a research results compilation about substitution of corn silage (CS) for alfalfa silage (AS) in diets for lactating dairy cows. Comparing AS with CS as unique source of forage for lactating dairy cows, receiving diets with 60% AS, 60% CS or 79% CS, Broderick (1985) concluded that AS is comparable to CS for

the milk production of approximately 25 kg day^{-1}, without affecting the dry matter intake, diet compounds digestibility and milk composition.

Dhimman & Satter (1997) evaluated the effect of different corn and alfalfa silage proportions (67:33; 33:67; 0:100) in diet (with 50% concentrate) in the productive performance and the efficiency of nitrogen compound utilization, in a complete lactation trial with 45 multiparous and 29 primiparous Holstein cows. The authors have not verified the effect on dry matter intake, milk production and composition. However, the ruminal ammonia concentration was lower and the N excretion on the environment was reduced between 6 and 15% with diets containing CS (33 and 67% of forage), which allowed the observation of an increase tendency between 6.8 and 13.6% in the efficiency of N utilization for milk production (N milk/N ingested). The authors concluded that CS can constitute one or two thirds of forage in diets containing AS for nutritional benefit maximization.

Brito & Broderick (2007), using 28 multiparous lactating Holstein cows, in a latin square design, investigated the effect of different proportions of AS:CS (100:0; 21:79; 47:53; 75:26) in diets containing 51% of concentrate and 16.7% of crude protein (mean), on milk production, efficiency of N utilization, digestibility and ruminal metabolism. The diet containing the highest CS proportion provided the lowest dry matter intake, neutral detergent fiber digestibility, ammonia concentration, ruminal acetate, milk production, 3.5% fat-corrected milk production, milk fat content and production. The effect in fiber digestion occurred, according to the authors, because of the increase in ruminal pH fluctuation and the time that ruminal pH was kept below 6 in the diet with higher CS level. The milk protein content and the efficiency of N utilization for milk production were higher in the diets containing higher CS proportions, but the milk protein production was not affected. The authors concluded that the best AS:CS proportion is 47:53, because it allows an increase in the efficiency of N utilization and support milk production.

Table 4 – Summary of research results on substitution of corn silage (CS) to alfalfa silage (AS) in lactating dairy cow's diet.

Author	CS:AS	FL (% DM)	DMI (kg/d)	MP (kg/d)	F (%)	P (%)	PUN (mg/dL)	NE (%)	N urine (g/dia)
Broderick	100:0	60	20.7	26.1 [a]	3.50	3.18			
(1985)	0:100	79	20.8	26.3 [a]	3.72	3.16			
	100:0	60	20.,0	23.9 [b]	3.74	3.21			
Dhiman &	67:33		21.1	31.4	3.65	3.19		33.5	
Satter	33:67	50	21,4	32.4	3.67	3.15		31.5	
(1997)	0:100		20.9	31.1	3.53	3.08		29.5	
Brito &	0:100		26.8 [a]	41.5 [a]			13.8	26.5[d]	217[a]
Broderick	21:79	51	26.5 [a]	42.0 [a]			13.9	28.5 [c]	215[a]
(2007)	53:47		25.4 [b]	41.5 [a]			14.1	30.0 [b]	201[b]
	75:26		23.7 [c]	39.5 [b]			14.4	31.7 [a]	188 [b]

Means followed by different letters in the same column and in the same trial are different (P<0.05)
FL= diet forage level; DMI= dry matter intake; MP= milk production; F= milk fat; P= milk protein; PUN = plasma urea-N; N= nitrogen compounds; NE= N efficiency (milk N secreted /N ingested); N urine= urine N excreted

4.1.3 Alfalfa silages vs. legume silages with lower proteolysis potential

Due to lower protein degradation extension in the silo for clover silages in relation to alfalfa silage, some researchers speculated that the substitution of alfalfa silage for clover silage (white or red) could increase the efficiency of N utilization and the productive performance in ruminants (Hoffman et al., 1997; Broderick et al., 2007).

Broderick et al. (2007) compared the productive performance and the utilization efficiency of the compounds (containing 50.3% forage, DM basis) in isonitrogen and isoenergetic diets (17.5% CP; 27.5% NDF; and 1.57 Mcal of ELp/kg DM), in Holstein cows with an mean initial milk production of 36 kg day^{-1} and 192 lactation days, containing alfalfa silage (41% DM; pH 4.94; 24.6 % CP containing 49% NPN; and 39.4% NDF) or red clover silage in initial stage (41.8% DM; pH 4.95; 23.3% CP containing 29.4% NPN; and 40.0% NDF) or at the end of maturity (43.4% DM; pH 4.86; 18.1% CP containing 27.1% NPN; and 41.4% NDF). The most advanced maturation stage of clover reduced diet DM, OM, CP and NDF digestibilities but did not affect dry matter intake, milk composition and production, intestine flow of rumen microbial protein and the efficiency of N utilization (N in milk/N ingested or milk production/N excreted in urine). The DM intake, the milk production and compound (fat, protein, lactose and total solids) were higher for diets containing alfalfa silages in relation to clover silages. The urea-N concentrations in plasma and milk, rumen ammonia and urinary excretion N were reduced with clover silages, suggesting better

efficiency in N utilization, due to a lower NPN fraction. According to authors, the efficiency of apparent N utilization (N milk/N ingested) tended to be higher for cows fed corn silage, but there was no difference when the efficiency was expressed as kg of milk/kg of excreted N.

4.1.4 Soybean silage

The nutritive value of soybean plant can be comparable to the alfalfa one in the start of flowering. Lactation cows and growing calves have similar performance when fed soybean hay or alfalfa (Garcia, 2002).

An interesting fact is regarded to the high ether extract content in soybean, approximately 10% (Muñoz et al., 1983, Griffin et al., 2000), once this nutrient inclusion, in levels higher than 6% in diets, can reduce the fiber digestion (Van Soest, 1994), by preventing the microorganisms adherence to feed particles (Devendra & Lewis, 1974) or by the toxic effect on cellulolytic organisms (Henderson, 1973). Besides, fat surplus in the diet can also reduce dry matter ingestion and passage rate (NRC, 2001). This way, soybean silage should not be offered only and exclusively in ruminant diets, once it compromises the assimilatory phenomenon. To avoid negative impacts of high ether extract content in diets, soybean as forage should not exceed 50% of the total dry matter (Wiederholt & Albrecht, 2002). Varner (1999) recommends that soybean silage should not exceed 30 or 40% of diets dry matter for dairy cows.

However, the information regarding to production and feeding with soybean silage are scarce. So that, in the text “A guide book for soybean silage production” (Undersander et al., 2007), the recommendations for silage production of this legume are based in farmer experiences who gave interviews about the silage production in the autumn 2005, in Wisconsin, United States. In these farms, the soybean silage constituted 15 to 20% of animal diets. It is observed, in Table 5, from the inspected farms, in only one there was an intake decrease. This way, although the soybean silage presents lower acceptability when compared to alfalfa or corn silage, it can be used in significant portion of diet, without influencing animal intake.

Table 5 – Soybean silage effects on milk production and food intake in eight farms of Wisconsin State, United States[1]

Number of inspected farms	Animal type	Food intake	Effects on milk production
6	Lactating cows	unchanged	None
1	Lactating cows	decreased	None
4	Dry cows and heifers	unchanged	Not aplly

[1] Undersander et al., 2007

Recently, Bello-Pérez et al. (2008) evaluated the nutritive value of soybean silage in relation to alfalfa silage, in diets for lactating dairy cows. Two isonitrogen diets (18.6 and 19% CP) and the same ether extract content (4.6 and 4.7%) were used, with forage:concentrate ratio of 48:52, being 72% from forage fraction constituted of soybean or alfalfa silages and, the other 28% from corn silages. The soybean silage resulted in lower ($P<0.05$) dry matter intake and milk production, in relation to alfalfa silage (Table 6), probably due to its lower ruminal NDF degradability (Table 7). Nevertheless, despite these negative effects of soybean silage, the 4% of fat-corrected milk production, milk composition (except fat content, higher for soybean silage), milk production efficiency and nutrients total digestibility were similar to both silages.

In Brazil, the first studies with soybean silage in animal feeding, soybean was consorted with corn, under different cultural arrangements, and its silage given to beef cattle as exclusive feed. It is observed on Table 8 that the mean weight gain of beef cattle fed these silages was 0.592 g day^{-1}. The greater weight gain in these animals compared to the ones fed only corn silage is due to the higher protein content of consortiated silages (Zago et al., 1985; Obeid et al., 1992b).

Table 6 - Performance and utilization efficiency of diet compounds in dairy cows fed soybean silage

Item	Diets		Significance level
	Soybean silage	Alfalfa silage	
Food intake (kg day^{-1})			
DM	23.5	25.1	0.033
CP	4.0	4.9	0.001
NDF	7.4	9.3	< 0.001
Production (kg day^{-1})			
Milk	35.5	37.2	0.002
Milk, 4% fat	34.3	34.8	0.31
Production efficiency	1.56	1.52	0.34
Milk composition (%)			
Fat	3.78	3.58	0.017
Protein	3.17	3.18	0.76
Lactose	4.69	4.69	0.89
Total solids	12.65	12.61	0.73
Digestibility (%)			
DM	71.1	70.9	0.88
OM	71.8	71.7	0.95
CP	70.6	69.5	0.74
NDF	57.8	53.5	0.27
Digestible energy, Mcal kg of DM^{-1}	3.11	3.01	0.17

[1]Adapted from Bello-Pérez et al. (2008)

Table 7 – *In situ* ruminal degradability of soybean and alfalfa silage nutrients

Item	Silages		Significance level
	Soybean	Alfalfa	
DM			
Soluble fraction, %	33.8	40.0	< 0.0001
Slowly degradable fraction, %	39.5	37.7	0.16
Degradability rate % h^{-1}	6.0	8.0	< 0.02
Latency time, h	0.62	0.54	0.69
Effective degradability, %	53.3	63.9	< 0.001
CP			
Soluble fraction, %	60.1	59.6	0.18
Slowly degradable fraction, %	30.4	31.2	0.73
Degradability rate % h^{-1}	6.5	9.4	0.034
Effective degradability, %	76.9	79.9	0.004
NDF			
Slowly degradable fraction, %	43.9	45.1	0.63
Degradability rate % h^{-1}	4.8	6.1	0.04
Latency time, h	1.0	0.8	0.85
Effective degradability, %	31.2	40.6	< 0.001

[1]Adapted from Bello-Pérez et al. (2008)

Table 8 – Dry matter intake (DMI), crude protein (CP) and weight gain of steers fed exclusive corn silage (CS) or in association with soybean silage (SS), sunnhemp (SuS) and velvet bean (VBS).

Treatments	Food intake (kg day^{-1})		Weight gain (kg day^{-1})	Author
	DM	CP		
CS[1]	10.6 c	0.662 b	0.265 b	Zago et al.(1985)
CS + SS[2]	14.1 a	0.979 a	0.596 a	
CS + SS[3]	13.1 ab	0.855 a	0.526 a	
CS + SS[4]	12.3 bc	0.873 a	0.566 a	
CS [1]	10.8 b	0.648 b	0.248 a	Obeid et al. (1992b)
CS + SS[5]	12.0 a	0.973 a	0.682 a	
CS + SuS[6]	12.8 a	1.152 a	0.698 1	
CS + VBS [7]	8.3 c	0.879 a	0.382 b	

[1] exclusive corn silage, [2] CS + SS (5:20 seeds m^{-1}), [3] CS + SS (5:30 seeds m^{-1}), [4] CS + SS (5:40 seeds m^{-1}), [5] CS + SS (6:20 seeds m^{-1}), [6] CS + SuS (6:10 seeds m^{-1}), [7] CS + VBS (6:6 seeds m^{-1}).

Rigueira (2008) evaluated the productive performance of 32 HxZ steers, with 355 kg initial weight fed diets containing soybean silage (SS), soybean silage with microbial inoculant (SSI), soybean silage with inoculant and molasses (SSIM) and soybean silage with molasses (SSM). The inoculant used was Sil All C4 (Alttech, Brazil). The powder molasses was used in 2.5% proportion as fed. The isonitrogen diets, presented 13% crude protein. The forage:concentrate ratio was 70:30 (DM basis), being 40% soybean silage and 30% corn silage. Four reference animals were slaughtered after the adaptation period for carcass gain estimative. The intake of all analyzed nutrients was lower ($P<0.05$), in the diet containing soybean silage without additives compared to the one with soybean silage with inoculant and molasses (Table 9). This is probably due to better fermentation of soybean silage treated with inoculant and molasses when comparing with the others, resulting then, in a higher acceptability, mainly, regarding the control silage, which presented strong acetic acid and ammonia odor, characteristics of undesirable fermentations, as well as higher pH and N-NH_3/total-N values.

Table 9 – Mean dry matter intake (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), non-fiber carbohydrate (NFC) and total digestible nutrients (TDN) of diets and respective variation coefficient (VC%)[1]

Item	Silages				VC (%)
	SS	SSI	SSIM	SSM	
	Intake (kg/day)				
DM	7.60b	8.46ab	9.55a	9.05ab	12.29
CP	0.80b	1.05a	1.24a	1.14a	12.78
EE	0.42b	0.50ab	0.56a	0.56a	12.80
NDF	3.03b	3.36ab	3.86a	3.65ab	12.65
NFC	2.74b	3.09ab	3.33a	3.17ab	11.70
TDN	4.72b	5.29b	6.60a	5.57b	11.45
	Intake (% BW)				
DM	1.83b	2.00ab	2.16a	2.10ab	9.54
NDF	0.73b	0.79ab	0.87a	0.85ab	9.98

[1]Adapted from Rigueira (2008)

Although it has been observed differences ($P<0.05$) in nutrients intake, this has not reflected in animal performance, once the weight and carcass gain, carcass dressing and feed conversion were not influenced by diets (Table 10). The author emphasizes that daily weight gain, which varied from 1.32 (exclusive soybean silage) to 1.68 kg day^{-1} (soybean silage with inoculant and molasses), can be considered high for the animal type applied. It is important to highlight also that the pH and ruminal ammonia concentration were not influenced ($P>0.05$) by diets. This study shows that soybean silage can become an interesting alternative when associated to other forage, in complete diets.

Table 10 – Obtained means for daily live weight gain (DWG), carcass gain (CG), carcass dressing (CD) and feed conversion (FC) for the different experimental diets and respective variation coefficient (VC%)[1]

Item	Silages				VC (%)
	SS	SSI	SSIM	SSM	
DWG(kg)	1.32	1.45	1.68	1.50	17.83
CG (kg)	0.84	0.87	0.99	0.96	15.70
CD (%)	54.41	53.62	53.59	54.69	2.54
FC	5.86	5.99	5.71	6.03	10.18

[1] Adapted from Rigueira (2008)

5. Economic evaluation of soybean silages based diets

On Table 11 is the chemical composition and forage income of soybean silage compared to corn and sorghum silages, most used forages for cattle feed in Brazil. The

total digestible nutrients value (TDN) was obtained by prediction equations described at NRC (2001), from feed chemical composition (sorghum and corn silages) described in Valadares Filho et al. (2006) and Magalhães (2007) (soybean silage). The soybean silage is presented as a competitive forage option facing the traditional corn and sorghum silages, mainly in situations with high protein demand diets because of lower cost of crude protein production (Table 12).

Table 11 – Chemical composition and forage yield of selected forages

Forages	Chemical composition				Forage yield ($t\ ha^{-1}year^{-1}$)		
	DM (%)	CP (%DM)	TDNp est[1] (% DM)	OM	DM	CP	TDN
Corn silage	30.9	7.26	64.87	40.0	12.4	0.90	8.04
Sorghum silage	30.8	6.69	61.02	45.0	13.9	0.93	8.48
Soybean silage[2]	31.0	20.18	68.00	24.6	7.63	1.54	5.19

Source: Adapted from Pereira et al. (2007). [1/] TDNp estimated from chemical composition using the predictive equations describe at NRC (2001), considering DMI = 2.5 times the maintenance. [2/] Mean forage yield (ton DM ha^{-1}) obtained from 22 varieties and 2 lineages, in the R6 stage, in Viçosa-MG, according to Mello Filho (2006).

Table 12 – Production costs per area, per organic matter (OM) unit, dry matter (DM), crude protein (CP) and total digestible nutrients (TDN) from the selected forages.

Forages	Total production costs				
	R$/ha/ year	R$/ton OM	R$/ton DM	R$/ton CP	R$/ton TDN
Corn silage	2.870,00	71.75	232.21	3.198,48	357.96
Sorghum silage	2.907,00	64.60	209.75	3.135,28	343.74
Soybean silage	2.511,17	10.08	329.32	1.631,91	484.29

Source: Adapted from Pereira et al. (2007c). Prices in July, 2008.

The economic analysis of soybean silage diets for feedlot beef cattle was performed, using data from the study of Souza et al. (2008). The authors evaluated the dry matter intake and the performance of 30 steers, weighing around 372.3 kg, during 84 days, receiving five diets with different substitution levels of corn silage to soybean silage (0, 25, 50, 75 e 100%, DM basis), both with 40% concentrate, DM basis. The DM intake, weight gain, carcass gain, carcass dressing and feed conversion were not influenced ($P>0.05$) by treatment (Table 13).

It is observed that the diet cost by carcass gain (R$ per each 15 kg) reduced as the corn silage was substituted by soybean silage, reaching the lowest value in the 75% level of substitution. However, total substitution was not beneficial because of the crude protein surplus in the diet when using soybean silage as an exclusive forage source.

Thus, due to the competitive production cost of soybean silage crude protein (R$ 1,631.91/ton of CP) compared to soybean meal (R$ 2.193,32/ton of CP), currently it is seek the potential use of it in partial substitution (between 50 to 75%) to corn silage.

Table 13 – Diet ingredients, animal performance and feed costs for finishing beef cattle fed diets with different substitution levels of corn silage to soybean silage (DM basis)

Items	Substitution levels (%) of corn silage to soybean silage					
	0	25	50	75	100	Costs R$/ t DM[2]
Diet[1]						
Soybean silage		15.00	30.00	45.00	60.00	329.32
Corn silage	60.00	45.00	30.00	15.00		232.21
Finely ground corn	27.68	32.71	37.74	39.60	39.70	526.99
Soybean meal	11.13	6.14	1.14	0.00	0.00	1096.66
Urea/ammonia sulfate (9:1)	0.60	0.60	0.60	0.00	0.00	1,249.50
Mineral premix	0.59	0.55	0.52	0.40	0.30	1,365.00
Total	100.00	100.00	100.00	100.00	100.00	
Diet CP (% DM)	13.57	13.57	13.57	13.57	15.53	
Diet EE (% DM)	2.89	3.94	4.98	5.95	6.87	
Animal performance[1]						
DM intake, kg/d	8.96	8.92	8.95	8.75	8.67	
DM intake, % BW/d	2.17	2.12	2.02	2.07	2.09	
Daily weight gain, kg/an/d	1.17	1.10	1.21	1.14	1.21	
Daily carcass gain, kg/an/d	0.678	0.698	0.690	0.688	0.665	
Feed conversion ratio	7.77	8.13	7.45	7.65	7.30	
Feed costs (diet)						
Fed diet, R$/t DM	422.80	408.61	394.44	397.17	410.90	
Animal - R$/an/d	3.79	3.64	3.53	3.48	3.56	
R$/@ of body weight	97.14	99.40	87.53	91.45	88.33	
R$/@ of carcass	83.81	78.33	76.74	75.77	80.36	

[1]Data obtained from Souza et al. (2008). *P* value (>0.05) from F test.
[2]Prices in July, 2009.

However, it is important to point out that the present economic evaluation was made considering soybean meal price of R$ 1,096.66/ton DM, which represents 3.33 times the cost of the soybean silage DM ton. As the bioeconomic value of diets depends on the used feed prices, in Figure 2 the feed costs per 15 kg produced of the five diets are presented with five different DM prices ratio of soybean meal DM:soybean silage DM.

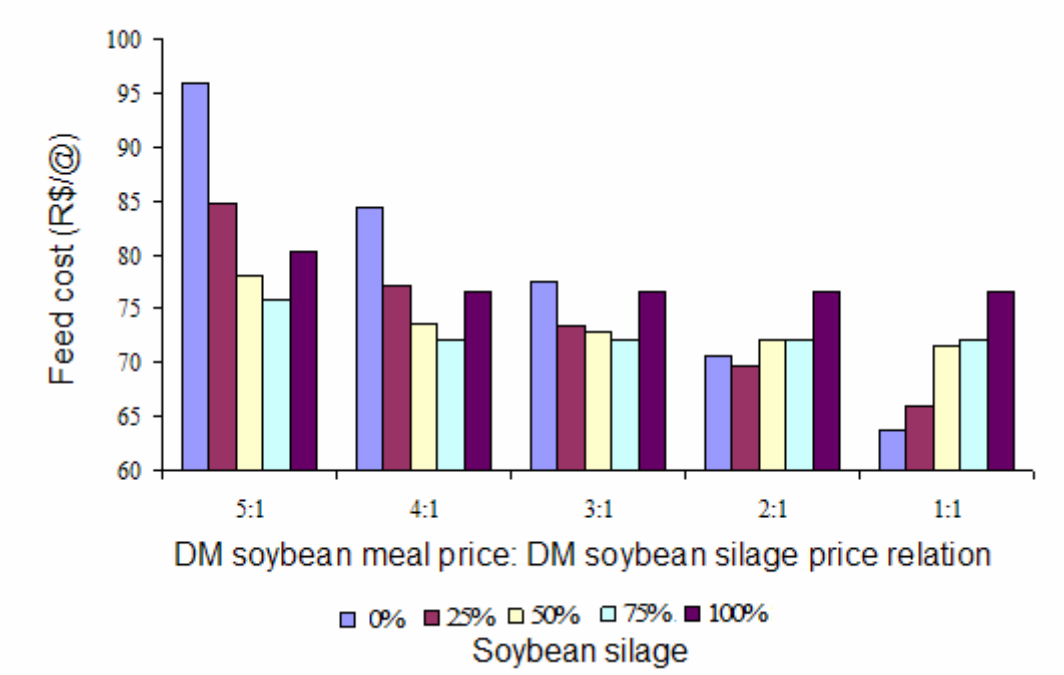

Figure 2 - Diet costs (R$/@ carcass gain) with different levels of corn silage substitution to soybean silage (0, 25, 50, 75 e 100%), according to different DM soybean meal price: DM soybean silage price ratio (@ = 15 kg).

It can be seen that as the price of soybean meal DM is reduced compared to soybean silage DM, the optimum level of corn silage substitution by soybean silage is affected. In price range of soybean meal DM between 5 and 3 times the price of soybean silage DM, the 75%level of substitution presented the lowest cost per 15 kg of carcass. When the price ratio reduced to 2:1, 25% was the optimum substitution level. Nevertheless, with 1:1 price ratio, the substitution of corn silage to soybean silage increased the cost per 15 kg of carcass, in all levels, not making the use of soybean silage economically possible.

6. Final Remarks

The harvest in adequate growth stage associated to the use of additive are strategies that enables producers to obtain good quality legume silage.

Legume silage should not be the only source of forage in ruminant diets, once it can limit the intake and, consequently, the animal performance. This way, the best strategy for legume silage use in ruminant feed is it association with other forage feed such as corn silage, to promote diet bioeconomic optimization.

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Chemical additives and the aerobic stability of silages

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1. Perspective

The most crucial step when ensiling any feedstuff is that it is stored in an air-free environment, thereby inhibiting the activity of the micro-organisms that cause aerobic deterioration. For as long as anaerobic conditions are maintained the aerobic activity of a wide range of micro-organisms is prevented. However, a harvested feedstuff will be exposed to air if silo filling and sealing are delayed, if the sealing of the silo is imperfect, or if the duration of exposure to air during feedout is too long. It will also be exposed to air when presented to livestock for consumption. In this latter case it may have been mixed with other dietary ingredients and be fully exposed to air. Since the first requirement when making and feeding silage is to manage all components of the process so as to minimise or prevent exposure to air, being successful in these tasks is the most cost-effective way to reduce the scale of aerobic losses that could occur. Silage additives, applied at ensiling or at feedout, should only be considered as a means to control aerobic losses once the relevant management practices for limiting aerobic deterioration have been undertaken correctly. Additives should not generally be relied upon to alleviate inadequate management.

There are circumstances where aerobic losses can be severe and where management practices cannot easily be altered to alleviate the problem. These are more likely when silage feedout and feeding occur during warm weather conditions and where the rate of use of silage is slower than optimal. Silages made from low buffering capacity feeds are often more susceptible.

Yeast are considered common initiators of aerobic deterioration, but bacteria can also be at fault, and both are usually succeeded in the deterioration process by moulds. Hence, the focus of chemical additives for limiting aerobic deterioration is to inhibit the aerobic activity of these micro-organisms while avoiding negative carryover effects to the rumen.

2. Organic chemical additives

2.1 Acids

Organic acid additives were originally used mainly to inhibit clostridial fermentations. This use has diminished as the general standard of silage-making has improved as well as due to issues relating to corrosion of metal, plastic and concrete and severe damage if they come into direct contact with animal tissue.

Formic acid. The main use of this additive has been to aid silage preservation, with the rate of application depending on the scale of the preservation challenge presented and the extent to which it was intended to restrict fermentation. Its use has been mainly with unwilted or lightly wilted herbages. When herbage ensiled without additive undergoes a clostridial fermentation then the even application of sufficient formic acid (2-4 l/t) helps prevent clostridial activity and stimulates a lactic acid fermentation instead. In contrast, when applied to herbage that would undergo a lactic acid dominant fermentation it restricts the extent of that fermentation thereby resulting in the silage having a higher concentration of unfermented water-soluble carbohydrates (WSC). Higher application rates (4-6 l/t) essentially prevent fermentation.

The data in Table 1 summarise a series of 78 laboratory silo experiments in which the conservation characteristics of ensiled unwilted grass silages were studied. In each experiment there were silages (4 silos/treatment) made without additive and with formic acid (850 g/kg) applied at 3 ml/kg (= 3 litres/tonne). The data have been segregated into those experiments where the herbages ensiled had 0-9 (n = 46 experiments), 10-19 (n = 17 experiments) and 20-30 (n = 15 experiments) g WSC/kg aqueous phase of the pre-ensiled herbage. In experiments where the herbage was in the lowest WSC category silage made without additive preserved very badly, and the standard of silage preservation improved as grass WSC category increased. There was a tendency for badly preserved silage to be more stable (smaller duration until pH or temperature rise commenced) when exposed to air than excellently preserved silage, and for it to have a smaller extent of aerobic deterioration (larger accumulated temperature rise during five days aerobiosis). This outcome is in accord with Ohyama *et al*. (1975). Formic acid significantly improved aerobic stability and reduced aerobic deterioration, having a larger effect where the extent of instability or deterioration was more severe (i.e. where silage

made without additive was well preserved). This agrees with Crawshaw *et al.* (1980) who also showed that aerobic deterioration was progressively reduced as the rate of formic acid applied to grass was incrementally increased from 0 to 6 l/t. Similar benefits from formic acid have also been reported by Salawu *et al.* (2001), Adesogan and Salawu (2002) and Randby (2002), while Conaghan *et al.* (2009) has shown ammonium tetraformate (640 g formic acid + 70 g ammonia/kg; density 1.18 g/ml) also capable of enhancing aerobic stability.

Table 1. Silage fermentation and aerobic stability and deterioration characteristics - statistical summary of 78 laboratory silo experiments at Teagasc Grange comparing unwilted grass ensiled with no additive (NA) or with formic acid (FA)

Grass juice WSC g/kg		pH	NH_3-N g/kgN	Days to pH rise	Days to pH max.	Days to ^{O}C rise	Days to ^{O}C max.	Acc. ^{O}C rise to day 5[3]
20-30	NA	3.9	80	3.2	5.8	2.1	5.3	95
	FA	4.0	61	4.6	6.8	2.4	6.6	67
	s.e.	0.05	2.6	0.37	0.25	0.10	0.29	6.1
	P[1]	0.03	<0.001	0.002	0.002	0.007	<0.001	<0.001
	n[2]	15	15	15	13	15	11	15
10-19	NA	4.3	121	3.0	6.7	2.3	5.0	61
	FA	4.2	77	4.4	7.6	2.4	6.4	44
	s.e.	0.08	5.3	0.37	0.29	0.18	0.36	6.2
	P[1]	0.23	<0.001	0.002	0.013	0.71	0.002	0.015
	n[2]	17	17	15	11	15	11	17
0-9	NA	5.4	283	3.9	5.3	2.4	4.8	47
	FA	4.8	151	4.0	6.3	2.5	5.9	37
	s.e.	0.08	14.7	0.37	0.35	0.15	0.31	3.7
	P[1]	<0.001	<0.001	0.84	0.012	0.29	0.001	0.008
	n[2]	46	45	32	28	32	26	46

[1]2-tail probability; [2]number of experiments with pair of treatments compared; [3]Accumulated ^{O}C rise to day 5

Propionic acid. Although this additive has been widely used for preventing fungal growth on aerobically stored moist grain or hay it can also be applied to herbage at ensiling to help prevent yeast or mould activity at feedout. Its anti-fungal character derives from the undissociated molecule, and the anti-fungal effect increases as forage pH declines (Woolford, 1975a) reflecting its reduced state of dissociation. The anti-mycotic effect was further highlighted by Crawshaw *et al.* (1980) who found that propionic acid (990 g/kg) applied at progressively higher rates (from 0 to 10 l/t) restricted yeast and in particular mould growth, and reduced respiration (CO_2 production), during the exposure of silage to air. This effect reflected the progressively higher input of the active ingredient interacting with the correspondingly lower pH that ensued.

Propionic acid is rarely used as the sole agent in silage additives (at least partially due to its cost), and is more usually applied in a mixture with other acid ingredients. These acids are often applied as salts (in liquid or solid form) rather than as straight acids. Arbabi *et al.* (2008) compared four additives [(a) propionic acid, (b) propionic acid (0.85) + formic acid (0.15), (c) calcium propionate and (d) propionic acid (0.8) + formic acid (0.15) + ammonia (0.05); each additive applied at 0.1%; propionic acid in each additive was buffered] applied to forage maize at ensiling. Each additive gave a major improvement in silage aerobic stability, with the effects of propionic acid and to a slightly lesser extent propionic acid + formic acid giving the most consistent benefits.

Propionic acid or propionate based additives have been shown to improve aerobic stability or reduce aerobic deterioration when an adequate rate is applied (Ohyama *et al.*, 1975; Woolford and Cook, 1977; Kung *et al.*, 1998; Stacey *et al.*, 2001) but not when insufficient additive is used (Ohyama *et al.*, 1975; Kung and Ranjit, 2001; Kleinschmit *et al.*, 2005). The critical rate required will depend on the susceptibility of a particular silage to aerobic deterioration, as well as the ambient environment and management factors. It will also depend on the chemical nature of the propionate compound(s) used.

It has often been suggested in cases where “heating” is occurring in the exposed face of silage during feedout that spraying propionic acid on the face would stop the aerobic deterioration process. Besides the dangers (to whoever does the spraying) associated with such an application process, Pitt and Muck (1993) concluded that although the extent of deterioration and associated losses would decrease at the sprayed silage face, there would

still be extensive deterioration in the silage beyond this treated surface layer because air would move freely through the treated zone. In fact, Ruxton and Gibson (1994) concluded that inhibiting deterioration at the exposed silage face leads to a deeper penetration of air into the silage with the result that no overall benefit accrues. Hence, spraying a silage face with propionic acid is not a recommended strategy for preventing aerobic losses during feedout. Any apparent benefit is therefore largely cosmetic. Similarly, spraying the surface of herbage in a silo immediately before sealing it beneath plastic film is unlikely to make a marked difference at feedout - Castor *et al.* (2006a; 2006b) sprayed wilted grass with buffered propionic acid (0.011 ml/cm^2) immediately before sealing it beneath plastic film and recorded no reduction in aerobic deterioration at feedout due to this treatment.

Propionic acid, or additives based on propionate, can be mixed with silage during the production of a total mixed ration (TMR). This was investigated by Kung *et al.* (1998) who reported that the adequate mixing of sufficient additive reduced yeast numbers and improved the aerobic stability of the TMR.

Other organic acids. Woolford (1995a; 1995b; 1998) screened the C_1 to C_{12} straight-chain fatty acids (formic, acetic, propionic, butyric, valeric, caproic, heptylic, caprylic, pelargonic, capric, hendecanoic and lauric, respectively), as well as lactic, acrylic and glycollic acids, for their anti-mycotic effects as silage additives. The longer straight-chain fatty acids from C_6 to C_{12} (but with the apparent exception of hendecanoic acid) were strongly antimycotic across a range of pH values. In contrast, acrylic, glycollic and lactic acids had a relatively modest impact on inhibiting yeast or mould activity.

Sorbic and benzoic acids are frequently used as food preservatives and have been considered as constituents of silage additives. Woolford (1975b) investigated the anti-mycotic effects of potassium sorbate and sodium benzoate. Both compounds were more effective at lower pH's and, although they were equally effective at pH 4, potassium sorbate was progressively more effective (on a molar basis) than sodium benzoate at pH 5 and 6. In practice, their effectiveness depends on the rate at which active ingredient is applied and the scale of aerobic stability challenge present. Thus, Kleinschmit *et al.* (2005) recorded major improvements in aerobic stability from potassium sorbate (plus EDTA; 0.1% addition rate of a 50:50 mixture) or sodium benzoate (0.1% addition rate).

Meanwhile, Pedroso *et al.* (2008) recorded a much larger benefit from sodium benzoate (1 g/kg) than potassium sorbate (0.3 g/kg) when added to sugarcane pre-ensiling. In contrast, Bernardes *et al.* (2003) recorded little benefit after applying sodium benzoate (at up to 3 g/kg) to marandu grass at ensiling.

More recently these additives have been co-applied with some lactic acid bacterial inoculants, partially to counteract the tendency of *Lactobacillus plantarum*-based additives to sometimes disimprove aerobic stability. Applying sodium benzoate (Saarisalo *et al.*, 2006; Pahlow *et al.*, 2004; O'Kiely *et al.*, 2008) or potassium sorbate (Pahlow *et al.*, 2004; Stryszewska and Pys, 2006) under such circumstances can improve aerobic stability or restrict the extent of aerobic deterioration at feedout. However, benefits to aerobic stability/deterioration from applying potassium sorbate or sodium benzoate are not guaranteed (O'Kiely *et al.*, 2006), with the critical application rate required presumably changing with the scale of aerobic deterioration challenge presented.

Additives such as sodium benzoate and potassium sorbate can also be mixed with silage at feedout. Saarisalo *et al.* (2006) applied a series of rates of these compounds (0, 15, 30 and 45 g/kg silage) to a range of silages and found that higher rates of application lead to a progressively larger curtailment of aerobic deterioration, and that the effects of potassium sorbate were more pronounced than those of sodium benzoate.

Caproic acid (added at 1.2 g/kg forage) applied to grass (Ohyama *et al.*, 1979) or maize (Ohyama and Hara, 1979) at ensiling or applied (at feedout) to the resultant silages (made without additive) improved aerobic stability, and including hydrochloric acid (to reduce pH and thus increase the undissociated nature of the caproic acid) conferred no measurable benefits. When caproic acid was applied at 1.2 and 6.0 g/kg the low rate greatly extended aerobic stability while the high rate prevented aerobic deterioration, and the effects were greater when the additives were applied at ensiling rather than at feedout (Ohyama *et al.*, 1977).

Woolford (1978) evaluated the effects of ammonium isobutyrate and concluded that although it might protect silage from aerobic deterioration the amount required for low DM silage was likely to be excessive.

Mixture of acids. For a variety of reasons mixtures of acid or acid-based compounds are used as silage additives. Table 2 summarises a series of eight laboratory silo experiments

comparing unwilted grass ensiled with no additive, formic acid (850 g/kg; density 1.192 kg/l; applied at 3 ml/kg) or a partially neutralized blend of aliphatic organic acids (860 g ammonium formate, 100 g ammonium propionate and 20 g caprylic acid/kg; density 1.172; applied at 6 ml/kg). Within each experiment there were four silos per treatment. Although the effects of the additives on aerobic stability (duration until pH or temperature rose) were not significant (P>0.05) the extent of aerobic deterioration (accumulated temperature rise) was halved by the blend of aliphatic organic acids. Thus,

Table 2. Silage fermentation and aerobic stability and deterioration characteristics - statistical summary of eight laboratory silo experiments at Teagasc Grange comparing unwilted grass ensiled with no additive (NA), formic acid (FA) or a partially neutralized blend of aliphatic organic acids (Mx)

Additive	pH	NH_3-N g/kgN	Days to pH rise	Days to ^{O}C rise	Acc. ^{O}C rise to day 5[1]
NA	4.49	121	3.5	2.8	49
FA	4.28	78	3.8	2.6	43
Mx	4.29	91	5.4	2.9	22
sem	0.101	7.8	0.49	0.11	6.9
Sig.	0.278	0.005	0.033	0.321	0.034

[1]Accumulated ^{O}C rise to day 5

whereas the duration of aerobic stability was not altered, the extent of subsequent deterioration once instability commenced was greatly restricted by the blend of organic acids.

Improvements in aerobic stability have been widely reported in response to applying mixtures of organic acids to forage pre-ensiling – e.g. Stryszewska and Pys (2006; 0.59 formic acid + 0.2 propionic acid + 0.043 ammonium formate + 0.0255 potassium sorbate) and Randby (2002; 0.64 formic acid + 0.093 propionic acid + 0.019 benzoic acid). O'Kiely *et al.* (2007) applied a mixture of formic acid, ammonium formate, propionic acid, benzoic acid and ethyl benzoate (A1) or a mixture of acetic acid and iso-butyric acid (A2) to a series of harvests of high-moisture wheat and barley grain, and more frequently recorded aerobic stability benefits for A1 compared to A2. Adesogan *et al.* (2003) recorded similarly beneficial effects when A1 was applied at ensiling to high-moisture wheat grain. In contrast to the above, Lorenzo and O'Kiely (2008) recorded either no

benefit or a disimprovement in aerobic deterioration when a mixture of potassium formate (0.82) + sodium bisulphate (0.1) + sodium benzoate (0.07) was applied (at 3 g/kg) to grasses at ensiling.

Ohyama and McDonald (1975) showed that under conditions where formic acid stimulated aerobic deterioration then including isovaleric acid or caproic acid with formic acid prevented the deterioration while including lauric acid provided no such benefit [3.8 g of each acid added to 15 g formic acid and applied to 1 kgDM].

Ohyama and Hara (1979) evaluated a mixture of calcium formate (0.75) + sodium benzoate (0.1) + sodium bisulphite (0.1) + minerals (0.05) [applied at 2.5 g/kg forage maize pre- or post-ensiling] and found only a relatively small benefit to aerobic stability from applying the additive at ensiling but a substantial benefit form applying it to the silage at feedout.

2.2 Aldehydes

Woolford (1975b) evaluated the anti-mycotic effects of both formaldehyde and paraformaldehyde and found a markedly greater effect with formaldehyde. Ohyama and McDonald (1975) confirmed that applying formaldehyde to wilted grass (at 6.9 g/kg) inhibited yeast activity and produced aerobically stable silage. Hexamine (hexamethylenetetramine) releases formaldehyde under acidic conditions and Woolford (1975b) found it to be much less anti-mycotic than paraformaldehyde at pH 6 or 5, but to be equally anti-mycotic as formaldehyde at pH 4. These compounds are not used as the sole ingredients in silage additives, and only hexamine has been used to any extent in recent years. It has most frequently been combined with sodium nitrite, as well as with some other salts.

3. Inorganic chemical additives

3.1 Acids

Sulphuric acid (450 g/kg) has been used as a lower cost alternative to formic acid when seeking to achieve satisfactory silage preservation. However, it is less inhibitory in its anti-microbial effects than formic acid. Using immature forage maize, O'Kiely (1998a) showed that whereas aerobic stability was improved and aerobic deterioration reduced by

formic acid (850 g/kg; applied at 3 ml/kg), the reverse outcome occurred when sulphuric acid (applied at 3 ml/kg) was used. O'Kiely (1997a) applied sulphuric acid at 0, 1.5, 3.0, 4.5 and 6.0 ml/kg and found that even though the 4.5 and 6.0 ml/kg application rates progressively reduced aerobic deterioration, the biological magnitude of this statistically significant effect was relatively small. Thus, sulphuric acid applied alone has little to contribute to providing aerobically more stable silage.

The above agrees with Woolford (1978) who evaluated the anti-microbial effects of hydrochloric acid, orthophosphoric acid, sulphuric acid and sulphamic acid and, other than their direct pH effect, found little direct inhibition of deleterious yeast, mould or bacteria.

3.2 Alkali

Ammonia has strong anti-microbial characteristics, being particularly effective against yeast and mould. Its use as a silage additive is generally restricted to crops with a high content of fermentable substrate and a low buffering capacity (e.g. forage maize), usually of moderately high DM content, since these will readily undergo a lactic acid dominant fermentation and where it is most unlikely that the alkali effect of ammonia will neutralize an excessive proportion of lactic acid and thus promote a clostridial fermentation. Under these conditions (chopped forage maize of approximately 350 gDM/kg) the addition of 1% ammonia at ensiling prevented aerobic deterioration when silage was exposed to air at feedout (Glewen and Young, 1982). Similarly, Buchanan-Smith (1982) recorded a large benefit from cold flow ammonia treatment (+1% on a DM basis) of forage maize ensiled at 280 and 420 gDM/kg, while Phillip *et al.* (1985) reported benefits with high-moisture ear maize (+1% to fresh weight).

Urea can be used as an indirect source of ammonia due to the ubiquitous availability of urease on ensiled forage. This has been shown to improve the aerobic stability of maize or whole-crop cereal silage (Pahlow, 1979; Stacey *et al.*, 2001), and O'Kiely (1998a) repeated these effects (using 3 g urea/kg forage maize) when even immature (182 gDM/kg) maize was ensiled. However, the effects of urea treatment are not universally guaranteed and Pedroso *et al.* (2008) recorded no benefit when urea (5 g/kg) was applied to sugarcane pre-ensiling.

3.3 Other chemical agents

Propylene oxide had relatively weak anti-mycotic effects (less than paraformaldehyde) whereas pimiracin (anti-mycotic), tylosin (anti-bacterial) and bronopol (synthetic anti-microbial) were effective at inhibiting yeast and mould activity in a laboratory assay (Woolford, 1975b). Woolford and Cook (1978) added the anti-mycotic pimaricin and the anti-bacterial chlorotetracycline + chloramphenicol + streptomycin B + bacitracin C + polymyxin B + rose bengal alone or as a mixture to maize silage (i.e. at feedout), and reduced heating only with the anti-bacterial product. However, the use of such compounds in commercial silage additives is unlikely.

Woolford (1978) concluded that chlorine dioxide and sodium formaldehyde bisulphite were unlikely to have a role inhibiting yeast and mould activity in silage. Sodium bisulphite had the ability to limit yeast and mould growth at pH 4, but Woolford (1978) speculated that a high rate of application would be necessary in practice.

When sodium metabisulphite is added to herbage at ensiling it reacts with moisture or acids, releasing SO_2 (beneficially scavenging O_2 in the process) and salts of the acids. Woolford (1978) showed it has potential to reduce yeast and mould growth, particularly at pH 4, but Kleinschmit *et al.* (2005) detected no aerobic stability benefit with maize silage made using a mixture of sodium metabisulphite and amylase.

Sulphite salts in a mixture with lactic acid bacteria (Regulator Live [Thomas & Fontaine Ltd., London] at 1 ml/kg), as well as quebracho tannins, were investigated by Adesogan and Salawu (2002). Using pea/wheat bi-crops these authors found no benefits to silage aerobic stability from applying these products pre-ensiling. This agrees with the findings of O'Kiely *et al.* (1997) who applied sulphites (Regulator [Thomas & Fontaine Ltd., London]) to grass and immature maize at ensiling (at up to 0.4 ml/kg) and found no improvement in silage aerobic stability or reduction in aerobic deterioration – in the case of grass this outcome occurred following 0, 24 and 48 h durations of wilting and where silos were sealed after a 0 or 24 h delay. Since the anti-mycotic effects of sulphites are pH dependent, O'Kiely (1997a) ensiled grasses at pH 5.3, 5.0, 4.5, 3.9 and 3.2 (following addition of sulphuric acid at 0, 1.5, 3.0, 4.5 or 6.0 ml/kg, respectively) but recorded no benefit to aerobic stability from sulphites (and no interaction between herbage pH at ensiling and the application of sulphites). When forage maize of high starch content was

treated with sulphites pre- or post-ensiling (at up to 1.2 ml/kg) the additive considerably reduced aerobic deterioration when intimately mixed with silage at feedout but had a lesser or no effect when applied pre-ensiling (O'Kiely, 1998b). The benefits of applying the sulphites-based additive to silage was confirmed for a series of grass and maize silages by O'Kiely (1996), with the effects being largest with the aerobically least stable silages and with an application rate of 4 ml/kg appearing adequate. At this rate of application a negative impact on subsequent rumen digestion would not be anticipated, but such effects could occur at or above 1.2 ml/kg silage (O'Kiely and Moloney, 1997).

Although sodium nitrite has been used as a food preservative, it was much less effective against yeast and less effective against mould (on a molar basis) than potassium sorbate or sodium benzoate in the study of Woolford (1975b). Nevertheless, its anti-mycotic effect increased with a reduction in pH. Sodium nitrite is usually used in combination with hexamine, calcium formate or other compounds, and the nitrite component can degrade during ensilage. Lingvall and Lattemae (1999) sought to identify the optimal application rate of additives containing hexamine and sodium nitrite together with sodium benzoate and sodium propionate, and concluded that the mixture of hexamine and sodium nitrite was unreliable at producing aerobically stable silage when sodium benzoate was included at only 400 g/tonne herbage. However, stability was achieved by the inclusion of sodium benzoate at 800 g/tonne or by 690 g sodium benzoate + 210 g sodium propionate per tonne. In agreement with these findings, Conaghan *et al.* (2009) concluded that a mixture of 80 g hexamine + 120 g sodium nitrite + 150 g sodium benzoate + 50 g sodium benzoate (total density 1.17 g/ml) needed to be applied to wilted grass at 5.0 rather than 2.5 ml/kg to ensure aerobic stability, while McEniry *et al.* (2007) recorded a significant benefit with wilted grass when this additive was applied at 3 ml/kg. However, occasions do occur when this mixture does not give a marked improvement in aerobic stability (O'Kiely *et al.*, 2006). That much of the aerobic stability enhancing effect comes from compounds such as sorbate, benzoate or propionate rather than from hexamine + sodium nitrite was suggested by Ohyama *et al.* (1975) (who used propionate).

Sodium chloride applied at the equivalent of up to 35 g/kg showed little ability to limit yeast or mould growth (Woolford, 1978), and the results of O'Kiely (1996) confirm the

absence of an effect on silage aerobic stability or deterioration when NaCl was applied (2 or 4 g/kg) to silage at feedout. However, whereas Harpur *et al.* (1999) also found no benefit from adding NaCl at 10 g/kg silage, they recorded a progressive improvement from mixing NaCl at 20, 30 and 40 g/kg silage. Similarly, O'Kiely and O'Brien (2007) did not obtain a significant reduction in aerobic deterioration by adding NaCl at 8.3 g/kg silage but minimized deterioration with rates of ≥16.7 g/kg silage.

4. Feedstuffs

Sugar-rich materials are sometimes co-ensiled with forage to influence silage preservation, effluent production or nutritive value. A range of outcomes have ensued, with glucose, sucrose or molasses having little impact on subsequent aerobic stability (Nkosi *et al.*, 2009; O'Kiely, 1997b; O'Kiely *et al.*, 2000; O'Kiely and O'Brien, 2007) or improving aerobic stability/deterioration (Arbabi and Ghoorchi, 2008; McEniry *et al.*, 2007; O'Kiely, 1998a), and with materials such as unmolassed or molassed beet pulp and citrus pulp being associated with a disimprovement (Cummins *et al.*, 2007; O'Kiely, 1992; O'Kiely 2002b), no effect (O'Kiely, 1992; O'Kiely, 2002a; O'Kiely, 2002b) or an improvement (Arbabi *et al.*, 2008; O'Kiely and Moloney, 1999) in aerobic stability/deterioration variables. Among other ingredients, applying a blend of essential oils to forage maize at ensiling had little effect on aerobic stability (Kung *et al.*, 2008).

Silages are often mixed with concentrate feeds immediately prior to feeding livestock. This fully exposes the silage to air as well as potentially providing more respirable substrate or aerobic microbial inoculum. When a range of energy- and protein-rich concentrate feeds (wheat, barley, maize, beet pulp, citrus pulp, molasses, soyabean meal, maize gluten, sunflower meal, rapeseed meal, distillers grains and sunflower oil) were individually mixed with an aerobically unstable well preserved grass silage (at 67 g/kg) they had no impact on the aerobic stability/deterioration of the mixture (O'Kiely, *et al.*, 2001). However, when a concentrate feed (498 g barley, 120 g soyabean meal, 100 g palm kernel expeller meal, 125 g citrus pulp, 80 g maize gluten, 50 g molasses, 25 g mineral/vitamin premix. and 2 g oil blend/kg) was mixed (75 g/kg) with ten different grass silages the outcome was that whereas the concentrates did not make silage aerobically more unstable, once deterioration commenced they increased the extent of

deterioration (they provided more respirable substrate) (O'Kiely, 2007). In order to determine the impact of the form of the grain, Clancy *et al.* (2000) mixed a series of rates (67, 133 and 200 g grain/kg silage) of five forms of wheat grain (whole grain, rolled grain, milled grain (2mm apertures in sieve), NaOH-treated whole grain (30 g NaOH/kg grain) and urea-treated whole grain (30 g urea/kg grain)) with an aerobically stable and unstable silage. Silage aerobic stability was not compromised by the addition of whole grain, however rolling and milling grain increased the extent of aerobic deterioration without shortening the duration until heating commenced. NaOH-treated whole grain resulted in at least as much aerobic deterioration (i.e. heating) in the silage + grain mixture as had rolled and milled grains, but without altering the initial duration of aerobic stability. Urea-treated whole grain improved aerobic stability when mixed with aerobically unstable silage and increased the extent of aerobic deterioration with the aerobically stable silage.

Overall, the above results suggest that mixing energy- or protein-rich feeds with silage does not shorten the duration until the mixture starts to heat (compared to the same silage after 'mixing' but with no other feed added) – thus, aerobic stability is not shortened. However, once deterioration commences then the overall extent of heating increases due to the greater amount of readily respirable substrate present. This suggests that the supply of readily respirable substrate in silage is not a factor impacting on the aerobic stability of that silage (i.e. most silages already have enough readily respirable substrate to support measurable heating). It also suggests that, because the provision of aerobic microbial inoculum on the concentrate feed does not initiate deterioration sooner, the aerobic stability of the silage was not due to a simple shortage of indigenous micro-organisms capable of respiring substrate.

Sodium bicarbonate is sometimes included in TMRs that include silage and are constituted immediately before feeding. Harpur *et al.* (1999) mixed sodium bicarbonate with grass silage at 0, 10, 20, 30 and 40 g/kg - besides increasing silage pH, sodium bicarbonate also made it aerobically less stable although the effect was progressively less amplified at the two higher rates of addition (the general increase in instability was likely due to the rise in pH; the progressive lessening in the rise in instability was probably due to the corresponding reduction in water activity). In contrast, the same authors found that

whereas applying sodium hydroxide at 10 g/kg silage disimproved aerobic stability, increasing the application to 40 g/kg silage prevented aerobic deterioration for 7 days. Bolsen (1981) reported that adding NaOH (+12.2 kg/tonne sorghum at ensiling) improved aerobic stability.

Although O'Kiely (1991) found that mixing (35g/kg) a mineral + vitamin mixture with grass at ensiling ultimately reduced the extent of aerobic deterioration during feedout, this effect was mediated through a major disimprovement in silage preservation and thus would not be a viable option in farm practice.

5. Final comment

- The optimum application rate of a chemical additive that will restrict or inhibit aerobic deterioration of silage is not a constant value. It changes with a range of factors and depends on the scale and nature of the aerobic stability challenge presented by a particular silage under prevailing management and environmental conditions.

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Improved efficiency of sugarcane ensiling for ruminant supplementation

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1. Introduction

Sugarcane is among the main Brazilian agricultural products. It is estimated that by the year 2012 the country will be producing around 685 million tons in nine million ha (Agrianual, 2007), destined to alcohol and sugar production. Also, the use of sugarcane as forage for dairy and beef cattle in Brazil is increasing, becoming popular among traditional users of corn and sorghum silages.

The main advantage of sugarcane as forage for cattle is its high productivity of biomass production (over 100 t/ha) which results in low cost per unit of dry matter (DM), and high energy production per unit of area (15 a 20 t TDN/ha). Also, sugarcane maturation occurs in the same period as forage shortage in pastures. And the technology generated by the alcohol and sugar industry permits agronomical improvement in the production of this forage, in different environments.

Popularization of sugarcane among cattle farmers is due to studies of research and extension sectors that search for solutions to enhance the use of this forage. Among recent progresses, the selection of varieties for ruminant feed, the development of processes to enhance product quality and the use of additives to reduce losses in the sugarcane ensiling process.

Although sugarcane is most commonly used fresh, daily cut and chopped before provided to animals, sugarcane ensiling permits enhancing the activities on the farm and reducing the daily need for labor force, and time spent with feeding. This option has been used especially in large herds.

Due to ensiling, reduction of DM voluntary intake and decrease of nutritive value of sugarcane, are discussed by many researchers, being this effect caused by the typical alcoholic fermentation of yeasts (Pedroso et al., 2005).

In non controlled fermentation conditions (absence of additives), the pool of sugarcane water soluble carbohydrates, especially sucrose, is converted into ethanol, with losses of two CO_2 molecules for each fermented glucose molecule. This type of fermentation, characteristic of this species of forage, results in high DM losses in the

form of gas, over 30% of initial DM, according to several authors (Pedroso, 2003; Sousa, 2006). The biochemical equation of ethanol production catalyzed by the fermentative pathway of yeasts can be described as:

$$\text{Glicose} + 2\ ADP + 2\ Pi \rightarrow 2\ \text{etanol} + 2\ CO_2 + 2\ ATP + 2\ H_2O$$

Experiments in large scaled silos (Pedroso et al., 2006; Schmidt, 2006) have evidenced low recovery of the produced ethanol, probably due to fast volatilization of this component, which increases DM and forage energy losses. In addition, Schmidt's (2006) evaluation of feedlot beef cattle intake behavior evidenced inhibition of voluntary intake of silages with higher content of ethanol, with stronger effect in the first six hours after animal were feed.

The use of additives aims to reduce losses, elevate nutritive value or improve the aerobic stability of the final product. Kung Jr. (2000) affirms that in the ensiling process, additives have been used as a way to elevate nutrient and energy recovery in the preserved forage, with a consequent improvement in animal performance. Many factors can interfere in the efficiency of additive use in forages such as, characteristics of the used species, humidity content, mass temperature and pH, content of soluble carbohydrates and epiphytic microorganism population.

As any other technology, the use of additives in forage must be evaluated based on cost and economical profit in the final product. According to Weiss (1996), the cost of an additive, the equipment and labor force necessary for its application are easy to determine; however, the benefits, or investment are difficult to measure. Also, it is difficult to understand when comparing results of an application of a same additive, in the same forage species, in different trials, due to the variability of the obtained results.

In this report, the role of chemical and microbial additives in fresh or ensiled sugarcane conservation will be discussed, based on updated data presented in our previous report (Schmidt, 2008).

2. Additives and sugarcane composition

The use of additives in sugarcane ensiling aims to inhibit the growth or reduce the activity of epiphytic yeasts that produce alcoholic fermentation during the process. Therefore, a reduction of DM losses, that often overcome 30%, is expected. According

to Balieiro Neto et al. (2009), these losses can represent approximately 53 kg of TDN per ton of ensiled sugarcane.

Although many additives tested for these purposes seem to have a beneficial effect on qualitative characteristics of ensiled sugarcane, comparison of the magnitude of additive response, in different trials, is made more difficult by the low consistency and large variability of the results. Probably part of these differences in the result pattern are related to differences in the chemical composition of sugarcane used in experiments which alter the population and growth rate of epiphytic microorganisms, therefore influencing the additive effects. As an aggravating factor for result comparison**,** few studies present the complete chemical composition of sugarcane, especially regarding the content of sucrose or total soluble carbohydrates.

An idea of the variability in sugarcane chemical composition was presented by Nussio et al. (2006) based on samples of sugarcane forage sent to the Ruminant Nutrition Lab USP/ESALQ (Table 1).

Table 1. Chemical composition and variation range of sugarcane samples, 2000 – 2006

Item	n	average	minimum	maximum
Dry mater (%)	21	27,7	20,4	33,9
Non-nitrogen extractive (% DM)	26	68,0	53,3	75,5
TDN estimate (%)	26	64,5	53,9	69,5
NDF (% DM)	23	47,3	37,9	63,9

Source: Adapted from Nussio et al. (2006)

The large variation in the component contents listed in Table 1 can be due to factors such as sugarcane maturity (Fernandes et al., 2003), variety used (Rodriguez et al., 2006) and other soil, environment and management factors.

Therefore the effects of additives applied on sugarcane must be evaluated in trial groups, outlining response trendy lines, respecting the natural data variability already mentioned. It is important to remember that the reliability of tendencies depends directly on the number of available evaluations (n), being of the scientific community the responsibility to expand data about sugarcane additives.

According to Lovatto et al. (2007), the transformation of research results into usable knowledge requires dozens or even hundreds of experiments and that, in traditional reports, such as this present one, differences in experimental conditions among studies are ignored. The authors suggest the use of Meta-analysis to obtain a more precise estimative of a treatment effect, if the value of “n” is not low. This methodology was used by Kleinschmit e Kung Jr. (2006), who applied Meta-analysis to

evaluate the effect of the *L. buchneri* bacterial additive in corn silage and other grasses, using data from 43 experiments, with different doses of additive.

3. Additives in sugarcane ensiling

Studies on sugarcane ensiling in Brazil started in the end of the 90s and has been of great interest of researchers from several institutions ever since (Figure 1). Almost all studies published evaluate some kind of additive related to ensiling process. However there is still much to clarify about fermentative dynamic and the role of additives in the conservation of this forage.

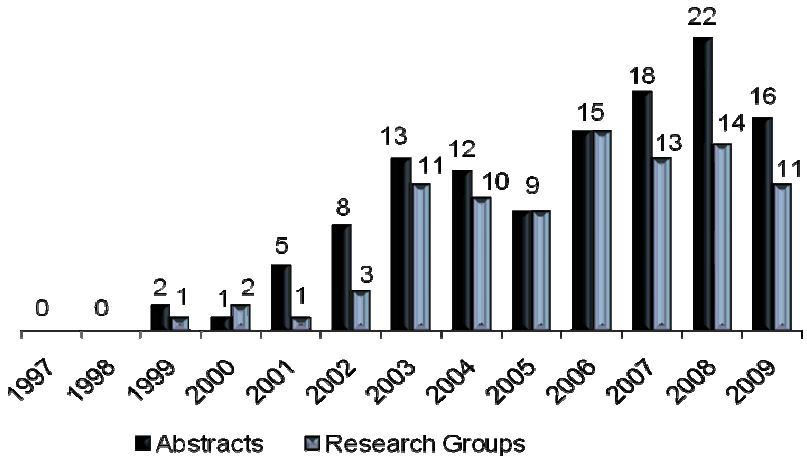

Figure 1. Number of abstracts and research groups working with sugarcane silage, published on the Proceedings of Annual Meeting of Brazilian Society of Animal Science, 1997 - 2009.

The experience, to this present day, allows affirming that the use of additives that control metabolic activity in yeasts is required in sugarcane ensiling. However the type and dose of the "ideal" additive for ensiling of this forage is still questionable due to the variations in the response patterns verified in different trials.

Main additives. A large diversity of additives has been tested in sugarcane ensiling as a way to preserve nutritive value of the preserved forage. In many cases, applying certain additives to sugarcane is based on positive results verified in silages of other forage crops. Also, the experience and intuition of Brazilian researchers, the availability and cost of chemical products used as additives and the partnership with companies that produce microbial additives has aimed researches to look for products

capable of blocking alcoholic fermentation inside the silo and guaranteeing good aerobic stability in post-opening.

Among the main additives tested to this present day, we can highlight the chemical ones: urea, Sodium hydroxid (NaOH), sodium benzoate, lime; and the microbial, composed by heterolatic bacteria (especially *Lactobacillus buchneri*) and homolatic bacteria (especially *L. plantarum*). In addition, the association of additives has been tested in order to obtain complementary effects.

Most trials evaluate additive response in relation to silage without additives (control). However, there are cases where the silage without additives behaves in an unexpected way, with low yeast activity and reduced fermentative losses, annulling possible positive additive results.

Factors such as sugarcane variety, maturity, cut season, weather, among others, have effect over epiphytic microorganism population, influencing the magnitude of response to determined additive.

Table 2. Comparison of control *versus* treated sugarcane silages on animal performance and total DM losses

Author	Month/Year	Average daily gain[1] (kg/day)	
		Control	Treated
Pedroso (2003)	Oct/02	0,94	1,14
Schmidt (2006)	Oct/02	0,82	1,00
Mari (2008)	Sep/04	0,88	0,88
Mari (2008)	Sep/05	1,16	1,03
Author	Month/Year	Total DM losses[2] (%)	
		Control	Treated
Pedroso (2003)	Aug/01	18,2	13,4
Pedroso (2003)	Jul/02	6,1	6,4
Junqueira (2006)	Sep/03	22,7	16,2
Sousa (2006)	Sep/04	32,5	27,9
Queiroz (2006)	Jul/05	31,4	29,2
Mari (2008)	Sep/05	6,7	10,7
Santos (2007)	Oct/05	34,3	23,9
Muñoz-Maldonado (2007)	Jul/06	15,7	20,7

[1] Beef Cattle Feedlot. Silage control or treated were the only variation among diets.
[2] Total DM losses evaluated in experimental silos

In Table 2, sugarcane silage data obtained by the *Equipe de Qualidade e Conservação de Forragens da USP-ESALQ* (Quality and Preservation of Forage Group from USP-ESALQ) are presented. The group analysis of the trials allows visualizing the

variability in the responses of control silages, due to factors that are not contemplated in experiment outline. In three experiments of feedlot cattle performance, control silage provided moderate weight gain rates, considering that in two of the trials (Pedroso, 2003; Schmidt, 2006) the additive silages resulted in a significantly higher weight gain than the control silage. However, in one of the trials developed by Mari (2008), the diet containing control silage provided a higher gain weight than the average of treated silages. In trials of experimental silos, under the same execution conditions, total DM losses in silages without additives vary between really reduced values (Pedroso, 2003; Mari, 2008) to really elevated ones (Sousa, 2006; Queiroz, 2006; Santos, 2007).

As mentioned before, the interpretation of results of additive application requires a group evaluation of available data to determine response tendencies, respecting natural variability of data.

3.1 Urea

Appling urea as an additive in sugarcane ensiling has been usual, due to its availability, solubility in water (easy to apply) and correction of silage nitrogen content. The start of yeast population inhibition with the addition of urea is related to the formation of ammonia in the urea degradation reaction, ammonia being a strong inhibitor of microorganisms, through mechanisms that are not well known (Palkova et al., 1997).

The doses of urea often used vary between 0.5 and 1.0% of organic matter (OM), although there are studies evaluating higher doses, up to 8.0% of OM (Ítavo et al., 2004). Most studies used urea diluted in water between 30 and 40 liters per ton of OM. The summary of the main results obtained to this present day is presented in Figure 2. Studies that evaluated only chemical composition and/or pH of silages were not included.

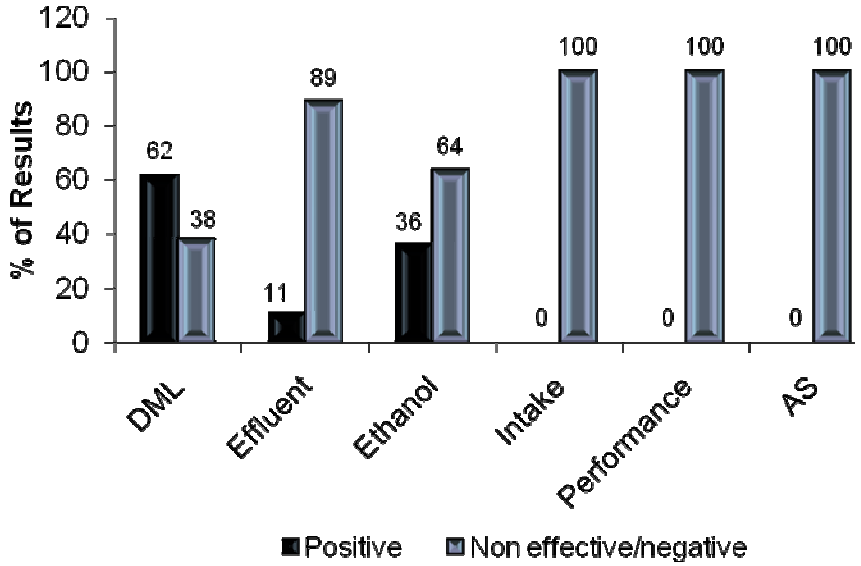

Figure 2. Results of published papers evaluating Urea on sugarcane ensiling, over the variables: Dry Matter Losses (n=13); Effluent production (n=9); Ethanol content (n=11); DM intake (n=6); Performance of cattle (n=2) and Aerobic stability of silages (n=6).

Thirteen studies evaluated DM fermentative losses in sugarcane silages added with urea. In eight studies a positive effect regarding loss reduction, which was in average of 5.9 percent units, was noticed. The highest loss reduction regarding application urea was verified by Pedroso (2003), 18.6% in control silage to 7.6% in silage with 1% of urea in the DM. However, this was the only author to verify, in another trial, a negative effect of a 0.5% dose of urea in OM, elevated losses from 6.8% to 12.6% in DM.

In eight out of nine studies evaluated, the effect of urea over effluent production was not verified and in only one trial urea elevated these losses from 15.1 to 32.2 kg/t OM (Pedroso, 2003).

The ethanol content in silages was evaluated in 11 trails and only in four of them the addition of urea was effective in reducing the production of this component, presenting no effect on the others.

Six studies evaluated the DM intake of ruminants and two evaluated the gain weight of heifers (Pedroso, 2003; Junqueira, 2006). In none of these studies there was an effect of urea addition over these variables, in relation to control silage. In none of the six studies that evaluated aerobic stability of silages with additives, was urea effective in enhancing this variable.

Discussion. In studies which evaluated the contents of urea addition in sugarcane ensiling (Pedroso, 2003; Siqueira et al., 2004; Roth et al., 2005, Junqueira, 2006), no benefit in using contents higher than 1%, over variables of DM losses or ethanol content was noticed. As discussed by Nussio et al. (2006), the average content of ammoniacal nitrogen in silages with urea additive is low, indicating reduced conversion of urea into ammonia, which limits the efficiency in the use of this additive.

The tendency to elevate effluent production due to application of urea mentioned by Nussio et al. (2003) is not confirmed by the data available to this present day, except those of studies where effluent in control silage was shown to be much reduced.

The benefits of easy access and usage, and improvement in the protein content of silage must be evaluated regarding moderate safety in preventing fermentative losses and silo panel losses.

3.2 Sodium benzoate

The dissociation of sodium benzoate during sugarcane ensiling produces benzoic acid, an antimicrobial agent with inhibiting action over yeasts and mold (Woolford, 1975). Benzoic acid is permeable to cellular membranes of yeasts, making the entrance of protons into the cell easier, which leads to internal pH reduction in more than one unit and induces yeast to waste energy in order to balance pH (Krebs et al., 1983).

The application of sodium benzoate was evaluated in eight experimental trials with sugarcane, in doses varying from 0.05 to 0.2% of OM; in six of these trials the used dose was of 0.1%. Additive cost estimative by Nussio e Schmidt (2004) indicates that doses over 0.1% can be economically impracticable. In all trials the product was applied diluted in water, in doses varying from 4.5 to 15 liters per ton of OM.

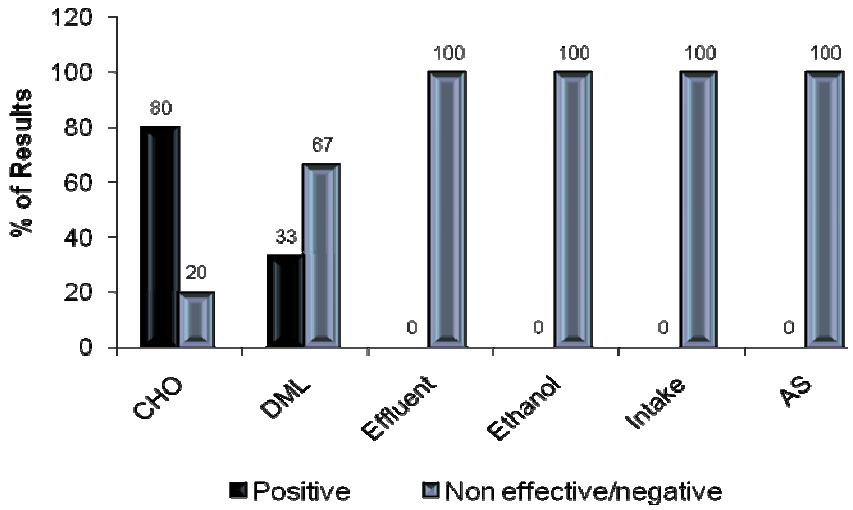

Figure 3. Results of published papers evaluating Sodium benzoate on sugarcane ensiling, over the variables: Soluble carbohydrates content (n=5); Dry matter losses (n=6); Effluent production (n=4); Ethanol content (n=6); DM intake (n=2) and Aerobic stability of silages (n=2).

In four out of five studies that evaluated soluble carbohydrate (CHO) content in silages, this fraction was higher with the application of sodium benzoate. Evaluating chemical and microbial additives in sugarcane ensiling, Schmidt (2006) observed that sodium benzoate was the most efficient additive in preserving the CHO fraction of silages, in relation to original sugarcane.

The total loss of DM was evaluated in six studies, presenting two positive results, one negative result and no verifies effect in three studies. In Siqueira et al. (2007), the addition of sodium benzoate reduced in 7.3 percent units the loss of DM. In two of the four trials that evaluated effluent production, this variable was elevated by the addition of sodium benzoate and in the other two there was no effect. However, the variation magnitude was small and of non concern for none of the trials.

Six trials evaluated the ethanol content of silages with sodium benzoate additive, and in none of them this variable was affected by the additive. However, in two trials the ensiling was done on large scale silos, what results in low detection of the ethanol produced, as evidenced by Schmidt (2006). In addition, it was not possible to verify a positive effect of sodium benzoate addition on both trials that evaluated aerobic stability in silages.

Two trials evaluated the DM intake of cattle and one evaluated the weight gain of heifers, fed with a sugarcane silage diet. In none of the trials there were statistic differences due to sodium benzoate over these variables, although daily average weight

gain of heifers in the Pedroso et al. (2006) trial was 200 grams higher in silages with sodium benzoate additive than in control silage.

Discussion. The number of studies that evaluated sodium benzoate in sugarcane ensiling is small, making better interpretations impossible. Only one trial evaluated cattle performance, obtaining highly satisfactory results. In trials were there was no effect of this additive over the DM losses, none of the other additives tested were efficient, due to the reduced level of loses in control silage, what shows feasibility in the use of this additive.

The use of sodium benzoate as an additive remains restricted to research, due to the difficulty of obtaining such product and lack of more conclusive results. In the last two years, research has not progressed in understanding the effect of this product in sugarcane ensiling.

3.3 Sodium hydroxide (NaOH)

Few studies have evaluated the use of Sodium hydroxide (NaOH) as an additive in sugarcane ensiling, based on positive results of this product in hydrolytic treatment of fresh sugarcane. The ensiling trials carried out used doses varying between 0.25% to 4% of OM, commercial products with concentrations of 33, 40 or 50%. The main results obtained are presented in Figure 4.

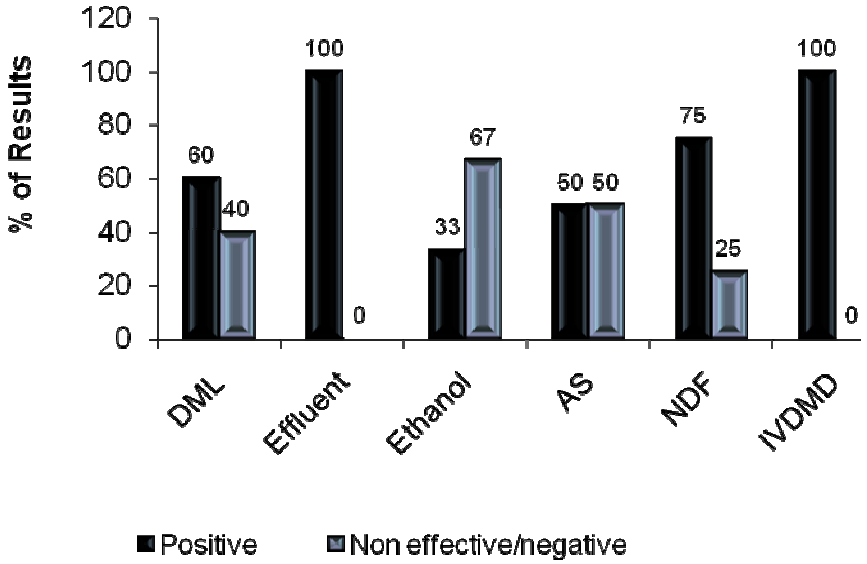

Figure 4. Results of published papers evaluating Sodium hydroxide on sugarcane ensiling, over the variables: Dry Matter losses (n=5); Effluent production (n=3); Ethanol content (n=3); Aerobic stability of silages (n=2); NDF content (n=4) and *In vitro* DM digestibility (n=3).

The use of NaOH in ensiling was effective in reducing DM losses in three of four trials that evaluated this variable; in one of the remaining trials there was a numerical tendency of loss reduction. In average this variable was reduced in 7.1 percent units. A reduction in effluent production was verified in the three trials that evaluated this variable, highlighting Siqueira et al. (2007) who verifies reduction of 76.2 (control) to 3.2kg/t OM (NaOH 1%).

Three studies measured the ethanol content of silages, and only Borgatti et al. (2008) verified a significant reduction, 15.3% in control silage to 1.57% in silage with 1% NaOH additive.

The evaluation of aerobic stability of silages showed antagonistic effects, with strong increase observed by Pedroso (2003) and tendency of stability reduction due to additives mentioned by Siqueira et al. (2007).

Fairly consistent results were evidenced for the NDF content and the *in vitro* dry matter digestibility, with marked effect from NaOH in the reduction of these variables. NDF was reduced in average 6.0 percent units and IVDMD was elevated in 15.6 percent units.

Discussion. Few studies evaluated the addition of NaOH in sugarcane ensiling, resulting in quite limited "n" value. Trials with animals using sugarcane silages with

this additive were not found in technical literature. However, good results seem to be obtained with medium doses, around 1% of OM.

Although little information apparently shows technical feasibility in the use of NaOH, there is low interest of researchers in new studies with this additive due to safety, economical and environmental limitations (Berger et al., 1994).

3.4 Calcium oxide

The use of calcium oxide (lime) as an additive for sugarcane ensiling is a new and growing technology, with 20 publications and a large number of research studies in progress. The first studies on the subject were recently published (Oliveira et al., 2004; Balieiro Neto et al., 2005) and interested the scientific community due to the results. The advantages in the use of lime as an additive is related to its low cost, effectiveness in reducing fermentative losses and possibility of fiber fraction hydrolyze.

The doses tested in trials vary between 0.5% and 2.0% of DM, applied as dry powder or diluted in water (20 to 40 L/t DM). The summary of the main results obtained is represented in Figure 5.

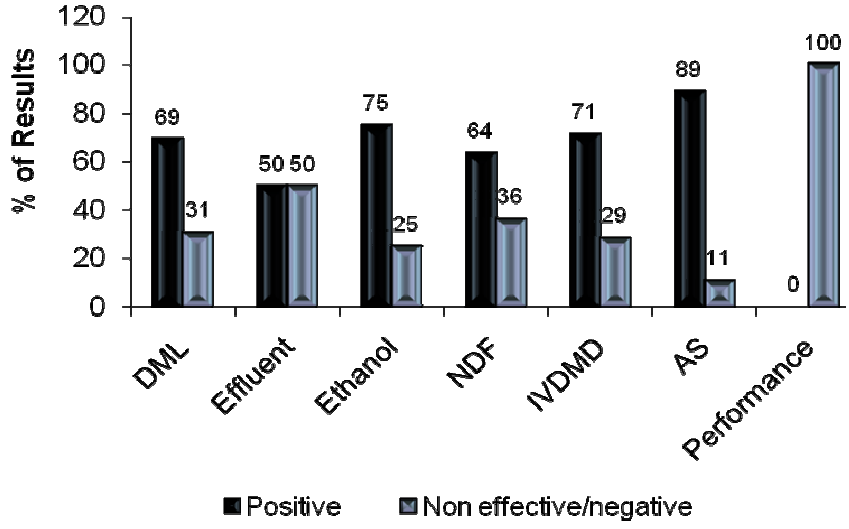

Figure 5. Results of published papers evaluating calcium oxide on sugarcane ensiling, over the variables: Dry Matter Losses (n=13); Effluent production (n=8); Ethanol content (n=8); NDF content (n=11); *In vitro* DM digestibility (n=7); Aerobic stability of silages (n=9) and Performance of ruminants (n=5).

The application of calcium oxide in the moment at ensiling reduced the fermentative DM losses in nine out of the thirteen trials evaluated. There was an average reduction of 11.3 percent units in total DM losses values, highlighting the data

obtained by Roth et al. (2006), that verified reduction of 38.7 (control) to 19.3% (CaO 1%). Only Mari (2008) verified elevation of DM losses, in 2.5 percent units.

The production of effluents in silages was reduced in four out of eight trials, the average reduction being of 14.8 kg/OM. Cavali et al. (2006) tested the doses 0; 0.5; 1.0; 1.5 and 2.0% of CaO and verified linear dose effect in reduction of effluent production.

In six trials the addition of lime was efficient in reducing the ethanol production in silage (average reduction of 6.9 percent units) and only in two trials no effect of lime was verified in this variable.

Hydrolytic activities of lime over the fiber fraction, during sugarcane ensiling, was well characterized in 11 trials that evaluated NDF content and in 7 trials that evaluated *in vitro* dry matter digestibility in silages. In seven studies, the NDF content was reduced under lime activity, being that, in average, the additive silages presented 11.2 percent units less in NDF fraction. A similar result was verified for DVIVMS, with increase of 13.7 percent units (average) due to lime application. However, in two studies (Balieiro Neto et al., 2008; Bergamaschini et al., 2008) the lime additive reduced the digestibility of matter in an average of 10.5 percent units.

Positive results were obtained in eight out of nine experiments that evaluated aerobic stability of additive silages, which, in average, slowed down heating in 65 hours, in relation to silages with no additives. Santos (2007) verified dose effect over this variable with 40, 131 and 240 hours for temperature elevation at 2ºC in control silages; 1.0 and 1.5% of CaO, respectively.

Five experiments with animals evaluated sugarcane silage with CaO additive, and in none of them a positive effect of animal performance was verified.

Amaral (2007) did not observe effect of silage over the DM intake in dairy goat or sheep. Mari (2008) and Bergamaschini et al. (2008) also did not observe effect of lime addition in ensiling over DM intake of cattle. In addition, the diet containing silage with 1% of CaO provided lower weight gain (0.97 kg/day) than the diet containing control silage (1.16 kg/day).

Discussion. Lime as an additive in sugarcane ensiling has been widely used in farms, with highly satisfactory results.

Although some variables show linear effect of additive doses (NDF, DVIVMS, effluents, the best results seem to be concentrated in the dose of 1% of OM. Higher content of additives imply in elevation of calcium content in the diet, reaching critical levels as discussed by Schmidt (2008).

The elevation of ash content in silages with CaO additive is noticeable. Santos (2007) verified ash values of 1.75; 6.09 e 7.29% of DM, for control silages, 1.0 and 1.5% of CaO, respectively. Therefore the practical recommendation of calcium oxide content over 1% must be cautious. Regarding application forms, data from Mari (2008) suggest higher effectiveness of calcium oxide diluted in water (40 L/t OM), in relation to applied in dry form.

Research results to this present day have been very positive, with noticeable reduction in DM losses, effluent production and ethanol content, besides marked increase in aerobic stability and DVIVMS. However, the few experiments with animals do not reflect these benefits, making it necessary for new trials to confirm if the results seen in chemical composition of forage translate into performance enhancement.

3.5 Lactobacillus plantarum

The bacteria *Lactobacillus plantarum* (LP) is the main agent of the lactic acid bacteria (LAB) group or homolatic bacteria, microorganisms traditionally used as stimulators of fermentation in annual grass, legume and tropical grass ensiling, and that are characterized by the elevated production of lactic acid (Muck e Kung Jr., 1997). Most studies that evaluated the addition of these microorganisms in sugarcane ensiling applied the doses recommended by the manufacturer which is in general between $5x10^5$ and $1x10^6$ CFU/g wet forage. The main results obtained still this moment are summarized in Figure 6.

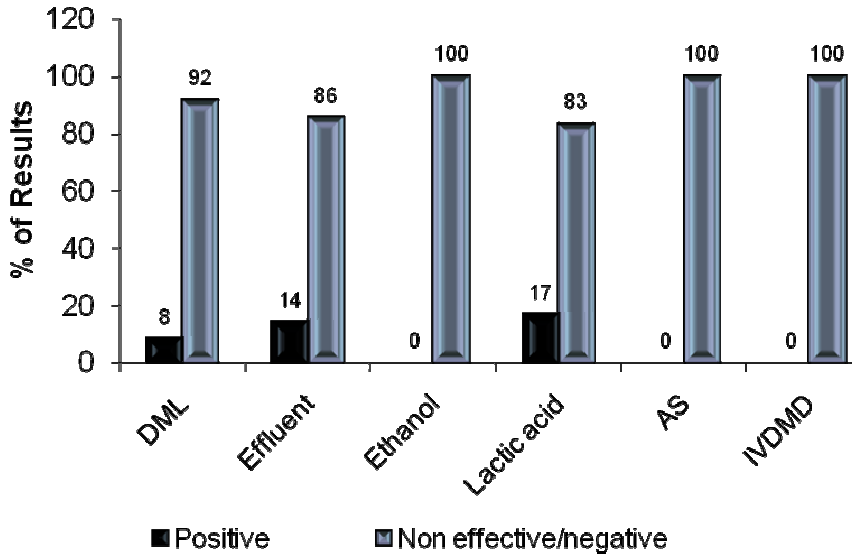

Figure 6. Results of published papers evaluating inoculation of *Lactobacillus plantarum* on sugarcane ensiling, over the variables: Dry Matter Losses (n=12); Effluent production (n=7); Ethanol content (n=10); Lactic acid content (n=6); Aerobic stability of silages (n=6) and *In vitro DM digestibility* (n=11).

In twelve studies that evaluated DM losses during sugarcane ensiling, only one presented reduction of 5.6 percent units (Ítavo et al., 2004), due to LP inoculation.

In ten studies higher numerical values were observed for total loss of DM in inoculated silages, in relation to control silages, and in Junges et al. (2009), the inoculation with LP elevated the losses of DM in 12.8 percent units.

Only one out of seven studies that evaluated effluent production in silages verified beneficial effects of this additive. (Pedroso, 2003)

Of the tem studies that evaluated ethanol content in silages, five did not present LP inoculation effect, and in the other five the additive elevated the ethanol content in silages (% of DM), in relation to control (Pedroso, 2003; Castro Neto, 2003; Pedroso et al., 2007; Bergamaschine et al., 2008; Junges et al., 2009).

The lactic acid content in silage was elevated by the addition of LP in only one (Junges et al., 2009) of the six trials that evaluated this component, although Castro Neto (2003), Freitas et al. (2006a) and Muñoz-Maldonado (2007) had observed low numerical elevation in this variable.

In six trials that evaluated aerobic stability in silages, five did not present significant additive effect, and one trial verifies stability reduction (Pedroso, 2003) due to LP inoculation.

The *in vitro* DM digestibility analyses of LP inoculated silages was carried out in 11 trials; in nine there were no significant treatment effects, and in two the LP inoculation reduced the DVIVMS. The average values observed for digestibility were 46.4% of DM for silages without additives, and 45.8% for silages with LP additive.

Discussion. The analysis of the studies shows the inefficiency of lactic acid bacteria in altering the fermentative pattern of sugarcane. The negative results obtained are probably due to the fact of yeasts not being efficiently inhibited by lactic acid (Moon, 1983; Danner et al., 2003) and use this acid as a substrate for ethanol production (McDonald et al., 1991), stimulating fermentative losses of DM.

Only one trial (Freitas et al., 2006b) evaluated levels of inclusion above manufacturer recommendations, without verifying elevation effect of 20 and 40% in doses, over the evaluated variables.

The lack of response in lactic acid content of LP inoculated silages, in almost all trials, can be related to the fast development of epiphytic microorganisms in sugarcane, due to high content of soluble sugar in this forage. Data from Pedroso (2003) show pH stability of silages without additives on the third day after ensiling, and confirm that, in sugarcane silages, the pH value does not indicate fermentative quality (Schmidt, 2006). The values detected in lab analysis show only the punctual content of lactic acid in sample harvesting, not considering that part of the lactic acid produced could have been metabolized by other microorganisms.

As mentioned for other additives, trials of animal performance using LB inoculated sugarcane silage were not found in literature

The use of additives containing LAB for sugarcane ensiling in farms is very common and it is related to the commercial opportunity and lack of technical knowledge of the inefficiency of this product.

3.6 Lactobacillus buchneri

The bacteria *L. buchneri* (LB) is an agent in the group of Heterolatic bacteria, microorganisms that produce significant quantities of other organic acids such as the acetic and propionic, besides lactic acid.

Moon (1983) affirms that the acetic acid is and acknowledged inhibitor agent of yeast growth, and according to Danner et al. (2003), the antimicrobial effect of an organic acid depends of its pKa and of the environment pH. The lactic acid has lower

pH than the acetic acid making it stronger for this reason. However, in the pH range normally found in silages, the acetic acid is less dissociated than the lactic acid, allowing it to penetrate the plasma membrane of yeast, inactivating them.

Inoculation with LB in sugarcane ensiling was first suggested by Nussio et al. (2003), base done positive results of yeast population control in corn silages (Driehuis et al., 1999). In Figure 7 the main results of studies which evaluated this additive in sugarcane are shown.

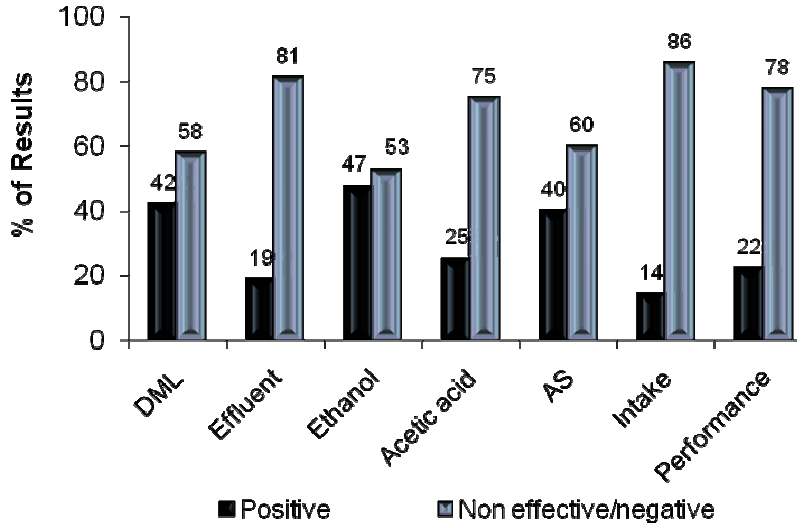

Figure 7. Results of published papers evaluating inoculation of *Lactobacillus buchneri* on sugarcane ensiling, over the variables: Dry Matter Losses (n=19); Effluent production (n=16); Ethanol content (n=19); Acetic acid content (n=12); Aerobic stability of silages (n=15); DM intake by ruminants (n=14) and performance of ruminants (n=9).

Of 31 experiments evaluated, 27 used the LB dose recommended by manufacturers, of $5x10^4$ CFU/g wet forage. Only the initial trials of Pedroso (2003) and Schmidt (2006) worked with a $3,6x10^5$ CFU/g wet forage dose.

Of the 19 trials that evaluated fermentative losses of DM, the LB inoculation was capable of reducing them to eight. In 9 trials, treatment effect was not verified, and Mari (2008) and Junges (2009) observed elevation in DM losses in LB inoculated silage. In average, the values of fermentative loss of DM were 26.7% ± 10.7 for silages without additive and 23.0% ± 11.4 for silages with LB additive.

The production of effluents in experimental silos was evaluated in 16 trials, presenting no treatment effect in 13 trials. The values observed vary between 6.0 a 95.9

L/t OM, however without showing themselves critical or excessive. The LB inoculated silages presented average production of 38.3 ± 19.9 L/t OM.

The ethanol content was reduced by the LB inoculation in nine of the 19 trials which evaluated this component. In average, the inoculation with LB reduced 2.9 percent units in the ethanol content, in relation to silage control, which presented average content of 6.7% of DM.

Only three among 12 trials verified significant elevation in the acetic acid content of LB inoculated silages, in relation to control. The average content of this acid for silages without additive and LB inoculated silages were 3.19 and 3.77% of DM, respectively.

Aerobic stability of silages inoculated with *L. buchneri* was evaluated in 15 studies. In six studies the bacteria was effective in significantly elevating stability, and in other five studies there was a numerical tendency of elevation. The average time for temperature elevation of 2ºC in silages was of 49 ± 15 hours and 63 ± 23 hours, for control silages and LB inoculated silages, respectively. For inoculated silages the longest stability time was 106 hours, verified by Mari (2008) and the shortest was 35 hours (Santos, 2007).

Ten authors carried out trials with animals, evaluating metabolism, milk production and weight gain. Fourteen studies evaluated the DM intake of goats (Mendes, 2006), lambs (Gentil, 2006), dairy heifers (Pedroso, 2003; Junqueira, 2006), dairy cows (Queiroz, 2006; Azevedo et al., 2007; Pedroso et al., 2009) and beef cattle (Schmidt, 2006; Mari, 2008; Balieiro Neto et al., 2008; Bergamaschini et al., 2008). Twelve trials did not verify effect of the silage type over voluntary intake, and the inoculation with LB was effective in elevating this variable only in Queiroz (2006) and Junqueira (2006) studies. In both studies the comparison was made in the remaining additive silages, for not evaluating silages without additives.

The performance of animals which intake diets containing silage of sugarcane inoculated with *L. buchneri* was evaluated in nine trials. Only two initial trials verified positive effects of inoculation. Pedroso (2003) observed increase of 0.3 kg in daily weight gain of Holstein heifers, in relation to control silage (0.94 x 1.24 kg/day). Schmidt (2006) observed elevation of 0.18 kg/day in weight gain of Nelore and Canchim beef cattle, due to inoculation with LB in ensiling.

Discussion. The bacteria *Lactobacillus buchneri* is the inoculant for sugarcane with the largest number of information available, probably due to good results obtained

in initial researches (Pedroso, 2003; Schmidt, 2006). However, animal performance trials carried out afterwards do not confirm these results for unknown reasons.

The variability in response patterns of this inoculant seems to be superior to those of other additives. Possibly, this effect occurs as we are dealing with live microorganisms, which depend on a great number of factors for a good development. The elevated variation coefficient inside the same trial reduces the possibility of an additive to show significant differences in relation to another additive. This effect is well marked for the "aerobic stability" variable.

The verified results in acetic acid content and aerobic stability differ from observations made by Kleinschmit and Kung Jr. (2006), who observed significant increase of LB additive over these variables, in an average of 43 trials with corn and grass silages.

New investigations, including on factors that determine high variability in responses of sugarcane silages to LB inoculation, are necessary to confirm the potential of controlling ethanolic fermentation and increase in nutritive values of silages shown in some trials. The strains of LB and the doses commonly inoculated need to be reviewed to assure more consistent results.

4. Additive association

Several authors have tried to increase the benefits of additives in sugarcane silages, associating products to possible synergic effects. Commercial additives containing different bacterial strains, associated to enzymes or chemical products have tried to minimize losses and elevate fermentative efficiency in silages.

Siqueira (2005) evaluated the association of chemical additives (urea, sodium benzoate and NaOH) and microbial (LAB and *L. buchneri*) in sugarcane ensiling and verified that the joint additive with LB enhanced the efficiency of chemical additives.

The association of *L. buchneri* and lime was evaluated by Roth et al. (2007) who verified positive results for the variable DM recovery, gas losses and effluent losses under lime addition (1% of OM), independent of inoculation or not with LB.

Muñoz-Maldonado (2007) evaluated the association of two microbial additives base don LAB with sodium benzoate, in sugarcane ensiling, without verifying any positive effect of inoculation with microorganisms, exclusive or associated to benzoate.

Recently, Junges et al. (2009) evaluated the association of microorganisms *Lactobacillus brevis*, *Enterococcus faecium* and *L. plantarum* in sugarcane ensiling, and verified prevailing lactic fermentation, with no benefits over the losses and stability of silages.

Although technically interesting, the association effects of different additives are not necessarily complementary, being able to elevate the variability in response patterns and making it even harder to comprehend obtained results. However, the use of two associated additives implies in elevating costs and logistic demands inside the farm, which limits its practical terms use.

5. Implications

The responses top additive use in ensiling present great variability due to non contemplated effects in experimental models, possibly due to epiphytic microorganisms populations in the material taken to the silo. These variations were observed by other authors (Muck e Kung Jr., 1997), especially when the animal component is analyzed (Kung Jr. e Muck, 1997).

The choice for an additive must be done analyzing the total cost of product application and the potential benefit in nutritive value and loss reduction. Available data indicate lime as the best additive option for sugarcane ensiling, although the benefit in animal performance has not yet been proved.

It is up to researchers to continue studies of additive evaluation in sugarcane ensiling, considering fermentation patterns, inherent losses to ensiling process, the mechanism of additive addition and variables of responses in animals.

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METHODS TO CHARACTERIZE MICROORGANISMS OF INTEREST PRESENT IN SILAGES

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INTRODUCTION

Silage is a major component of the diet fed to ruminants and the ensiling process relies on the anaerobic conversion of fermentable water-soluble carbohydrates to organic acids in forage crops with adequate moisture (McDonald et al., 1991). Lactic acid bacteria (LAB) usually dominate the ensilage and produce mainly lactic acid, though fermentation is also dependent on a variety of other epiphytic microbes (often detrimental); for instance, forage dry-matter content, water-soluble carbohydrates concentration, hybrid selection or stage of maturity (Bolsen et al., 1992). Additionally, enterobacteria, clostridia, yeasts and molds can be involved depending on the conditions at the time of ensiling. Hence, the use of microbial inoculants to suppress these microbes and ensure good silage quality is a recommended practice.

Traditional inoculants, which normally contain species of classical homofermentative LAB, often claim to promote a faster fermentation, shifting the end-products towards lactic acid and away from ethanol and acetic acid, thus preserving nutrients by inhibiting plant enzymes and detrimental microorganisms. In contrast, other bacteria are specifically marketed as enhancers of aerobic stability such as *Lactobacillus buchneri* and species of Propionibacteria, primarily by the production of acetic and propionic acids, respectively (Kung et al., 2003).

For a long time, the populations of these microorganisms that dictate the ensilage were enumerated by classical plating, where the microorganisms are grown on a medium that supports its growth. However, one important shortcoming of plating is that the cultivable fraction of most microbial ecosystems is still a minority.

Microscopic counts were done as means of comparison to the total viable counts done by plating, and the higher total microscopic counts were believed to be caused by the number of dead cells (Apatalahti et al., 2003). Nonetheless, molecular techniques

indicated that the majority of the bacteria could not be cultured. Unknown nutrient and/or growth factor requirements, dormant or latent organisms, the selectivity of the media that are used, the stress due to the cultivation procedures, the need of anaerobic conditions, and inability to reproduce the interactions with other microorganisms and host cells are some explanations for the unsuccessful cultivation (Zoetendal et al., 2004).

Therefore, in order to answer the most essential questions for environmental microbiology – "Who are there? What are they doing?" – It is essential to precisely investigate the microbial groups present in that environment and their roles.

The development of culture-independent techniques based on the analysis of nucleic acids has been increasing in popularity. The 16S rDNA encoding gene is a highly conservative region that can be amplified with universal primers and compared with a reference-sequence database to evaluate phylogenetic relationships. Since the bases of this conserved region are not neighboring each other, there is flexibility to design a universal or specific prime pair (Baker et al., 2003). Molecular protocols to identify, quantify and access the microbial population in silages such as denaturing gradient gel electrophoresis, terminal restriction fragment analysis, 16S rDNA cloning and sequencing are replacing phenotypic methods (Figure 1).

This short review aimed to provide a summary of the most common techniques to identify and compare the composition and structure of microorganisms in silages.

THE USE OF TRADITIONAL METHODS TO MONITOR MICROBIAL POPULATIONS IN SILAGES

Classical or traditional methods used for identifying microbial species have been based on microbial culturing, microscopy or phenotypic traits such as substrate utilization, fermentation products and enzyme activity. Nevertheless, these techniques are expensive and time-consuming, and often cannot clearly differentiate among the LAB species (Mackay, 2004).

Dutkiewicz et al. (1989) studied the concentration and species composition of the microflora found in the surface and upper layers of bulk and aerosolized corn silages. The authors used selective media, morphology, Gram reaction, substrate preference tests,

and microscopic, biochemical, and serological methods selected from *Bergey's Manual of Systematic Bacteriology* (Sneath et al., 1986) as tools for differentiation of the microorganisms. *Aspergillus fumigatus* prevailed among the fungi, while *Bacillus* and aerobic gram-negative organisms predominated among bacteria.

Lin et al. (1992b) reported that lactic acid bacteria comprised only a small fraction (< 0.5%) of the epiphytic microbial population in whole-plant corn and alfalfa, measured by traditional plating. The group *Enterobacteriaceae*, enumerated on violet red bile agar, was predominant in both standing crops, and yeasts and molds were also major epiphytic microbial groups but only in whole-plant corn.

Bolsen et al. (1992) evaluated the impact of additives on the microbial succession of alfalfa and corn silages using selective media. Overall counts of LAB were higher for one of the two treatments with microbial inoculants when compared to control; other microorganisms – *Enterobacteriaceae*, yeasts and molds, and clostridial spores – were not affected by treatment for either crop.

Lin et al. (1992a) estimated only the lactic acid bacterial population at species level during the pre-ensiling and ensiling periods of alfalfa and maize. *Lactobacillus plantarum, Pediococcus pentosaceus, Enterococcus faecium and E. faecalis*, enumerated on Rogosa SL medium, were predominant on both standing crops and, shortly after ensiling, the species most involved in the microbial succession were *L. plantarum* and *P. pentosaceus*. After 7 d of storage, *L. brevis, L. gasseri* and *L. homohiochii* became prevalent depending on the crop. In addition to selective media, Gram reaction, morphology, catalase production, soluble protein patterns, deamination of arginine, and tests for lactic acid configuration, optimum growth temperature, gas production from glucose and fermentation of 32 carbohydrates were conducted.

Grazia and Suzzi (1984) harvested LAB colonies from MRS agar plates and used the same phenotypic tests as the previous authors. They reported that lactobacilli were the predominant lactic acid bacteria, with *L. buchneri* being the most frequently recovered bacterium in 13 samples of maize and 10 samples of alfalfa silages. Among the homofermentative LAB, strains of *L. plantarum* and *L. casei* were the most recovered.

Torriani et al. (1992) studied the natural populations of LAB in growing maize and lucerne. The phenotypic characterization indicated *L. plantarum* and *Leuconostoc*

paramesenteroides as dominant species for both forages and *L. buchneri* as a major species in lucerne. The identification of strains was done by analysis of total cell proteins with sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE).

USING MOLECULAR TECHNIQUES TO MONITOR MICROORGANISMS IN SILAGES

The use of phenotypic methods for species-specific identification and quantification of bacteria are labor intensive and time-consuming and, furthermore, there is currently no selective media available to enumerate microorganisms in mixed cultures; therefore, genetic approaches are required. Specifically, ribosomal RNA genes are extensively used to classify bacteria and have already been used to identify and quantify lactic acid bacteria present in silages (Klocke et al., 2006; Schmidt et al., 2008).

Moreover, several methods were developed for the assessment of genetic diversity and, particularly, random amplification of polymorphic DNA (RAPD), terminal restriction length polymorphism (T-RFLP), temperature or denaturing gradient gel electrophoresis (TGGE or DGGE) and length heterogeneity polymerase chain reaction (LH-PCR) have been used to determine the microbial diversity of silages. Each technique has its advantages and limitations and these factors need to be considered when choosing which method is best suited for a particular study (Table 1).

The extraction of the microbial DNA dictates the success of the analysis

The variability in DNA extraction efficiencies and the presence of inhibitory compounds are the major obstacles for successful PCR analysis of environmental samples (Mumy and Findlay, 2004). Inconsistency in the yield and purity of the DNA can significantly affect the result of the microbial community analysis (vonWintzingerode et al., 1997).

Specifically, lactic acid bacteria are Gram-positive rod or cocci, hence the cells are more resistant to lyses than and DNA extraction from Gram-negative bacteria. Furthermore, the isolation and purification of the microbial DNA from silage samples can magnify this challenge.

Classical protocol for extraction of DNA from Gram-positive bacteria relies on lysis of the cells by physical, enzymatic and/or chemical treatments. Lysozyme and mutanolysin function by attacking peptidoglycans, a component of the cell walls of Gram-positive bacteria that is organized as a multilayer network (Kämpfer, 1995). Krsek and Wellington (1999) observed beneficial effects of lysozyme on purity of isolated DNA by acting directly on humic acids. Furthermore, they reported high yields of DNA by treatment with SDS and precipitation with ethanol. DNA isolated using lysozyme and SDS lysis is of higher molecular weight and can further be used for a wide variety of applications, including microbial community and diversity analyses. The drawbacks of this protocol are the labor and the time required.

Commercial DNA extraction kits currently available enable rapid recovery of PCR-ready nucleic acids from a large number of samples, but most researchers prefer to use more traditional methods to obtain higher yields of purer DNA, especially from complex samples (Wilson, 1997). Schmidt et al (2008) compared commercial kits to lysozyme and SDS, aided by overnight incubation with proteinase K and mutanolysin, and purified with phenol and chloroform. Compared to commercial kits, the DNA yield from lysozyme/SDS methodology was the highest and with good purity.

PCR and Real-time quantitative PCR

Polymerase chain reaction is an *in vitro* technique for the enzymatic synthesis of specific DNA sequences, using two oligonucleotide primers that hybridize to opposite strands and flank that region of interest in the target DNA (Saiki, 1995). Primarily used to produce copies of a specific sequence, PCR is also a powerful and precise way of altering a particular template sequence.

Utilization of PCR technology has proved to be sensitive and specific for rapid detection of lactic acid bacteria (Dubernet et al., 2002) and the 16S rDNA targeted end-point PCR technique has been shown to be a valuable technique to detect the presence of specific bacteria in silages (Romanov et al., 2004). Further down, the quantification of PCR products with the aid of the non-specific double-stranded DNA binding dye SYBR Green I can be accomplished with only minor changes in the protocol from the classic PCR. In addition, the SYBR Green I method allows high resolution of DNA fragments

100-1000 bp in size and has a large linear detection range, which is an essential requirement for quantification (Skeidsvoll and Ueland, 1995).

Real-time quantitative polymerase chain reaction (real-time qPCR) has been applied to monitor the dynamics of the specific LAB populations rapidly in a single process (Deng et al., 2008) but more research needs to be conducted to evaluate specific bacterial species, minimize the variations during preparation and purification of genomic DNA, and determine the influence of different types of silage crops under investigation.

Klocke et al. (2006) monitored the dynamics of selected bacteria (*L. plantarum*) in grass silages for the first time with a real-time qPCR. However, after 20 and 40 d of ensilage large variations were observed between the 16S rDNA copy number calculated by real-time qPCR and the LAB colony-forming units.

In contrast, Stevenson et al. (2006) quantified seven LAB species in alfalfa, treated or not with commercial silage inoculant formulations, and corn stover silages using primers designed based on *recA* gene sequences. At 4 days of storage, *L. brevis* was the most abundant LAB in alfalfa silages while modest populations of *L. plantarum* and *P. pentosaceus* were observed. The authors noted a lack of persistence of the silage inoculant strains, which varied according to the inoculant and the environmental conditions. High epiphytic populations of *L. plantarum* and *L. brevis* were detected in corn stover after 9 months of storage. *Lactococcus lactis* and *L. buchneri* comprised a small but consistent LAB population in all silages.

Schmidt et al. (in press) measured the population of *L. buchneri* in corn silage treated or not with it at the moment of ensiling, with the study replicated in different locations. After 120 days of storage, the specific fermentation profile from silages treated with *L. buchneri* matched the treatments and sites that had higher populations of this organism.

Schmidt et al. (2007) observed a small natural population of *L. buchneri* in alfalfa silage, not able to dominate the fermentation process. When applied at the moment of ensiling, the population of *L. buchneri* increased markedly after 2 and 5 days of ensilage. After 45, 90 and 180 days of storage, *L. buchneri* appeared to be the predominant bacterium in inoculated silages based on the fermentation profile of those silages.

DNA-DNA hybridization and DNA sequencing

DNA-DNA hybridization measures the degree of genetic similarity between complete genomes by measuring the amount of heat required to melt the hydrogen bonds between the base pairs that form the links between the two strands of the double helix of duplex DNA (Theron and Cloete, 2000). Under experimental conditions, "hybrid" double-stranded DNA molecules may be formed from the single strands of the DNAs of two species. The hybrid molecules are then dissociated in order to measure the melting temperature of the hybrid duplex. The experimental conditions are set so that only homologous sequences, i.e. sequences derived from a common ancestor, can form double-stranded structures.

Dellaglio and Torriani (1986) were some of the pioneers to use genetic tests to determine LAB populations in silage. The total 100 LAB strains that were identified in maize silages could be divided in four groups: *L. plantarum, L. casei* and *L. coryniformis*; *L. buchneri, L. brevis, L. fermentum* and *Le. paramesenteroides*; *P. pentosaceus* and *P. acidilactici*; and *E. faecium*, *Streptococcus lactis* and *S. bovis*. The authors found good correlation between the DNA-DNA hybridization and the phenotypic tests done.

Cai et al. (1998) harvested LAB colonies from forage crops and studied their impact on silage fermentation. DNA base composition and DNA-DNA hybridization were determined to identify the strains besides morphological, physiological and biochemical tests. Two epiphytic strains assigned as *Weissella paramesenteroides* and *Le. mesenteroides* and a commercial strain of *L. casei* were applied to alfalfa and Italian ryegrass at the time of the harvest. Only the commercial inoculant improved the silage quality and reduced fermentation loss, since it is a homofermentative LAB. A study with similar methodology and design was conducted by Cai (1999), evaluating *Enterococcus* spp. from forage crops and their effects on alfalfa and guine grass silages but no improvements in silage quality from inoculation were noticed.

Cai et al. (1999) repeated the previous model with *Pediococcus* spp. isolated from forage crops and applied as inoculant to alfalfa and Italian ryegrass. In addition to DNA-DNA hybridization, the authors used 16rRNA sequencing to identify 3 epiphytic strains used for the ensilage trial. Two strains of *P. acidilactici* were more efficient than the strain of *P. pentosaceus* and therefore, more suitable as silage inoculants. Initially,

methods to analyze microbial population relied on DNA sequencing from environmental samples. The rRNA genes can be amplified directly from the total sample DNA, cloned and sequenced. Universal primers can be used to amplify genes from all domains, or specific primers from a particular genus or species.

The automated fluorescent DNA sequencing machine relies on the use of a different colored dye for each of the four DNA bases. The Sanger method is based on the use of dideoxynucleotide triphosphates as DNA chain terminators and results in all fragments ending in one of the four labeled terminators correspondent to the dideoxy bases. The fragments are sorted by electrophoresis and the use of four different dyes permits the sequencing reaction to be performed in a single tube. The obtained sequence is then compared to sequences stored in online databases, which is done using a search algorithm such as FASTA (Temmerman et al., 2004).

Ennahar et al. (2003) sequenced 161 Gram-positive bacteria isolated from paddy rice silage to confirm the results obtained by phenotypic analyses. The molecular data was in agreement with the phenotypic results, except for one species. The homofermentative bacteria totalized 2/3 of total LAB, and the predominant species were *L. plantarum* (24%), *Lc. lactis* (22%), *Le. pseudomesenteroides* (20%), *P. acidilactici* (11%) and *L. brevis* (11%).

Although the majority of published microbial silage research has been done with LAB, Mansfield and Kuldau (2007) studied the mycobiota of fresh and ensiled maize with culturing techniques and a DNA sequence-based approach. Comparing the two methods, the molecular techniques detected a greater number of species than selective plating. Yeast was the majority of the detected fungi and *Fusarium* and *Penicillium* were the dominant mycotoxigenic fungi in silage. The abundance of *Penicillium* in spoiled silages is well-known. O'Brien et al. (2008) characterized the morphological, cultural and molecular characters of 237 isolates of *P. roqueforti* and 78 isolates of *P. paneum* from over 900 colonies cultured from baled grass silage. The molecular description was based on the partial sequences of β-tubulin and acetyl-co-enzyme A synthetase genes.

Random amplification of polymorphic DNA

RAPD protocols rely on the random amplification of anonymous DNA segments with short, arbitrary 8 to 10 bases-pair identical primers under low annealing temperature, in order to amplify multiple products that would represent different locus (Liu and Cordes, 2004).

Advantages of RAPD include: no previous DNA knowledge is required, the commercially available primers will bind somewhere in the sequence, although it is not certain exactly where. It is an inexpensive though powerful method for many bacterial species. Limitations of this technique include the inability to distinguish between homozygote (2 copies) and heterozygote (1 copy), the prerequisite of large and intact DNA template sequences, and low reproducibility due to the low annealing temperature used in the PCR amplification.

Rossi and Dellaglio (2007) studied the quality of farm-made silages in Italy by examining populations of LAB, clostridia, lactate-fermenting yeasts and propionibacteria using RAPD-PCR, sequencing of the V2-V3 16S rRNA gene region, 5.8 ITS rDNA RFLP and species-specific PCR. Among the LAB isolates, *L. plantarum* was the predominant species in alfalfa, maize and Italian ryegrass silages, but strains of *L. buchneri* were isolated in many samples. Anaerobic spore-producing organisms included 6 species of the genus *Clostridium* and *Paenibacillus macerans* and yeasts were identified as *Candida apicola*, *C. mesenterica* and *Pichia fermentans*.

Walker et al. (2009) used RAPD-PCR to discriminate between different strains of *L. buchneri*. Although some strain differences could not be picked by using 16S rDNA sequencing, RAPD-PCR was able to distinguish to the strain level.

Restriction fragment length polymorphism

This technique is based on an enzymatic restriction digestion of total DNA and separation by gel electrophoresis; the DNA fragments are transferred to a membrane via Southern blotting, hybridized to a labeled DNA probe to a final radioactive pattern. The RFLP analysis was developed before DNA sequencing techniques but it is still being used due to its low cost, although it is slow and cumbersome, and requires a large amount

of template DNA. More recent protocols utilize PCR instead of the Southern blot method (Liu and Cordes, 2004)

Chan et al. (2003) utilized specialized computer software to compare RFLP pattern from gels instead of the Southern blot of the total DNA digest. Electrophoretic patterns of DNA fragments from *Eco*RI digests of total DNA were utilized to analyze over 10,000 isolates of LAB from fresh and ensiled corn, alfalfa and ryegrass, and constructed a database of approximately 700 unique patterns. The database contained large homogenous clusters of *L. plantarum, E. faecium, L. buchneri, L. brevis* and *Pediococci*.

Denaturing gradient gel electrophoresis

Analogous to Temperature gradient gel electrophoresis (TGGE), DGGE is a form of electrophoresis on acrylamide gel where DNA fragments of identical length but different in sequence composition are separated by their denaturing profile (from double- to single stranded) when subjected to a chemical gradient. For each kind of application the optimum denaturing gradient has to be adjusted, although theoretically differences of a single base pair could be detected (Temmerman et al., 2004).

Major advantages of this well-established technique are low cost and simple interpretation of the results; furthermore, individual bands can be extracted from the gel and identified by sequencing. Shortcomings of this method include the presence of small fragments that contain insufficient information, lack of reproducibility and low sensitivity because of gel staining (Justé et al., 2008).

May et al. (2001) used DGGE on fungal rDNA amplified from total silage to identify fungi in maize silage; however, closely related rDNA sequences were complex to resolve. Yeasts were the predominant fungi and *P. anomala* was the dominant species present in silages after 2-3 months of fermentation.

Wang et al. (2006) evaluated a LAB community (Al2) from well-preserved alfalfa silage through continuous restricted subcultivation, containing *L. plantarum, L. kimchii, L. pentosus* and *L. faciminis*. The Al2 community was then used to inoculate alfalfa and, at different lengths of storage, the researchers studied the LAB diversity with DGGE. The DGGE bands showed that *P. pentosaceus* was the predominant species in untreated

silage; however, the components of the Al2 community, particularly *L. plantarum*, dominated the fermentation and were the prevailing bacterial species in treated silage.

Terminal restriction fragment length polymorphism

Similar to DGGE, T-RFLP is a technique based on PCR amplification that aims to produce a profile of an unidentified microbial community. In this method, the sets of primers used for the PCR assay have their 5' end labeled with a fluorescent molecule. The amplicons are subjected to a reaction using a restriction enzyme, and the resulting fragments are sorted using a DNA sequencer. Different from RFLP, only the terminal (and end-labeled) fragments are read because they are analyzed in a sequencer. The sizes of the different fragments are determined depending on the emitted fluorescence (Liu et al. 1997).

The use of a sequencer provides reproducible results for repeated samples and the digital output allows easy data storage for later comparison with other samples or experiments. Nonetheless, distinct sequences that share a terminal restriction site will result in only one peak on the electropherogram, and the possibility of false restriction fragments are some disadvantages. Another drawback is the impossibility to recover the sequences; thus, parallel analysis of clone libraries have to be done (Egert and Friedrich, 2003).

McEniry et al. (2008) described the bacterial profile of wilted perennial ryegrass ensiled as baled and precision-chop silage using culture-based and culture-independent T-RFLP with robust statistical analysis. The ensiling system had little effect on the overall community composition, except that *Enterobacteria* sp. was more abundant in the baled system compared to precision chop silage. *Leuconostoc mesenteroides, Le. carnosum, L. sakei and L. graminus* were present after 2 and 6 days of ensiling. Nevertheless, the authors suggested additional research involving the application of more-species-specific molecular techniques.

Length heterogeneity PCR

While DGGE/TGGE and T-RFLP target base changes in DNA sequence, LH-PCR uses naturally occurring sequence length-based differences to distinguish between

communities. The LH-PCR is a sensitive and reproducible technique that does not require post-PCR treatment; therefore the products can be directly loaded onto the genetic analyzer. Nevertheless, the software can impose a limitation when the profiles often present adjacent amplicon distributions complex to resolve; also, if one amplicon can represent more than one taxon that are distinct but produce the same length amplicon; moreover, clones libraries need to be constructed given that individual fractions or peaks cannot be collected (Mills et al., 2007).

Brusetti et al. (2006) monitored LAB succession in maize silages under ideal and spoilage-simulating conditions. During 30 days of storage, the LAB community peaked at 13 d in silages under optimal ensiling conditions. *Pediococcus* and *Weissella* predominated in the early days of fermentation, *Lc. lactis* and *L. brevis* were the dominant organisms after 6 d. The presence of *L. plantarum* was noticed at all stages of fermentation but it was only a fraction of the total LAB population.

CONCLUSIONS

The field of silage and environmental microbiology has observed a huge jump in qualitative and quantitative knowledge over the past 20 years. Due to limitation on probe/primers and the artifacts associated with the reaction, there is a need of improving accuracy, specificity and quantitative detection. Beneficially, the development of probes and primers is being reinforced by the increasing discovery of new sequences. Furthermore, to investigate the gene functions and the ecological purpose of the organisms that enclose them is a significant task.

Moreover, the development of new techniques for cultivation of microorganisms cannot be forgotten and has to be investigated. The successful cultivation of these microorganisms is still the most efficient way to study and characterize the genotypes and phenotypes of novel species.

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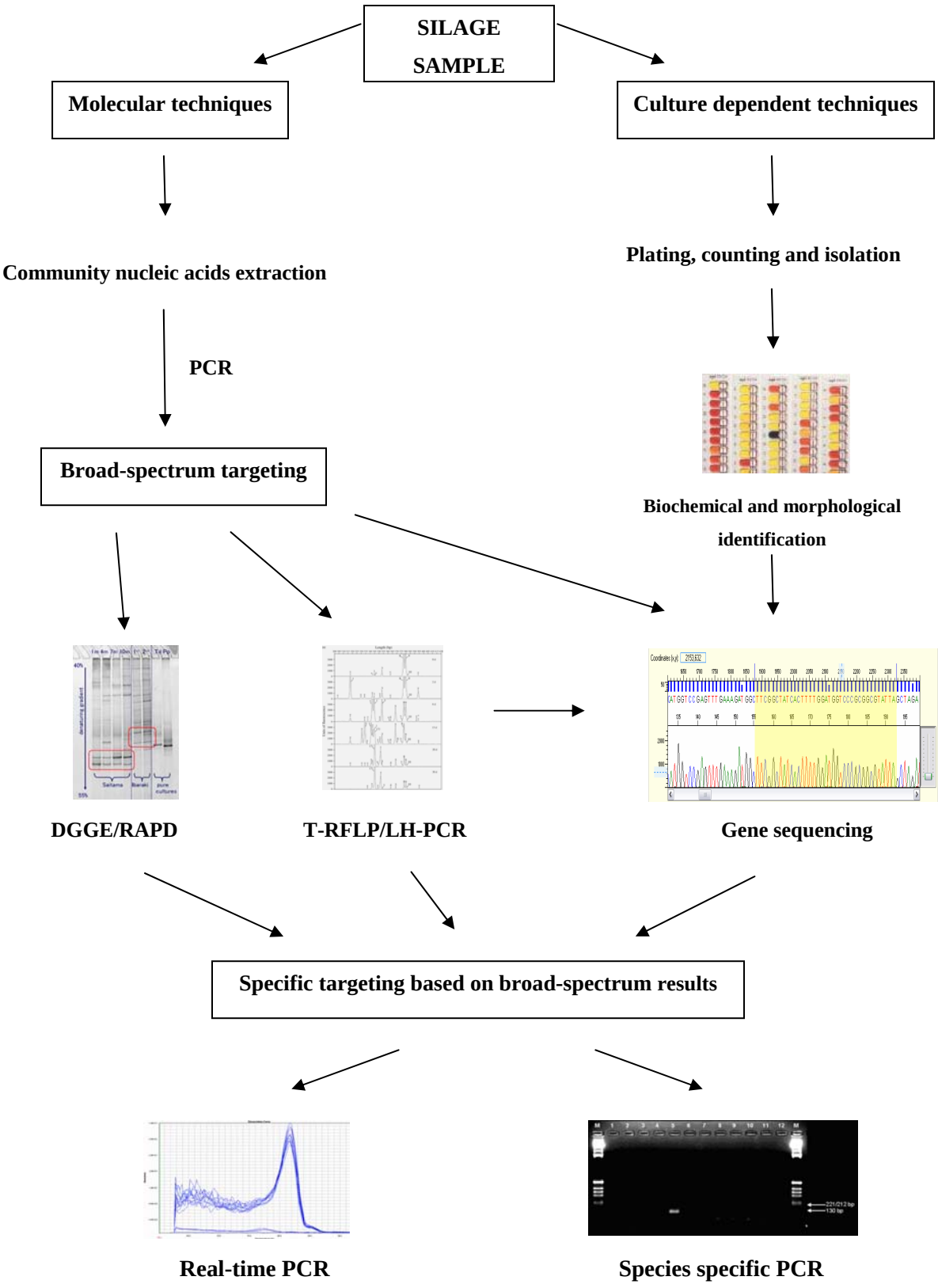
Table 1. Techniques that can be used to study silage microorganisms [adapted from Gilbride et al. (2006) and Zoetendal et al. (2004)].

Technique	Benefits	Limitations
Cultivation/	Easy to carry out	Not representative, laborious, slow
Media-based methods	Identification of individual microbes of interest	Majority of bacteria cannot be easily cultivated
		Selective media are not specific
Phenotypic test systems	Easy to carry out	Laborious, slow
	Identification of individual microbes of interest	Limited accuracy
PCR	Fast detection of specific microbes at species or genus level	Subject to DNA extraction biases
16S rDNA sequencing	Phylogenic identification	Subject to DNA extraction and PCR biases
		Laborious
RAPD	Knowledge of the DNA sequence of the target organism not required	Subject to DNA extraction and PCR biases
		Requires large, intact DNA template sequence
	Inexpensive and powerful	
LH-PCR	Allows a rapid analysis of a wide range of microbes and the monitoring of population shifts	Subject to DNA extraction and PCR biases
		Individual peaks cannot be separated
	Post-PCR treatment not required	Accuracy of the peak detection for longer domains
	Reproducible and fast	
DGGE	Allows a rapid analysis of a wide range of microbes and the monitoring of population shifts	Subject to DNA extraction and PCR biases
		Identification requires clone library
	Powerful and robust	
T-RFLP	Allows a rapid analysis of a wide range of microbes and the monitoring of population shifts	Subject to DNA extraction and PCR biases
		Identification requires clone library
	Potential for high throughput	

Figure 1. Flow diagram of methods used singularly or in combination to analyze microbial ecosystems [adapted from Justé et al. (2008) and Zoetendal et al. (2004)].



Opportunities for inclusion of tropical grasses silage in ruminant total mixed rations

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1 Introduction

The Brazilian livestock, nowadays, is one of the most powerful, supporting the largest commercial herd in the world. As it occurs commonly all over the globe, Brazil has a strong seasonality period of production in pastures during the year, with an increase in the production in the warm and rainy period, from October to December, followed by a decreasing or almost shortage period of production from the months of April to September, characterized by a dry and cold period and by smaller photoperiod, typical from the South-center region of the country, coincidentally the one which contains the largest bovine herd in the country.

The emphasized seasonality period of production in pastures has an important demand of feed to be used and conserved as silage and/or hay, of agro industry byproducts, differed grazing strategies, of adopting forage crops resistant to the winter conditions and of the irrigation of pastures (Rolim, 1986; Costa et al., 2008), among other resources, to the animal feeding supplementation in the period of the low forage availability.

The ensiling is often applied, overall to the corn silages (*Zea mays* L.) and sorghum (*Sorghum* spp), which are excellent but at a high cost, being the other species also applied, such as sugar cane (*Saccharum* spp.), the pearl millet [*Pennisetum glaucum* (L.) R. Br.] and the tropical perennial forage grasses, specially the ones belonging to the gender *Pennisetum*, *Panicum*, *Brachiaria* and *Cynodon*, among others.

The most applied perennial grass are the cultivars: *Pennisetum purpureum* Schum., *Panicum maximum* Jacq., *Brachiaria brizantha* (Hochst ex A. Rich.) Stapf, *Brachiaria decumbens* Stapf and *Cynodon dactylon* (L) species, and other species and varieties of *Cynodon* gender.

The tropical perennial grasses, or forages, have a high production of green forage, from a reasonable to a good quality, with major concentration in the summer mainly (rainy season), generating almost always a forage surplus which can perfectly harvested and conserved in a silage form, with a low cost, that will drastically reduce the problems coming from the small availability of green forage to the animals in the dry season of the year, if properly produced.

In this review the aim is to present part of the available strategies to the production of tropical perennial grass silages, of a good quality, and the opportunities of its inclusion in the ruminant ration.

2 Conditioning factors to the production and usage of tropical grass silages in the ruminant diets

The dry matter yield (DM) of the tropical grass can reach really high values, once they have the C_4 pathway and if their nutritional requirements were met. The ones from the species mentioned before are really high, except the ones from *Brachiaria decumbens*. Da Silva et al. (1996), approaching the Elephant-grass production potential (*Pennisetum purpureum* Schum.), the most productive among the tropical perennial, said that this grass intensively managed is capable of producing from 80 to 90 t ha^{-1} $year^{-1}$ of DM. The DM annual production of *P. maximum* grass (cvs. Mombaça, Tanzania 1, Tobiatã, etc.), *B. brizantha* (cvs. Marandu, MG-5 Vitória, Xaraés, etc.) and *Cynodon* spp. (cvs. Coastcross 1, Tifton 85, Tifton 68, Florona, etc.) can reach from 20 to 30 t ha^{-1}. Values of DM production of these grasses are showed on Tables 1 and 2.

Table 1. Dry matter yield (DM) of *P. purpureum* cv. Napier and *Cynodon* spp. cvs. Coastcross and Florona with different nitrogen (N), Potassium (K_2O) and phosphorus (P_2O_5) fertilization levels.

$N + K_2O$ (kg ha^{-1})	Napier Ir[1] DM (t ha^{-1})	Napier Ni[1] DM (t ha^{-1})	P_2O_5 (kg ha^{-1})	Coastcross[2] DM (t ha^{-1})	Florona[2] DM (t ha^{-1})
400 +320	21.65a	23.65a	0	15.09	14.85
300 + 240	21.38a	20.31a	40	16.07	14.12
200 + 160	17.15a	17.16ab	80	15.90	14.51
100 + 80	14.26b	12.20c	120	16.18	14.26
Average	18.61	18.39	Average	15.81a	14.44b

Adaptation from data by Mistura et al. (2006)[1], Napier forage irrigated (Ir) and no irrigated (Ni), in 2001 dry period (April until September), Viçosa-MG; Averages with different letters, in column, are statistically different according to Tukey test ($P<0.05$); Santos (2004)[2], Average production of three P sources (triple superphosphate, Arad phosphates and Araxá phosphates), for each P_2O_5 dose, in 2001/02 dry period (October until April), Lavras-MG; Averages with different letters, in line, are statistically different according to Tukey test ($P<0.01$).

Table 2. Dry matter yield (t ha^{-1}) of tropical forages *Cynodon* sp. cv. Tifton 85, *P. maximum* cv. Tanzânia 1 and *B. brizantha* cv. Marandu irrigated and no irrigated and combined analysis of means.

Forage	Water regime		combined analysis
	Irrigated	No Irrigated	(Mean)
Tifton 85	17.96	16.99	17.48a
Tanzânia	17.98	17.58	17.78a
Marandu	16.35	13.98	15.16a
combined analysis (average of harvest)	17.43A	16.18A	

Source: Santos et al. (2008), 2004/05 wet period (November until April), Itapetininga-BA; Averages with the same minuscule letter in column and capital letter in line are not statistically different according Tukey test ($P>0.05$).

Besides the photosynthetic characteristics, the soil and the climate have a great influence on the growth and development and, consequently, on the forage crops plants production (McKenzie et al., 2002). The climate can be understood as the mean and typical pattern of the weather in a certain region, settling the conditions to the plants growth and its area of distribution, imposing limits to its survival. (Larcher, 2000).

According to Volenec and Nelson (2003), the climate refers to the long term report of temperature, precipitation and radiation for a given region. Light (or radiation), temperature and humidity of the land are the three main environmental factors which affect the adaptation, the vegetative development and the forage crops species reproduction. Understanding how these environmental factors affect the forage crops is fundamental to develop management models and techniques which might help to assure the high productivity of the pastures.

In the Northeastern Brazil, where there is a semi-arid climate, the forage production shows a strong seasonality period due, mainly, to the unequal distribution of rain, limiting the corn production to the ensiling. However, the grass silages have been showing as an opportunity of forage production in the dry period, and due to their vegetative characteristics, these grasses present a high forage productivity of a good nutritive value, with relatively competitive costs.

On the other hand, in many regions during the rainy season, because of a more favorable climate conditions, the plants growth in some months is so intense that exceeds the herd DM feed intake demand, being necessary some stocking adjustments, mainly in the fertilized systems.

This management, varying the animal number for area unit also in the rainy season, sometimes becomes not so practical or not so interesting to the producer. In this context, the conservation of the pasture surplus by the ensiling and/or haylage has been shown as an interesting opportunity in the feed deficit control. This is a widely used technique in Europe, being part of the pasture management. This implicates in the manipulation of the stocking rate in the pasture areas, thus increasing the pasture controlling flexibility. Nevertheless, because part of the total pasture area is applied to the forage conservation and these areas are isolated from the grazing areas for a couple of weeks, the control is not precise (Hodgson, 1990). Futhermore, the forage accumulation to conservation probably reduce the adoption and the potential of future regrowth. On the other hand, if the goals are to reach more elevated levels of animal performance under variable conditions of forage supply, the maintenance of high stocking rates will only be possible by adopting a very flexible herd management and/or a predisposition to the supplemental feed usage.

The forage conservation is a key component in many animal production systems in those regions where in any time of the year the forage crops growth is very slow or almost null. In these conditions, the value of this management practice is incontrovertible.

Intermittent stocking is the grazing of two or more paddocks, in sequence, followed by a resting period to the grazed forage crops recovery. The principal advantages of this method, according to Matches and Burns (1995), are the best plants persistence, more pasture conservation opportunities and more time to use the forage. The intermittent stocking presents a bigger number of modalities than the continuous stocking, all of them involving the integration with the conservation. In the

conventional intermittent stocking the animals stay in the paddocks for a period of time (grazing period), followed by a resting period. In this modality, paddock number and occupation and fixed resting period and similar size paddocks are used, defined previously (Mayne et al., 2000). This method is one of the most found in Brazil when talking about systems of animal production in pastures which adopts the intermittent stocking, probably because it is presented as an easier way of management.

According to da Silva (2004), management recommendations like those are inefficient and inconsistent, causing qualitative and quantitative loss to the animal production. And yet, according to the author, the grazing management strategies must try to find an optimum balance between the growth process, senescence and feed intake, so that a forage production raise of good quality is possible, being these management actions specific for each condition of use and production. This indicates that the stocking rate must be flexible so that it adequate to the ecophysiological limits of the plant, while the paddock number can rise or not, using electric fence. In those situations when the pasture offer exceeds the demand, the forage surplus in the paddock can be conserved as hay or silage, in the beginning of the season, when the growth rates are high.

In Brazil, the pastures management associating grazing with forage conservation is not common. However some studies with silages of tropical forage, integrating grazing and forage conservation, seeking to keep the animal stocking more stable and raised all year long have been led. Corrêa et al. (2001), working with intensive intermittent stocking, checked that as forage surplus occurred, part of the paddocks was being reserved to the silage making. The harvest was made in the 55^{th} day of the plant growth of Tanzania-grass and in the 30^{th}, 35^{th} and 45^{th} day to the Coastcross grass. The silage supply started in June, when the produced forage in the pastures was not enough anymore to keep the stocking defined. Each animal fed with Tanzania-grass silage mixed to the silage, 0.5 kg of Soybean meal per day. During the dry climate, the resting periods passed to 60 and 48 days respectively, to the pastures of Tanzania-grass and Coastcross grass, having the animals free access to the pasture and to the silage

According to the authors, the use of the silage in the dry climate, integrated with the grazing, was possible to maintain both systems intensified all year long, with a relatively stable herd, fed practically only with forage from the rotational system. In the Tanzania-grass pasture case (background-finishing) was possible to obtain Canchim beef cattle with slaughter weight around 450 kg of live weight, 19 to 20 months old. In

the rainy season, the feeding consisted only of grazing forage, with an average gain of 850 g animal^{-1} day^{-1} and, in the dry season, grazing forage added with silage from the forage surplus from the rainy season and 0.5 kg of soybean meal, with an average gain of 440 g animal^{-1} day^{-1}.

3 Indispensable cares to the making of good tropical grass silage

To obtain good qualities silages of tropical perennial grass, special care are required regarding the restrictions such as the high concentration of humidity and low soluble carbohydrate content and the high buffering capacity presented by the green forage at the best nutritional moment and which must be ensiled. The standard corn silage shows DM contents around 30 and 35%, however for the perennial grass contents higher than 20% are acceptable. Lavezzo (1993), in a review of ensiling of elephant-grass, mentioned that in forages with less than 20% of DM the high humidity will reduce the conservative effects of the primary acid fermentations and the growth of *Clostridium* can be not restricting in pH values from 3.8 to 4.0. To the elephant-grass cultivars, as informed by Lavezzo (1993), several approaches can be used to determine the moment of harvesting to the ensiling such as the age of the plant being around 60 and 90 days (70 days as average); height varying from 1.5 to 1.8 m and the leaf: steam ratio is 1.0. Ávila et al. (2006), when working with cv. Tanzania, say that the tropical perennial grass must be harvested younger, with the age of 60 and 70 days, to obtain of the best nutritional value of silage.

The low soluble carbohydrate contents of the tropical grass, in general, will not provide silages rich in lactic acid. Lavezzo (1993), reviewing several authors, reported that the lactic acid content must be around 10%, preferably around 13 and 16% in DM. Associating the DM concentration to the soluble carbohydrate contents, McCullough (1977) mentioned that the ideal fermentations of the ensiled mass happen when DM is from 28 to 34% and values above 6 to 8% of soluble carbohydrate, since the buffering capacity is not raised. The soluble carbohydrate / buffering capacity ratio is determining in the ensiling process, according to Vilela (1997). The author adds that when this ratio decreases, a minimal increase in the DM content is required to prevent undesirable fermentation in the interior of the silo.

The buffering capacity, the resistance offered to the lower values of pH in ensiling, decreases while the forage crops get older. Lavezzo (1993), revising studies

with elephant grass, quotes values of buffering capacity reducing from 55.26 to 36.81 mg g^{-1} of DM when the age of cv. Napier raised from 51 to 121 days, while to other cultivars of elephant grass these values were reduced from 38 to 40 eq. mg of HCl by 100 g of DM, at the age of 37 days de MS, to 13 to 17 eq. mg of HCl by 100 g of DM at the 67^{th} day of growth.

Tropical perennial grass restrictions known to the silage production of good quality, techniques such as wilting and the use of different additives establish the tool applied to avoid such limits.

4 Opportunities to the application of byproducts in tropical grass silages

The tropical grass stands out for being DM high potential production plants and good chemical composition. However, there is a significant decrease in the nutritive value across the maturity. One of the techniques used to the management of these forage crops is the ensiling use of the material to a superior conservation of its nutritive value. This grass, however, presents a low content of soluble carbohydrates and DM contents of around 20%, insufficient to guarantee a good fermentation. An alternative to improve the fermentative standards and, consequently, the nutritive value of the tropical grass silage is the use of additive.

In Brazil, it has been increasing the use of agroindustrial byproducts or co-products as silage additive, mainly in the regions where there is a great availability of these substrates. Its application aims to reduce the ruminant feeding costs and to minimize the environment pollution. Several agroindustrial byproducts are being evaluated and have already been applied as additive in the perennial grass silages. Among these, the fruit byproducts, agricultural byproducts and the like stand out.

4.1 Byproducts from the fruit processing

4.1.1 Pineapple

During the pineapple industrialization, hulls, stem, crown and cylinders, considered as rejects and which correspond to, in average, 35% of the processed raw-material weight. The pressing of these rejects results in juice and pie, which are used in

animal rations in an empirical way. From the total pressed rejects, 75 to 85% are juice and 15 to 25% are meal (Py et al., 1984).

About the byproducts use in the pineapple juice production, Oliveira Filho et al. (2002) evaluated the nutritive value of the elephant grass silages containing different levels of pineapple byproducts and observed that the silage DM contents increased progressively with the sub product addition. The minimum level of 30% of DM, considered ideal by Lavezzo (1988), was not reached, but near values were observed with the addition of 20% of pineapple sub product (28.89% of DM). About the values in neutral detergent fiber (NDF), it was observed that with the addition of the pineapple sub product there was a reduction in their contents and for each addition of 1% it was registered a decrease of 0.35% in the NDF in the silages, reaching a minimal of 63.88% of NDF when adding 20% of the sub product.

4.1.2 Cashew

Ferreira et al (2002) observed that the addition of 36% of cashew bagasse in the elephant-grass silage caused a rising of 63% in the CP contents. While in the pure silages the values were 3.6%, in the ones containing 36% of cashew bagasse, they were 9.6%. The authors observed as well that the cashew sub product addition induced to a decrease in the pH values and in the ammoniacal nitrogen contents, showing this way that the fermentative process was improved.

Teixeira et al. (2003) evaluated the productive performance of ovine fed with elephant-grass silages containing dehydrated cashew bagasse (12% in DM basis) comparing to pure elephant-grass silage. On trial with confined the five months old ovine, approximately, receiving ration supplements concentrated in the proportion of 1.5 and 2.5% of live weight. The researchers observed that when the animals received supplements with the concentrate 1.5% of live weight (about 340 g day^{-1}), they showed a bigger weight gain (110 g day^{-1}) when the forage was the elephant-grass silage with cashew bagasse. The animals fed with the same quantity of concentrate ration, still with pure elephant-grass silage, presented a weight gain of 66 g day^{-1}. The authors also observed the increase in the feed conversion when it was used the pure elephant-grass silage plus concentrate.

4.1.3 Guava

According to Arraes (2000), the guava presents a juice yield of 75%, generating around 25% of waste. This waste can vary according to the methods used in the processing, production purpose (pulp, juice, purée, candies, etc.) the equipments and the efficiency of them. The generated waste can show the following compositions: pure seeds, seeds and rejected fruit, seeds and purée, seeds and purée and rejected fruit.

Neiva et al. (2002) evaluated the elephant-grass silages nutritive value containing different levels of the sub product of guava pulp production. The authors observed that the DM contents increased linearly when the addition of the guava byproduct was made, the way that for every 1% of byproduct addition, it was observed an increase of 0.5 in percentage in the silage DM contents. There was also an increase in the silage CP, NDF and ADF contents with the addition of guava byproduct.

4.1.4 Passion Fruit

The main waste component of the passion fruit is the hull, being that fundamental to the generation of more information about its nutritive value. The passion fruit hull which performs, in average, 50% of the purple variety and 50-60% of the yellow passion fruit, is considered as “loss factor”. However, its chemical composition and nutritive value have been given more attention in the past years.

Aquino et al. (2003) evaluated the fermentative and nutritional characteristics of the elephant-grass silages containing different levels (0.5; 10; 15 e 20%) of passion fruit juice production byproducts. The sub product addition promoted a raise of 0.44 percent units in the DM contents for each 1% of addition. However, the ideal DM content of 30-35% quoted by McDonald (1981) to the occurrence of a good fermentative process was not reached. Now the CP content increased 0.17 percentage unit for each 1% of sub product addition to the silages. It is pointed out that for this variable, the addition of 0.47% of sub product made the CP content reached the ideal level to a good ruminal performance (7%). The addition of passion fruit sub product did not altered ($P>0.05$) the silage pH values, but the observed values were kept within 3.8-4.2.

Reis et al. (2000), in a study performed in 1992, used different levels of passion fruit hull addition in elephant-grass ensiling, concluding that 75 and 50% were the best addition levels.

4.2 Agro industry byproducts

4.2.1 Potato

Approximately, 35% of the produced potato is rejected in the industrialization process. There are different forms of processing the potato for use in animal nutrition, including the potato meal (plant tubercle waste without being processed, dehydrated and floured); moist potato (potato processing waste to the human feed, composed, most of it, by the used potato hull with no dehydration); potato filter meal (representing 20% of the potato total waste resulting from the vacuous percolation); potato flakes (waste obtained from the cooked potato which is mashed and dehydrated) and potato pulp (waste from the leftovers after the starch extraction). The nutritional characteristics of this waste are similar to the ones from the raw potato. (Church, 1991).

Rezende et al (2007), working with elephant-grass silages added with various proportions of potato scraper, verified that as the quantity of potato was increased lower NDF and ADF and higher CP contents were registered, and also higher values of IVDMD were registered related to the testified silage.

Tavares (2009) worked with elephant-grass silages added with potato meal in the proportions of 0; 7 and 14% comparing to the corn silage in the milk cows diet. The addition of potato sub product in the elephant-grass ensiling improved the DM ingestion, as well as the CP, EE and the P element, while there was a reduction of the ADF consumption. The addition of 14% of the potato waste in the elephant-grass silage became similar to the corn silage for the production and composition of milk.

4.2.2 Coffee hulls

The coffee hull, waste from the coffee bean improvement, presenting high DM content and good capacity of humidity absorption has been applied to the tropical perennial grass ensiling.

Bernardino et al. (2005) worked with coffee hull added to the elephant-grass in five levels of inclusion (0, 10, 20, 30 and 40%). The coffee hull addition in the elephant-grass ensiling with 12% of DM improved the silage fermentative characteristics, reducing the pH values and the ammoniacal nitrogen contents. However,

its inclusion increased the NDF, ADIN and lignin concentration, besides the IVDMD reduction.

Carvalho et al. (2007) also worked with the coffee hull added to the elephant-grass, but with different inclusion levels (0, 6, 12, 18 and 24%) compared to the previous study. The authors verified some improvements in the silage fermentative characteristics, with in decrease in the pH values and in the ammoniacal nitrogen contents and in the butyric acid and an increase in the lactic acid contents, promoting this way a better prevention of the ensiled material. Also, the IVDMD values reduced with the increase of coffee hull addition.

4.2.3 Cassava

The cassava processing involves the making of flour and starch extraction, also called cassava starch. These processing results in a great variety of solid waste such as peels, rejection and bagasse, or liquid waste such as manipueira and the water used to wash the root. When released in the environment they can cause serious problems of pollution because, besides the high organic values, they present a compound which can generate cyanide, toxic compound to most of the aerobic species.

Ferrari Jr. e Lavezzo (2001) worked with elephant-grass silage wilted and added with potato meal, verifying that the withering is a feasible alternative to decrease the humidity, but the cassava meal can raise the DM and soluble carbohydrates contents of the silage. These authors, when evaluating the quality of the wilted elephant-grass silage with an inclusion level of cassava meal up to 12%, observed an increase of 7.5% in the DM contents (28.61%) of the silages with additives which are in higher levels of meal regarding the ones with no additives (26.61%). Though, this increase was not enough to improve the silage quality because there was no significant difference on the pH values, ammoniacal nitrogen and butyric acid.

4.3 Biodiesel production byproducts

Brazil presents great potentialities to the oil plants cultivation for the biodiesel production because of its climate and ecosystem diversity. The main oleaginous cultivated in Brazil, which could be used in the making of biodiesel, are the soybean (*Glycine max*), the sunflower (*Helianthus annuus*), the castor oil plant (*Ricinus*

communis), the palm oil (*Elaeis guineensis*), the jatropha (*Jatropha curcas*), the forage turnip straw (*Raphanus sativus*), the cotton (*Gossypium* spp.), the peanut (*Arachis hypogaea*), the canola (*Brassica napus*), the sesame (*Sesamum orientale*), the babassu (*Orrbignya speciosa*) and the macaúba (*Acrocomia aculeata*) (BiodieselBr.com, 2008).

Most of the oleaginous meals which have been used to the biodiesel production in Brazil are susceptible to use in animal feeding, but each one with their particularities regarding the cares before being supplied to the animals due to the presence of some toxic or anti nutritional factors. As examples, it is mentioned the castor oil plant meal whose application is limited by the ricin and allergenic principles (Castor Bean Alergen – CBA), which can go to the milk of the cattle fed with it (Evangelista et al., 2007). And the jatropha meal which presents a phorbol ester that has carcinogenic activities and inflammatory action. The jatropha and castor oil plant meal have a high CP content and after taking the intoxication out, they can be used in the animal feeding.

Van Cleef (2008), in an essay *in vitro* comparing the jatropha meal and the forage turnip straw addition in elephant-grass silages, verified a reduction in the IVDMD and an increase in the pH values, in the ammoniacal nitrogen and in the lignin, proportional to the addition of the meal increasing levels to the silages. These ones, added with jatropha presented worse quality and IVDMD compared to the testified silages and added with forage turnip straw.

5 Economical feasibility of the tropical grass silages

The usage of tropical grass silages to substitute the traditional corn silage is seen by many technician and producer as a technology capable to increase the efficiency in the pasture use, through the surplus harvesting produced during the rainy season. Also it is capable to minimize the feeding costs in feedlot, the milk cattle as well as the beef cattle, because of the greater possibility of optimize the land factor use, due to the greater productive potential which this forage resource presents regarding the corn and sorghum silages. However, as it was demonstrated by Igarasi et al. (2002), the high tropical grass productivity potential, capable to make them economically competitive forages, is not obtained in most cattle breeding settlements which use them. These authors observed a big variation extent of the production for these crops and very low values of the potential production preconized in literature. (Table 3)

Table 3. Tropical grasses yields

Production (t ha^{-1})	Range		Mean
	Minimum	Maximum	
Original matter harvest $^{-1}$	14	35	23
Dry matter harvest $^{-1}$	2.7	8.1	5.4
Original matter year $^{-1}$	42	105	65
Dry matter year $^{-1}$	8.1	24.3	17

Source: Igarasi et al. (2002)

Once the well managed corn farming produces from 13 to 14 t ha^{-1} of DM, able to reach 20 t ha^{-1} of DM in optimum conditions (Siqueira et al., 2007), the average productivity observed to the perennial grass silage (17 t ha^{-1} year^{-1} of DM) become not so attractive, even more considering that, in average, it is necessary to harvest three times so that the forage is produced in an efficient way, increasing the labor force to the silage making.

In the perennial grass silage case, it is believed that one of the principal causes of low productivity is the low fertilization used in the appropriate areas for its production. This way, once the decision is made to the silage production of perennial crop, the fertilization must be conducted to obtain high productions. (Siqueira et al., 2007).

The impact of the Tanzania grass productivity over the bio economic value of the diet formulated with this forage was shown in simulations made with feedlot cattle Muraro et al. (2008). Considering a weight gain of 1.25 kg animal^{-1} day^{-1}, these authors verified that the liquid profit per produced arroba (15 kg) has only been favorable to the ration containing Tanzania grass silage when this grass presented high production of DM (29 t ha^{-1} year^{-1}). But, considering the income per area, all the ration containing Tanzania grass silages presented a superior income if compared to that presented to the ration containing corn silage of low productivity (12 t ha^{-1} year^{-1} of DM) (Table 4), suggesting then being an advantageous strategies to make the use of these silages possible, that is, exploring the production scale to maximize the profit per area and not the performance of the animals individually.

Table 4. Charge and income simulation on finishing beef cattle fed with corn silage or Tanzania silage, estimated gain of 1.25 kg animal $^{-1}$ day $^{-1}$

Forage	Corn silage		Tanzania silage			
	DM Yield, t ha^{-1} year^{-1}					
	12	14	17	21	25	29
Forage, % DM diet	41.9	41.9	26.8	26.8	26.8	26.8
Silage cost, R$ t^{-1} DM	247.50	212.00	305.50	247.00	208.00	179.00
Diet cost, R$ t^{-1} DM	369.54	354.63	409.98	394.28	383.81	376.03
@ (15 kg) Cost, R$	59.49	57.08	65.99	63.47	61.78	60.53
Liquid profit, R$ t^{-1} diet	89.52	104.43	49.08	64.78	72.25	83.03
Liquid profit, R$ ha^{-1} year^{-1}	2563.18	3488.50	3108.76	5068.73	7000.20	8971.68
Liquid profit, R$ @$^{-1}$	14.41	16.82	7.91	10.43	12.12	16.82
Relative index, RL ha^{-1}	100	-	121	198	273	350
Relative index, RL @$^{-1}$	100	-	55	72	84	117

Source : Muraro et al. (2008)

Although fundamental, the productivity is not the only factor which might be considered when it is intended to produce tropical grass silages. Another important aspect when choosing the forage is the nutritive value, since this factor determines the degree of its participation in the formulated diet. As the corn silage presents an energetic content higher than the tropical grass silages (10% more of TDN, in average), the TDN kilogram cost of the corn silage is lower than the same TDN kilogram of a perennial grass silage. This way, diets taking as basis corn silage present a bigger forage participation, while diets formulated based on tropical grass silages present the biggest participation of concentrate, because of the lower TDN content (Pereira et al., 2007). Thus, ration made with grass silages reach the cost, in average, 15% higher than the ones obtained with the other forages in the same weight gain rate (Muraro et al., 2008). Although these observations do not make the technology use impossible, they serve to demystify the idea of silage low costs for tropical grass. (Pereira et al., 2007; Muraro et al., 2008).

According to Pereira et al. (2007), the bio economic value of conserved forage diets depend of the concentrate feed price. That is, as the relative price of the energy source is reduced, the bio economic value of the high performance forage crops of DM per area and of a lower production cost, even with a lower nutritive value (lower TDN), as the tropical grass silages, become more attractive than the sorghum and corn silages. This way, the nutritive value loses its importance and increases the weight of the forage profit and the production cost of DM in the conserved forage bio economic value. Now in an opposite situation, where the relative price of the energy source is high, the forage nutritive value is the main factor which determines its bio economic value, even

considering the area, because it permits to minimize the use of the concentrate in the diet.

When simulations of the bio economic value of diets with conserved forage to beef cattle feedlot were made, with live weight starting on 400 kg, weight gain of 1.5 kg animal^{-1} day^{-1} and ton cost of the energy source feed of R$ 319.46 Brazilian real, Pereira et al. (2007) noted that the most economically attractive options, under the animal performance aspects or per area, were the corn and sorghum basis diets. (Table 5). The higher energy value of these silages (mainly the corn ones), associated to lower TDN production costs favored them, considering the valid prices of the energy source feed. Regarding the animal performance, the tropical grass silages have shown little attractive with an average cost of diet production, per arroba, being 19% higher than the corn silage, and, in spite of the high revenue of DM per area, the tropical grass silage use resulted in an economical loss, considering the feed analysis only.

Table 5. Simulation of economic values of conserved forages in diets for feed lot cattle

Item		**Silage**				
	Unit	**Corn**	**Sorghum**	**Elephant**	**Tanzania**	**Braquiarão**
TDN	%	65	61	56	56	57
Forage on diet	%	51	42	36	35	35
Cost of						
forage	R$ t^{-1} DM	189.25	171.90	176.52	186.35	206.59
concentrate[1]	R$ t^{-1} DM	319.46	319.46	319.46	319.46	319.46
@ selled (A)	R$ @$^{-1}$	55.00	55.00	55.00	55.00	55.00
Income per animal	R$ animal/day	2.75	2.75	2.75	2.75	2.75
Income per area (B)	R$ ha^{-1}	7084.80	9553.20	23426.70	16850.30	11787.70
Cost of						
@ produced (C)	R$ @$^{-1}$	53.43	54.30	56.51	56.90	58.30
feedlot animal	R$ animal/day	2.67	2.71	2.83	2.85	2.92
DM t	R$ t^{-1} DM	283.00	287.60	299.30	301.50	308.82
per area (D)	R$ ha^{-1}	6882.80	9431.50	24068.90	17444.10	12496.00
Feed balance						
@ Produced						
E = (A - C)	R$ @$^{-1}$	0.08	0.04	-0.08	-0.10	-0.17
Area F = (B - D)	R$ ha^{-1}	202.00	121.60	-642.20	-593.80	-708.3
Cost-benefit ratio	**%**	**2.9**	**1.3**	**-2.7**	**-3.4**	**-5.7**

Source: Adapted from Pereira et al. (2007)
[1] Corn + citrus pulp (1:1)

Besides, according to the authors, for the gain of live weight of 1.0 kg day^{-1}, the difference in the production costs, per arroba, between diets with the sorghum and corn silages and the tropical grass, are higher than for the gain of 1.5 kg day^{-1} due to the bigger nutritive value of the sorghum and corn silages, reflecting in a bigger forage participation in the diet to a lower level of performance. Concentrate high price and low levels of performance conditions, the advantages of the sorghum and corn silages when

compared to the tropical grass silages are enlarged because of the lower energy source concentrate, of high cost, in the diet.

In simulations carried out with dairy cattle and with the same prices of the energy source, Pereira et al. (2007) obtained the same conclusions observed to the beef cattle, that is, higher profitability, measured by the benefit-cost ratio in favor of sorghum and corn silages and the difference enlargement against the perennial grass silage, for the lower level of production simulated (30 kg versus 15 kg of milk cow^{-1} day^{-1}). Of course the price relations simulated by Pereira et al. (2007), of 5.8 @ t^{-1} of concentrate or 0.6 kg of milk kg^{-1} of concentrate, are not the same ones observed at the moment, however, what is clear is that it is not enough getting high productivities so the tropical grass silages are economically feasible, the energy source cost is a determining factor when deciding to adopt these forages.

Another issue which deserves to be commented is regarded to the ideal level of concentrate inclusion in diets formulated with tropical grass silages. To illustrate the importance of the diet adaptation and the animal performance on the bio economic feasibility of tropical grass silages, Pereira et al. (2008) evaluated the earning cost of the beef cattle carcass, finished in feedlot, from the experimental data obtained by Vieira (2007), with formulated diets with four levels of concentrate (20, 35, 50 and 65%, in DM basis), of which used forage was the silage of Tanzania-grass harvested in the 100^{th} day, and they observed that the bio economic levels of concentrate introduction in the diet were located between 50 and 60% of DM. It is important to point out that the bio economic value of the forages is not static because it depends on the animal production level, the availability of feed and its price (Ely, 1992). This way, at the moment of the forage choice, factors like animal production potential, concentrate availability, production area availability, crop regional ability and producer ability are the main aspects to be considered (Nogueira, 2004).

To be able to ally production with quality, the tropical grass can not be ensiled in advanced vegetative stage. Nevertheless, its ensiling in a earlier vegetative stage presents some limits due to DM low contents, high buffering capacity and low contents of soluble carbohydrates, which justify the use of conditioning additives (Coan et al., 2008).

As the bacterial inoculants have not presented consistent results (Pereira et al., 2002), the additive usage which absorbs humidity and, at the same time, supplies carbohydrates represents a potentially important alternative. Since the expectancy of an

improvement of the fermentative standard of ensiled forage, resulting in a better quality silage, also with a higher energy content, and guaranteeing that the loss during the fermentative process are reduced, which would result in a higher efficiency of the ensiled forage. .

The pelleted citrus pulp, possibly, is the best example to characterize this additive group previously mentioned. This additive has the humidity absorption potential of 145% of its weight and besides that the introduction of 10% of pulp (natural matter base) in tropical grass silages can increase the energy content of the forages from 62 to 65% of TDN, making the material similar to good quality corn silage, in energy point of view.

Despite the favorable characteristics of this additive group, most of the studies limits itself to the qualitative aspects of the resulting silages and does not include economical feasibility analysis of adopting technology, which prevents from a more discerning evaluation concerning its use in cattle business on a large scale. (Coan et al., 2008).

The economical evaluation of finishing feedlot beef cattle, comparing to Tanzania and Marandu grass silages, harvested respectively on the 97^{th} and 106^{th} day, and corn silage, with diets formulated to attend to weight gain of 1.0 or 1.2 kg animal^{-1} day^{-1}, showed that, in a scenario with R$ 58.50 arroba price and R$ 751.67 thin animal price, in spite of the tropical grass silages ensiled with 10% of pelleted citrus pulp (PCP) (natural matter base) have presented satisfactory animal performance, the benefit/cost relation did not justify the substitution of corn silages to silages added with PCP, since the diets composed by Tanzania and Marandu grass silages and PCP presented, in average, 79.5% of the weight gain provided by corn silage, although having a 3.94% higher cost per gained arroba (Coan et al., 2008) (Table 6). Another important observation regarding this study is that the diet formulated with marandu-grass silage without adding PCP was the one which provided the higher return among all the diets formulated with tropical grass silages, showing then that, in the study conditions, even with improvements in the qualitative attributes of the silages using additive, basing on the economic result, its application would not be feasible in the adopted inclusion level.

Table 6. Economic evaluation of feedlot beef experimental diets with 1.0 and 1.2 kg animal^{-1} day^{-1} of weight gain

Item	Treatment							
	STZ	**STZP**	**SMA1**	**SMAP1**	**SMA2**	**SMAP2**	**SMI1**	**SMI2**
Forage on the diet (%)	38.35	61.59	35.51	56.69	18.00	28.45	59.01	29.57
Feeding cost, (85%), R$	174.82	151.20	184.86	183.71	186.00	216.47	129.62	176.82
Other costs (15%), R$	30.85	26.68	32.62	32.42	32.82	38.20	22.87	31.20
Total cost, R$ (A)	966.53	938.48	978.46	977.09	979.81	1016.01	912.84	968.91
Total profit, R$ (B)	1029.60	1017.90	1070.55	1064.70	1041.30	1058.85	1017.90	1076.40
Result R$ (C = A – B)	63.07	79.42	92.09	87.61	61.49	42.84	105.06	107.49
Capital remuneration (%)a.m.	2.18	2.82	3.14	2.99	2.09	1.41	3.84	3.70
Rate of return, (%) a.m.	**6.53%**	**8.46%**	**9.41%**	**8.97%**	**6.28%**	**4.22%**	**11.51%**	**11.09%**

Source: Adapted from Coan et al. (2008)

* STZ = Tanzânia silage (1.0 kg day^{-1}); STZP = Tanzânia silage with 10% of pelletized citrus pulp (PCP) (1.0 kg day^{-1}); SMA1 = Marandu silage (1.0 kg day^{-1}); SMAP1 = Marandu silage with 10% of PCP(1.0 kg day^{-1}); SMA2 = Marandu silage (1.2 kg day^{-1}); SMAP2 = Marandu silage with 10% of PCP (1.2 kg day^{-1}); SMI1 = corn silage (1.0 kg day^{-1}); SM2 = corn silage (1.2 kg day^{-1}).

6 Conclusions

Only the high yield is not a guarantee of high bio economic value of diets formulated with tropical grass silages. The opportunities of these forages inclusion in the ruminant feed also depend on the energy source price, price of additives which improve the fermentative standards and their level of inclusion, animal genetic merit, and capacity of resources usage and possibility of agro industry byproducts use, mainly the energy sources such as the additives.

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Silage pathogenicity and implications for the ruminant production chain

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The objective of this paper is to discuss pathogenic organisms and other harmful agents that may be present in silages, consider their implications for ruminant animal production, and to recommend management practices that are critical to ensuring the hygienic quality of silages.

Pathogens and harmful agents in silages

Yeasts

Unlike those on fresh crops that are nonfermentative, silage yeasts are facultative aerobic fungi that can tolerate oxygen deprivation and low pH for prolonged periods. Although, these conditions inhibit their growth, yeasts remain viable during the anaerobic fermentation phase by deriving energy from fermentation of plant sugars into ethanol and carbon–di-oxide predominantly, especially when small quantities of oxygen are present (McDonald et al., 1991). This fermentation depletes some of the energy and fermentable substrates that would have been available to ruminants feeding on the forage, but the main adverse effects of silage yeasts occur after the ensiling phase. Active yeast growth and respiration resumes when oxygen infiltrates the silage mass after silo opening. Pahlow et al. (2003) reported that yeasts of the genera *Candida* and *Hansenula* (*Pichia*) are particularly prolific during the aerobic phase because of their lactate assimilating ability and their strong affinity for glucose. These yeasts typically outcompete *Sachharomyces cerevisiae*, which does not assimilate lactate but is the dominant yeast at the end of ensiling in sorghum and corn silages (Sanderson, 1993). In most cases, yeasts are the initiators of aerobic deterioration (Pahlow et al., 2003). Lactate assimilation by yeasts increases the pH after silo opening, leading to a more conducive environment for the proliferation of pathogenic organisms like molds, *Listeria*, and *Bacilli* that worsen aerobic deterioration.

An additional major problem arising from aerobic spoilage is the development of inedible waste due to silage oxidation. McDonald et al. (1991) cited studies showing that up to 75% of the original forage DM was lost as waste; furthermore, waste-associated DM losses ranged from 3.4 to 51.5% across studies. Certain yeasts have also been associated with the incidence of mastitis in cattle. For instance, Elad et al. (1995) implicated yeasts from wheat silage as the cause of the onset of acute mastitis in cattle though the condition only persisted when milking hygiene was inadequate.

Molds and mycotoxins

Silage molds are filamentous aerobic fungi that promote silage aerobic deterioration thereby depleting nutrients and enhancing DM losses. Except for *Penicillium* spp, common silage molds (*Monascus, Fusarium, Aspergillus, Rhizopus,* and *Geotrichum* spp) are less tolerant of low oxygen and low pH conditions than silage yeasts (Pahlow et al., 2003). Consequently, in well-prepared intact silos, molds are found mainly in parts exposed to oxygen infiltration such as the top layer or sides; whereas in open silos, molds proliferate throughout the silage in areas where the pH is high (McDonald et al., 1991). Like yeasts, molds degrade sugars and lactic acid in silages but they also degrade cellulose, which is one of the most important energy sources for ruminants.

Although several molds produce mycotoxins, *Aspergillus, Penicillium* and *Fusarium* molds are the most frequent causes of decreased feed value and livestock poisoning. *Aspergillus fumigatus* molds are responsible for causing aspergillosis in livestock and man. This disease is characterized by inflammation and nodulation of various nasal and intestinal passages and it occurs in cattle, sheep, horses, pigs and man. Smith and Lynch (1973) reported that feeding moldy, *A. fumigatus*-contaminated corn to pregnant beef cows resulted in irritability, incoordination, and orange-colored livers and about 8% of cows died at parturition or shortly afterwards. Certain species of *Aspergillus* as well as thermophilic *Actinomyces* also cause Farmers Lung. This is a type of hypersensitivity pneumonitis resulting from inhalation of moldy forage or silage and it causes respiratory problems and occasional mortality (Wild and Chang, 2009).

Another disease of immunocompromized dairy cattle often associated with silage contaminated with *Aspergillus fumigatus* is hemorrhagic bowel syndrome (HBS), also known as hemorrhagic jejunal syndrome (HJS) or bloody gut disease. This recently discovered syndrome causes sudden reductions in feed intake and milk production, intestinal hemorrhaging, and sudden death of cattle. It is responsible for the death of about 2% of dairy animals in the US (Baker, 2002). *Aspergillus fumigatus* has been proposed as the agent causing with HBS in dairy cattle because the incidence of the disease has been associated with ingestion of moldy hay containing the pathogen; also high populations of the fungus are often present in the blood of affected cows (Puntenney et al., 2003). However, others have implicated *Clostridium perfringes* Type A in the etiology of the syndrome because the bacteria has been found in tissues and feces of affected animals (Kirkpatrick et al., 2001; Dennison et al., 2002). Acidotic diets are thought to contribute to the problem, because acidic conditions predispose *C. perfringes* to toxin production and the classical symptoms. However, this pathogen is also found in the intestines of healthy animals therefore its role in the pathogenesis of the syndrome is unclear (Berghaus et al., 2005). Although, the exact cause of the disease remains unknown, suggestions that moldy or clostridial silages are involved in the etiology of the disease emphasize the importance of ensuring good silage management practices to minimize outbreaks.

The most notorious pathogenic effect of molds probably occurs via the mycotoxins they produce. Mycotoxins are poisonous secondary metabolites that can reduce feed intake, growth, and milk production and also cause diseases, reproductive problems and death in livestock. The most common and most problematic mycotoxins are those produced by Penicillium (PR toxin, mycophenolic acid, roquefortine C, patulin), Fusarium (deoxynivalenol, zearalenone, T-2 toxin), and Aspergillus (aflatoxin, gliotoxin, fumitremorgens, fumigaclavines), but others may also be

present (Whitlow, 2009). However, little is known about conditions that optimize mycotoxin production by molds.

Kiessling et al. (1984) showed that zearalanone, ochratoxin A, diacetoxyscirpenol, and T-2 toxin were rumen degradable but deoxynivalenol and aflatoxin B_1 were not. However, these authors noted that some of these were not completely degraded and some of the degradation products remained toxic. Therefore, the extent of ruminal degradation of such toxins appears to be variable; situations resulting in a faster rate of ruminal feed passage or a low population of protozoa in the rumen may reduce mycotoxin degradation in the rumen (Whitlow and Hagler, 2009).

Driehuis (2000) indicated that little or no dietary zearalenone, deoxynivalenol ochratoxin A is transmitted into the milk of dairy cows. In contrast, aflatoxin B_1, the most toxic (carcinogenic) and widespread of the aflatoxins, can be passed from contaminated silage into milk where it is excreted as aflatoxin M_1. On average, milk aflatoxin M_1 concentrations are approximately 1.7% of the aflatoxin B_1 concentration in the total ration dry matter (Whitlow, 2005). Dietary levels of B_1 above 100 ppb can compromise the performance of dairy cattle, and cause kidney damage in beef cattle (Garett et al., 1968; Whitlow, 2005). Aflatoxin is the only mycotoxin with Food and Drug Administration (FDA) action levels in the US. These are 20 ppb in most feeds and 0.5 ppb in milk.

Mycotoxin production can occur during plant growth in the field leading to uniform contamination of silage, or in hotspots where oxygen pockets allow mold growth during ensiling or on the silo face at feedout (Adesogan, 2006). Delayed harvesting, slow or delayed filling, inadequate packing and sealing, slow feedout rates and damaged bunker or bag plastic can lead to pockets of mycotoxin production (Whitlow and Hagler, 2009). Recent work also showed that aflatoxin levels in rust-infested corn silage exceeded actionable levels stipulated by the US Food and Drug Administration (Queiroz et al., 2009). Other factors such as insect damage may have similar effects. Therefore, it is imperative that management practices that avoid these predisposing factors are implemented to minimize the risk of mycotoxicoses in ruminants and humans.

Bacilli

Bacilli are gram positive, facultatively anaerobic, sporulating bacteria; and their endospores tolerate harsh environmental temperatures including milk pasteurization or boiling temperatures (Pahlow et al., 2003). *Bacilli* are probably the first groups of microorganisms to develop in deteriorating silages after yeasts initiate the process (Pahlow et al., 2003). However, *Bacillus* species may initiate spoilage in certain instances such as under high temperatures, in big bale silages, or after treatment with formaldehyde or antibiotics (McDonald et al., 1991).

Bacillus cereus is particularly notorious because its spores can pass through the digestive tract intact, contaminate the milk of dairy cows, survive pasteurization temperatures, and decrease the shelf life of milk and cream (Christiansson et al., 1999; Pahlow et al., 2003). Furthermore, enterotoxins produced by this bacteria cause foodborne illnesses, notably emesis and diarrhea (Ankolekar et al., 2008).

Listeria

Listeria are opportunistic gram-positive aerobic or facultatively anaerobic bacteria that cause high mortality rates and a wide range of diseases in immunocompromized animals and humans including meningitis, encephalitis, septicicaemia, gastroenteritis, mastitis and abortions (McDonald et al., 1991). *Listeria monocytogenes* (formerly *Bacterium monocytogenes*) is the main causative agent and the main source of the pathogen in ruminants is spoiled silage (Wiedman, 2003). This facultatively anaerobic bacterium is ubiquitous as it can tolerate refrigeration temperatures, low water activity, and a wide range of pH. Previous studies indicated that the organism required pH values above 5 (McDonald et al., 1991) but Ryser et al. (1997) demonstrated that some strains survived pH values below 4. Vilar et al. (2007) conducted a cross-sectional study on the prevalence and source of *L. monocytogenes* in milk from 98 dairies in Spain and statistically verified the relationship between low silage quality (as indicated by high pH) and presence of *Listeria* spp. in silage (29.5 vs. 6.2% for pH above or below 4.5, respectively). The pathogen is commonly found in baled silages because of their relatively low density, high pH and high surface area to mass ratio (McDonald et al., 1991). In well-prepared bunker silages, *L. monocytogens* only thrives in areas exposed to a prolonged low rate of oxygen infiltration because they are inhibited by low pH conditions. However, those that survive ensiling may experience a growth surge if the pH increases after silo opening (Figure 1). In Britain, outbreaks of listeriosis are more commonly associated with sheep because cattle are more resistant and sheep are more commonly fed baled silages (McDonald et al., 1991). Nevertheless, cattle are often asymptomatic carriers of the pathogen (Villar et al., 2007). *Listeria* monocytogenes can be transmitted from contaminated silages into milk. Fortunately, the pathogen is destroyed by adequate pasteurization but it may survive in soft cheeses and dairy products that are not subjected to such treatments (Griffiths, 1989).

Clostridia

Clostrida are gram positive, mostly obligately anaerobic, sporulating bacteria that thrive in low-sugar silages particularly when plant moisture (>70%), pH (>4.6), temperature (>30^{o}C) and buffering capacity are high. Consequently, they often dominate the fermentation of unwilted legumes ensiled without additives (McDonald and Whittenbury, 1973) and can also be common in unwilted tropical forages (Adesogan et al., 2004). Clostridial presence in silage is mainly from soil contamination or slurry application and this can lead to contamination of animal products (Figure 2). Those most commonly found in silage include saccharolytic types that ferment sugars and organic acids (e.g. *C. butyricum* and *C. tyrobutyricum*) as well as others that ferment both sugars and amino acids (e.g. *C. sporogenes* and *C. perfringens*); however, those that ferment amino acids exclusively are uncommon in silages (Pahlow et al., 2003). Certain saccharolytic *Clostridia* derive energy for growth by fermenting sugars and lactate into butyric acid, CO_2 and H_2. Although the antifungal properties of butyric acid can enhance aerobic stability (Adesogan et al., 2004), its pungent, acrid odor typically depresses intake in ruminants.

Depletion of lactate at feedout by saccharolytic *Clostridia* in silage increases the pH thereby enhancing the growth of proteolytic *Clostridia* that deaminate and catabolize amino acids into fatty acids (McDonald et al., 1991). Consequent increases in the ammonia concentration and protein solubility of silages make the silage protein less ideal for highly productive cattle and

enhance nitrogen pollution from cows fed such silages. Furthermore, biogenic amines such as cadaverine, glucosamine, histamine, putrescine, and tyramine can be produced during Clostridial proteolysis in silages. Although they are present in small quantities in all cells and can be ruminally degraded to a large extent (Van Os et al., 1995; Phuntsok et al., 1998), some are potentially toxic. Many of these putrefaction-associated compounds are malodorous and unpalatable, therefore they reduce feed intake by livestock (Table 1). Fusi et al. (2004) showed that oral administration of biogenic amines to kids reduced dry matter intake, growth rate, and body weight and adversely affected the histological characteristics and carcass quality. Furthermore, histamine is lethal at high doses, and when injected intravenously at low doses, it stopped ruminal motility and eructation (Dain et al., 1955). Intake of putrescine has also been associated with ketonemia and depressed milk production in cattle (Lingaas et al., 1992).

Although *Clostridia* are normal flora of ruminant digestive tracts, dietary stress, injury, management changes, and parasitism can make them produce potent toxins that cause sudden bouts of abdominal pain, diarrhea, ulceration and even death in calves (McGuirk, 2009). Enteric syndromes in cows, humans, lambs, and monogastric livestock are also common . *Clostridium perfringes* type A is frequently found in most cows with hemorrhagic bowel syndrome, consequently it is thought to be involved in the etiology of the syndrome. Rings (2004) cited studies in which outbreaks of botulism B in cattle were reported after wrapped small-grain haylages and ryegrass silage were fed and noted that *C. botulinum* grows and produces the neurotoxin when silage fermentation fails to achieve a pH less than 5.3. However, occurrence of *C. botulinum* in silages is rare (Driehuis and Elferink, 2000).

An added Clostridial problem is that spores transmitted from silage into milk can form outgrowths or gas pockets that double the size of cheese due to butyric fermentation. This phenomenon is called late blowing of cheese and the large quantities of butyric acid produced by clostridial fermentation in the cheese result in a rancid odor and taint the flavor (Cocolin et al., 2004).

Enterobacteria

Enterobacteria are gram positive facultatively anaerobic bacteria. Epiphytic enterobacteria including *Erwinia herbicola* and *Rahnella aquitilis* often dominate fresh crops, but these are superseded by others like *Escherichia coli, Hafnia alvei*, and *Serratia fonticola* during ensiling (Driehuis and Elferink, 2000). Although enterobacteria actively compete with lactic acid bacteria in the early stages of ensiling, they are inhibited once the pH drops below 4.5 (Pahlow et al., 2003) but those that survive ensiling can start growing actively when the pH increases after aerobic deterioration (Driehuis and Elferink, 2000). Like *Clostridia*, *Enterobacteria* deaminate and decarboxylate amino acids in silages, thereby enhancing ammonia and biogenic amine production and increasing the risk of depressed intake and ineffiecient N utilization by livestock.

Escherichia coli O157:H7, a shigatoxin producing gram-negative bacteria is the most notorious of the enterobacteria. It has emerged as an important cause of food borne disease. In children and the elderly, it initially causes acute bloody diarrhea but this may evolve into hemolytic uremic syndrome, a severe illness characterized by anemia and kidney failure. In ruminants, the pathogen can cause intestinal disorders and mastitis (Weinberg et al., 2004). Cattle are the main

reservoir of *E. coli* O157:H7 and the pathogen may be present in feces, milk, and feed of dairy cows (Armstrong et al., 1996; Mechie et al., 1997; Chapman et al., 1997; Lynn et al., 1998). Silage can be contaminated with *E. coli* O157:H7 via manure or irrigation water (Weinberg et al., 2004) but the pathogen disappears from contaminated silages when the pH drops below 4 - 5 (Bach et al., 2002; Chen et al., 2005; Pedroso et al., 2009). However, the pathogen has been found in decaying commercial silages with relatively high pH values and it survived for three weeks in grass silages (pH 4 to 4.6) recontaminated with the pathogen (Reinders et al., 1999). Therefore, it is critical that silage pH is kept below 4 during and after ensiling to prevent the growth of the pathogen.

Nitrates and nitrites

Plants transport nitrogen from roots to leaves in the form of nitrates but toxic levels can accumulate due to manure fertilization, plant photorespiration under high temperatures and moisture, injury from herbicides, and environmental stressors such as drought (Provin and Pitt, 2009). Nitrate toxicity causes reproductive problems and can be fatal to cattle or humans (Hill, 1999; Weinberg et al., 2004). Normally, ingested nitrates are converted to nitrite and then ammonia, such that they can be used to meet tissue N requirements. However, when ingested excessively, nitrites accumulate in the blood and bind to hemoglobin forming methmoglobin, which has a low oxygen carrying capacity (Provin and Pitt, 2009). Silage enterobacteria are usually effective at degrading nitrates (McDonald et al., 1991), but the product, nitrous oxide is also hazardous as it causes a respiratory problem in farm workers known as 'silo fillers disease' (Weinberg, 2004).

Strategies for ensuring the hygienic quality of silage

Preventing contamination of silage and animal products with pathogens requires the identification of critical control points (HACCP) that are related to contamination and replication of pathogenic silage organisms on the farm (Lynn et al., 1998). Silage contamination with pathogenic microorganisms can occur before, during, or after ensiling and it is critical that adequate control measures are used at each of these stages to prevent contamination.

Before and during ensiling, management practices that favor rapid homolactic fermentations should be ensured because a rapid pH drop is critical to inhibiting *Clostridia and Enterobacteria,* which cause proteolysis and secondary butyric fermentation. Specific measures include:

1. Choosing forages with a high WSC to buffering capacity ratio; where available hybrids resistant to fungi should be used.
2. Harvesting at appropriate moisture concentrations for ensiling and optimizing nutritive value and biomass yield
3. Wilting in a way that prevents proteolysis but reduces moisture concentrations to about 65% for grasses and 55% for legumes
4. Chopping forages to lengths that facilitate compaction but retain the physical effectiveness of the fiber
5. Unloading forages promptly into silos lined with appropriate plastic sheets

6. Compacting forages to a density of about 240 kg of DM/m^3 in the silo
7. Sealing the silo promptly with appropriate sheets and maintaining anaerobic conditions for the duration of ensiling by regularly sealing any holes that develop in the plastic cover.
8. Additives are not always necessary for good fermentation, but they are particularly useful for enhancing the fermentation of crops with high buffering capacities, low WSC concentrations or high moisture concentrations. Additives containing molasses, nitrate, inorganic and formic acids, buffered acids or least 10^5 cfu/g of specific lactic acid bacteria (*L. plantarum, Pediococcus acidilacti, P. pentosaceous, and Enterococcus faecium*) have enhanced homolactic fermentation by inhibiting undesirable bacteria, or increasing the rate of acidification, or dominating the flora. However, various additive and management-related factors determine additive efficacy. Therefore, more detailed reviews on the subject such as that of Kung et al. (2003) should be consulted before choosing an additive.

   Additives have also had secondary benefits for instance inoculation with L. casei has successfully reduced the biogenic amine concentration of different silages, but L. buchneri inoculation had inconsistent effects (Nishino et al., 2007).

Yeasts, molds, *Listeria*, and *Enterobacteria* that survive anaerobic fermentation grow rapidly when the pH is elevated during aerobic spoilage. Therefore, management practices that ensure the aerobic stability of silages and prevent increases in pH at feedout are critical. To ensure aerobic stability, the following steps should be implemented:

1. Silo design should minimize the size of the silo face as wider faces facilitate oxygen ingress.
2. Where appropriate, shavers should be used to ensure smooth silo faces to minimize the surface area exposed and reduce oxygen ingress into the silage.
3. Silages should be fed out at rates that minimize the length of time the face is exposed to the air; feedout rates of 5 to 10 cm/d from tower silos, 10 to 15 cm/d from bunker silos, and 30 or more cm/d from bag silos have been recommended in the US, whereas in Israel rates of 20 to 30 cm/d are recommended (Muck et al., 2003). Feedout rates in tropical areas should be at least 15 cm/d (Whitlow and Hagler, 2009) because warm humid conditions enhance the growth of spoilage organisms.

4. Silage aerobic stability can be been enhanced with propionic, acetic and sorbic acids and to a lesser extent benzoic acid because of their antifungal nature. These compounds are also sold as mold inhibitors. *Lactobacillus buchneri* degrade lactate to acetate, which inhibits the growth of yeasts and molds, thereby improving aerobic stability (Driehuis et al., 1999). Consequently, *L. buchneri* inoculants have been successfully used to improve the aerobic stability of several forages. Pedroso et al. (2009) reported that *L. buchneri* inoculants enhanced the aerobic stability of corn silages by increasing acetate production to levels that inhibited yeasts and minimized or prevented the attendant increases in pH. Therefore, these L. buchneri inoculants curtailed the growth of *E. coli* O157:H7 in silages contaminated with the pathogen after silo opening (Figure 3). All of these additives should be uniformly distributed in the silage for maximum efficacy.

5. Antioxidants like vitamin E and selenium, mold inhibitors like propionic, sorbic, acetic and benzoic acids, and mycotoxin binding adsorbents have been successfully used to reduce the risk of mycotoxicoses and prevent the transmission of aflatoxins into milk (Diaz et al., 2004; Whitlow and Hagler, 2009). *Lactobacillus buchneri* inoculation has also prevented aflatoxin synthesis in silages produced from corn plants infested with high levels of Southern rust.

6. When animals show symptoms of mycotoxicoses, mycotoxins binders should be added to the diet and the suspect silage should be withdrawn from the diet. Regular use of mycotoxins binders in healthy animal diets as a preventative measure may be cost prohibitive. The literature suggests that mycotoxin binders are specific in their activity and none binds all mycotoxins. Therefore, where possible, rapid identification of specific problematic mycotoxins should be ensured before addition of a binder.

7. Moldy samples of forage should be discarded and it is important to note that mold counts often do not correlate with mycotoxins levels in forages.

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Table 1. Effect of intake of high levels of biogenic amines from alfalfa silage on DMI, ruminal DM digestibility (RDMD), total tract DM digestibility (TTDMD), and ruminal outflow (RDMOF) in steers (Phuntsok et al., 1998)

	Alfalfa silage level				
Item	0%	33%	67%	100%	SE
Biogenic amine intake, g/d					
Putrescine[a]	1.10	3.10	4.91	6.45	0.16
Cadaverine[a]	1.22	4.01	6.39	8.52	0.21
Histamine[a]	1.32	3.40	5.31	6.16	0.20
Performance indices					
DMI,[a] kg/d	8.18	7.13	7.19	6.07	0.30
RDMD,[b] %	48.50	45.86	46.67	43.61	1.36
TTDMD,[c] %	67.14	73.46	71.21	66.75	2.00
RDMOF,[b] kg/d	4.25	3.89	3.84	3.41	0.22

[a]Linear effect caused by treatment ($P < 0.01$).
[b]Linear effect caused by treatment ($P < 0.05$).
[c]Quadratic effect caused by treatment ($P < 0.05$).

Figure 1. The effect of aerobic deterioration on silage pH and the survival of L. monocytogenes at two sites in laboratory-scale bagged silage (McDonald et al., 1991)

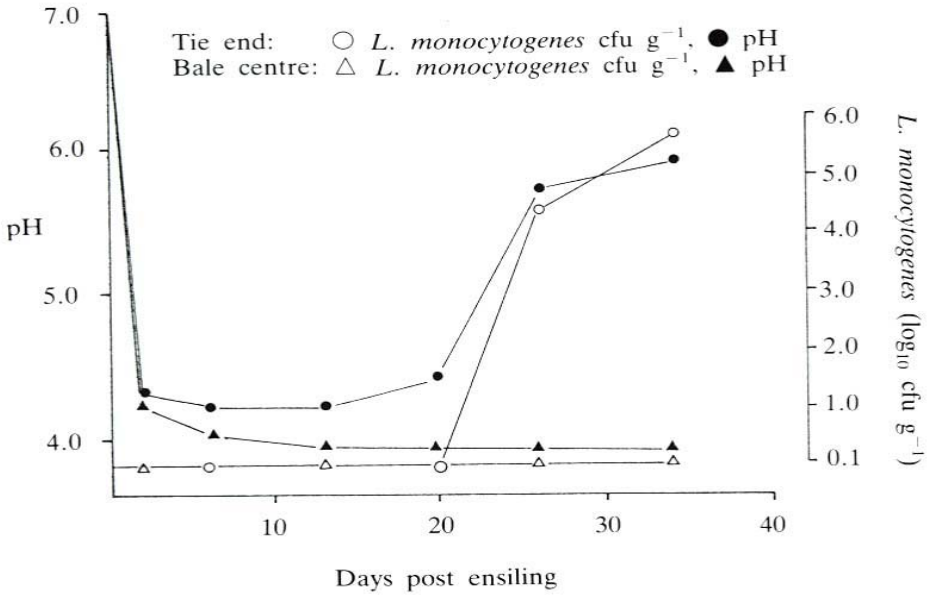


Figure 2. Production chain of a dairy farm with silage-based feeding; Main risk factors for Clostridia spore contamination (Weissbach, 2006)

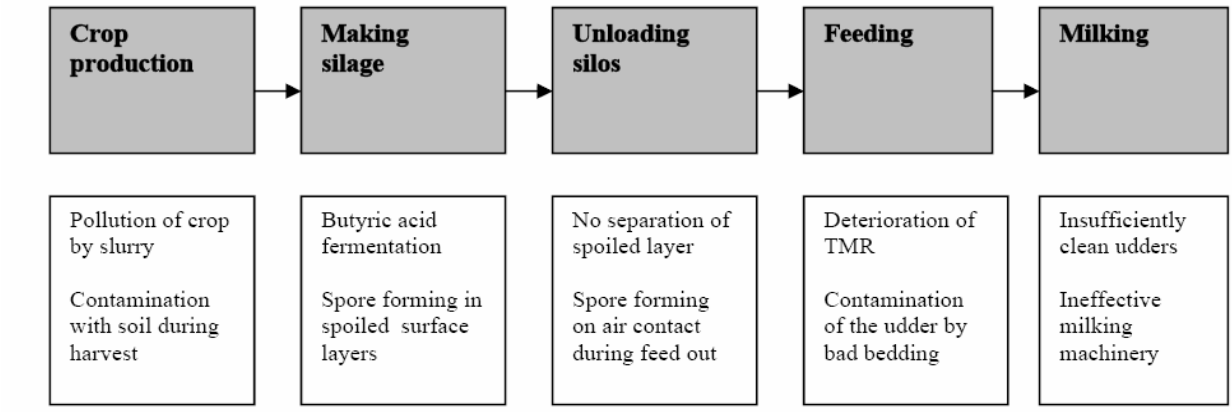
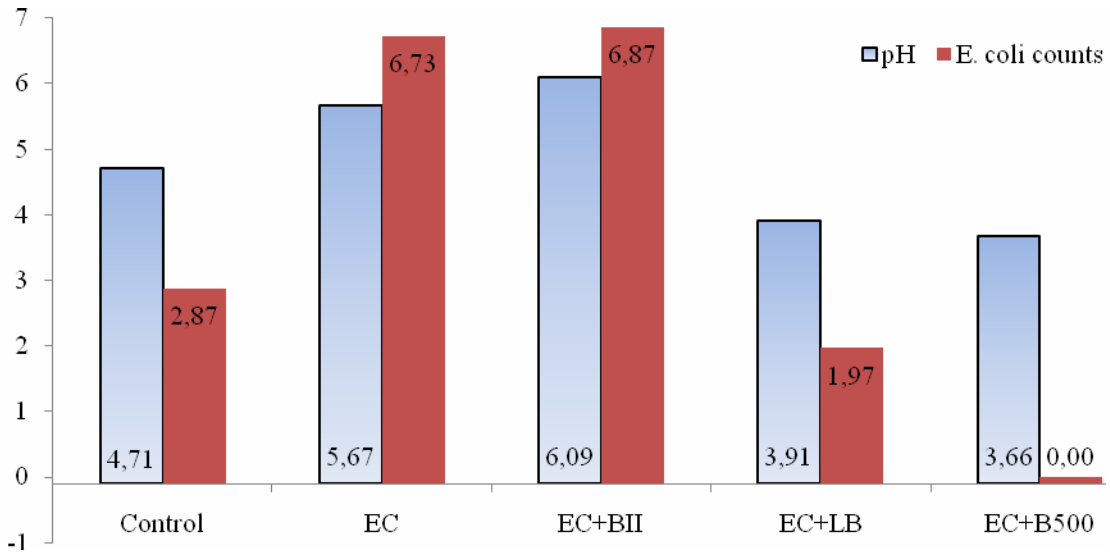


Figure 3. Effect of reinoculation of corn silages with 1×10^6 cfu/g of *E. coli* O157:H7 (EC) 144 h after silo opening (d 82) on pH and *E. coli* counts (EC; log cfu/g) 24 h later.
[1]BII = 1×10^6 cfu/g of *Pediococcus pentosaceus* and *Propionibacterium freudenreichii*; LB = 1×10^6 cfu/g of *Lactobacillus buchneri*; B500 = 1×10^6 cfu/g of *P. pentosaceus* and *L. buchneri*. S.E. values for pH and *E. coli* data were 0.41 and 1.04, respectively.



SEALING STRATEGIES TO CONTROL THE TOP LOSSES IN HORIZONTAL SILOS

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1. Introduction

Achieving and maintaining anaerobiosis is critical to successful ensilage. When no seal is applied, or when the seal is inadequate, air and moisture enter into the silo affecting both the ensiling process and the quality of silage during storing and feeding; therefore, silage is covered for two primary reasons (Holmes, 2006). The first is to exclude rainfall because precipitation washes organic acids and other soluble feed components from the forage and the second is to reduce exposure to air.

Oxygen enables various aerobic spoilage microorganisms to become active and to multiply themselves, resulting in aerobic deterioration (Pahlow et al., 2003) and substantial economic losses. The deterioration of the silage is indicated by temperature and pH increase, dry matter (DM) losses, lack of nutrient availability, surface mould growth and feed refusal by the animals.

Livestock farms can store silage in various ways such as horizontal silos (bunker and stacks), tower silos, bagged silos, or large wrapped bales. Several farms prefer horizontal silos due to relatively low construction costs, greater safety compared to tower silos (no toxic gases produced in closed areas, low risk of fall) and high work rates for filling and unloading (Savoie & Jofriet, 2003), but their design allows large areas of the ensiled material to be exposed to the environment and prone to spoilage, especially in the upper layer and near the walls (Ashbell & Kashanci, 1987; Borreani et al., 2007).

In horizontal silos, during the storage period, a spoilage layer is formed below the sealing sheet, known as "surface waste". Although there is also some evidence that invisible

oxidation losses occur throughout the whole mass of silage during the storage period. A large percentage of the silage mass (about 25%) can be within the top 1 meter depending on silo size and depth.

The most common material used to seal horizontal silos is the plastic film. The principal function of the film is to seal the forage and allow anaerobic conditions to establish. In Brazil, mainly films of 150-200 μm thickness are used for this purpose. Although polyethylene sheeting has been the most common method used to protect silage near the surface, the protection provided is highly variable and often changes during storage (Savoie, 1988). Thus, the effectiveness of covering methods is very important to limit aerobic deterioration and losses in the large mass being protected.

This paper presents the main factors related to sealing methods that affect the extent of aerobic deterioration in horizontal silos. Furthermore the review aimed to identify proper management strategies to improve silage quality on commercial farms.

2. Unsealed silos

Along with proper harvesting and filling techniques, it is also equally as important to properly cover a bunker silo. Previous studies have demonstrated that the quality and recovery of silage is compromised if horizontal silos are not covered with plastic film.

Berger & Bolsen (2006) summarized the research that documents DM and nutrient losses when bunker and stacks silos are not sealed (Table 1). From 1990 to 1993, the top 0.90 m of silage from 127 horizontal silos in Kansas was sampled at three locations across the width of the silos. Sampling depths were: 0 to 0.45 m from the surface (depth 1) and 0.45 to 0.90 m (depth 2). All sealed silos were covered with a single sheet of black or black-on-white 100 to 150 μm polyethylene, which was held in place with tires, sidewall disks, or soil.

In the top 0.45 m, additional organic matter (OM) losses (losses in addition to the losses in well-preserved silage) ranged from 17 to 60%, and losses were higher in bunkers and stacks that were not sealed. Applying a seal reduced OM losses in the top 0.45 m and also reduced additional spoilage losses in the second 0.45 m. Silage near the exposed surface of the unsealed silos had pH values ranging from 4.75 to 8.55, which were typical of severely deteriorated silage.

Table 1. Effect of sealing treatment on estimated additional spoilage losses of OM at the top of two depths in bunker and stacks silos (average 1990 to 1993)

Treatment	n of silos	Depth 1 (0 to 0.45 m)		Depth 2 (0.45 to 0.90 m)	
		average	range	average	Range
Sealed	9	23	17-31	5.3	1-12
Unsealed	54	50	44-60	14.0	9-19

Source: Adapted from Berger & Bolsen (2006)

The aerobic deterioration is initially limited to the top 15 to 30 cm in an uncovered silo. The reason for this is that aerobic microbial activity is great enough in the upper layer to remove all of the oxygen entering into the crop either by diffusion or convection. As the readily degradable components of the crop in the top layer are exhausted, the rate of microbial activity declines allowing oxygen to move deeper in the silo and cause deterioration at that level (Muck, 1988).

Economics evaluations indicate that the reduced losses from using a cover return more than $8.00 for each $1.00 invested in plastic and labor to cover a bunker silo (Rotz & Muck, 1993). In a 200-t bunker silo (6 m wide by 20 m long by 2.5 m deep), an effective seal to protect the top 1 meter of silage can prevent the loss 100 to 400 dollars worth silage, depending on the value of the crop. Proper sealing with a plastic cover is therefore essential to reduce losses and to prevent microbial deterioration, which may result in the presence of toxins.

3. Lining bunker walls with plastic

A large part of the silage stored in horizontal silos is exposed to air and is prone to spoilage, especially in the upper part near the walls (at the shoulders of the silo), which are difficult to seal properly. Ashbell & Kashanci (1987) reported silage DM losses near the surface of bunker silos to be highest (76%) near the silo wall and lowest (16%) in the core. Thus, a problem still not fully solved is the connection of the cover to the bunker silos.

The best results are achieved by putting an additional film 1 to 2 m deep (depending of the silo size) between wall and forage, and then over the forage, before the main sheet is attached (Figure 1). The result of this additional effort is that silage quality along the wall is similar as that throughout the silo (Honig, 1991).

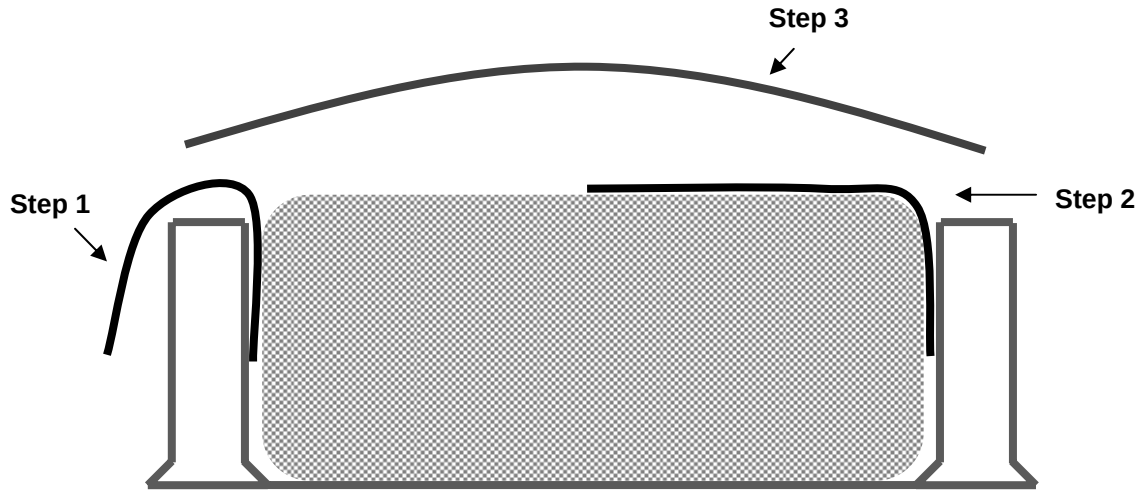

Figure 1. Bunker lining diagram. Step 1 = When filling, leave a flap of plastic over the silo walls; Step 2 = Fold the excessive plastic over the bunker after filling/packing; Step 3 = Cover the bunker with some additional plastic film.

4. Types of plastic film to cover silage

A plastic film to cover silage has to fulfill four essential functions. The characteristics of the films are of different importance for these functions. First, the film must prevent precipitation and damage caused by meteorological effects. Secondly, the film must offer a certain protection against animal attack. These functions require certain mechanical characteristics of plastic film because a failure is usually due to handling, wind, hail, rodents or birds. Thirdly, the silo film guarantees anaerobic conditions in the silage. The necessity of anaerobic conditions for the ensiling process is well known. Finally, the fourth function of the silo film is its influence on the temperature of the conserved forage. Moreover, the film should be UV resistant in order to withstand prolonged exposure to sunlight.

4.1 Effects of the colour and thickness of plastic film

The colour of sheet should affect the amount of air infiltration and subsequent aerobic losses because oxygen permeability into the silage is highly dependent on the temperature of the plastic. Only few data have been published about the thermal effects of covers on the upper silage layers. It is important to emphasize that these surface layers are highly susceptible to poor fermentation because of unsatisfactory compaction, the proximity of the

sheet and the high temperature of the microclimate influence strongly the growth of microorganisms.

This is consistent with the observations by Bernardes et al. (2009a), who found highest DM losses and yeast counts when corn silages were sealing with black polyethylene. Black sheet also shows higher temperature in relation to black-on-white film during storage period (Figure 2).

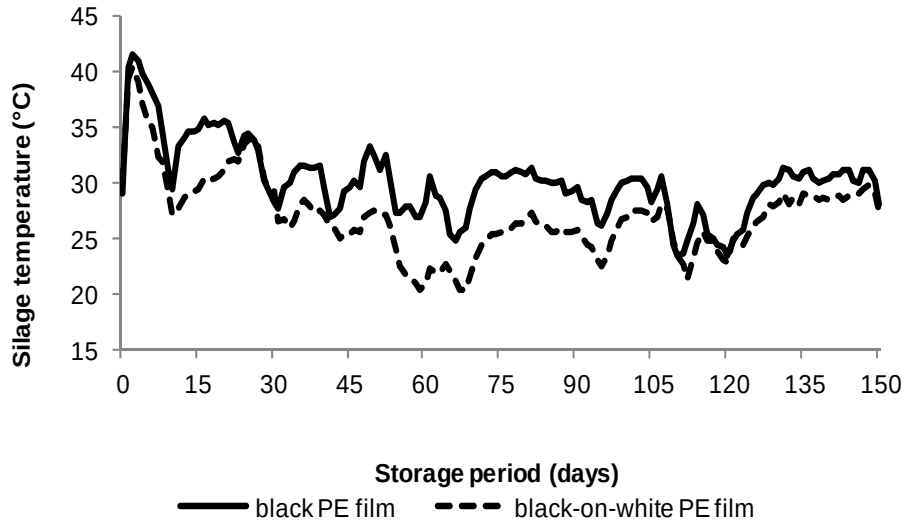

Figure 2. Effects of the colour of plastic film on temperature of corn silages during 150 days of storage

Snell et al. (2003) reported the effects of the colour on the temperature of the film surfaces. They found that in the morning hours, temperature peaks were up to 16°C higher for the black film in comparison with the white film. As expected, the highest values were reached at midday, with the black and green coloured films showing a very similar thermal behaviour. The same applied for the evening hours.

Savoie (1988) developed a theoretical model to predict the total cost of plastic and respiration losses because of air infiltration through the film. An optimal thickness was derived as a function of storage period, silage density and DM content, film permeability and the relative value of plastic and silage. Polyethylene silage bags of different thickness (100, 150, and 200 µm) did not produce significant differences in losses in 130d, averaging 0.2% loss/month when perfectly sealed (Savoie, 1988). However, modeling of different film thickness indicated that 100 µm was economically optimum on a stack silo for 3 months storage, 150 µm for 7 months, and 200 µm for 12 months.

A very thin plastic film may be inefficient due to excess air infiltration through the film during a long storage period. Mechanical failure and film thickness are interrelated because a very thin film will be a poor barrier to oxygen flow in addition to being more sensitive to tear and puncture.

4.2 Effects of the material properties on the oxygen permeability of plastic films

Air is the major cause of spoilage in silage. Polyethylene is not totally impermeable to oxygen diffusion and thus will not completely prevent oxygen ingress (O'Kiely & Forristal, 2003). There is a general agreement, therefore, that low oxygen permeability of the sheets has to be sought.

Borreani et al. (2007) reported that in the early 1990s, Daponte (1992) proposed the use of coextruded barrier films to seal silage, but at that time, plastic manufacturers had no commercial interest in these more expensive films. The situation is changing rapidly now, and new developments in sealing strategies have been reported recently, involving the use of a coextruded film with reduced oxygen permeability as an alternative to standard polyethylene.

A co-extruded polyethylene-polyamide film has been developed for covering horizontal silos (Borreani et al., 2007) by Industria Plastica Monregalese (Mondovi, Italy). It is 125 μm in thickness and comprises two outer layers of polyethylene with a central layer of polyamide (Silostop® 1-step).

Two trials were carried out in Brazil to evaluate a new black-on-white (125 μm) coextruded oxygen barrier film (OB) in a tropical climate. Bernardes et al. (2009a) studied eight covering systems and observed that the OB film had lower values of pH, yeasts and DM losses among the plastic films.

In a second trial (Bernardes et al. unpublished data), four black-on-white films were evaluated: 1) oxygen barrier film 125 μm thick (OB), 2) black-on-white (200 μm thick) polyethylene film (PE), 3) black-on-white (300 μm thick) polyvinyl chloride film (PVC), and 4) black-on-white (200 μm thick) polyvinyl alcohol film (PVOH). Table 2 shows the differences on oxygen permeability of the plastic sheets.

Table 2. Characteristics of the films used in the trial

Item	Plastic film			
	OB	PE	PVC	PVOH
Nominal thickness, µm	125	200	300	250
Measured thickness, µm	121	189	280	238
Oxygen Permeability, cm^3/m^2 per 24 h^1	75 ± 1	722 ± 19	289 ± 5	982 ± 32

[1]At 23°C, 85% relative humidity

Source: Bernardes et al. (unpublished data)

Oxygen permeability of the films influenced the DM losses in the upper 30 cm of the silage, as reported in Figure 3. The OB film affected the DM losses of the silage ($P < 0.05$). More lactic acid was produced in the silage sealed with the OB film and the yeast counts were always under the detection limit (1 x 10^5 cfu/g) in the OB corn silage (Bernardes et al., unpublished data).

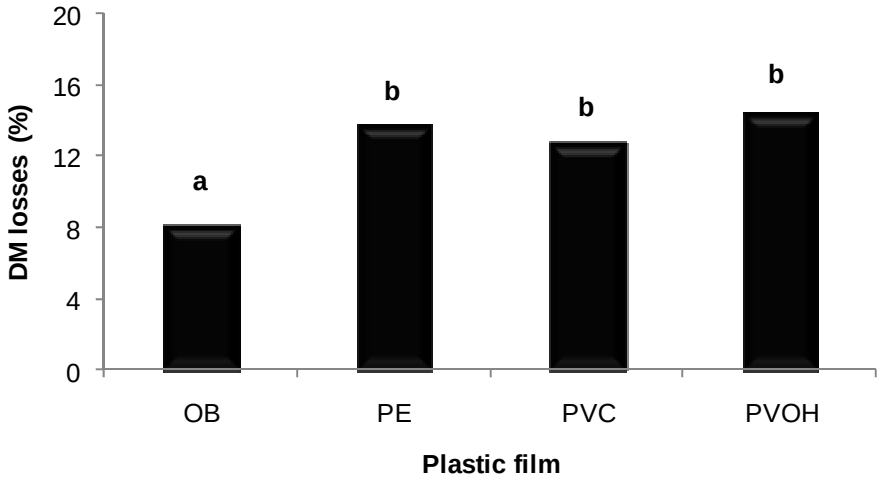

Figure 3. Effect of the plastic film on DM losses of corn silages. Bars with unlike letters differ ($P < 0.05$)

Source: Bernardes et al. (unpublished data)

The OB film has been tested on farm to assess the effects on the fermentation quality, the DM losses and the yeast and mold counts at opening of whole-crop corn bunker silos compared to conventional polyethylene (Borreani et al., 2007). Two trials were carried out in two commercial farms (Farm 1 and Farm 2). The bunkers were divided into two parts along the length so that half of the feed-out face was covered with PE film and the other with OB

film. The pH of the peripheral silages was different, for Farm 1 and Farm 2 under the two films with lower values in the OB treatment. The OB film in Farm 1, affected the silage DM losses, which were reduced 3.7 times in comparison to the PE film sealing. The results indicate that the new OB film is a promising tool to constrain spoilage and DM losses in critical farm conditions, when inadequate amounts of silage are removed daily. The OB film further improved the stability of the corn silage in the peripheral area of the silos even when a proper harvest-to-feedout management was implemented (Borreani et al., 2007).

The same company has also developed a 45 µm non-UV-stabilized translucent barrier film (Silostop® 2-step clear) with similar oxygen permeability characteristics as their Silostop 1-step. Usually, the Silostop 2-step is used in combination with a protective tarpaulin, but in Brazil studies have been tested in combination with a polyethylene sheet (black or black-on-white).

From 2006 to 2009, the top of corn silage from six commercial stack silos was sampled (Bernardes et al., 2009b; Basso et al., 2009; Basso et al., unpublished data). Two treatments were compared in all trials: 1) a sheet of 180 µm-thick polyethylene film (PE) and 2) a sheet of 45 µm thick transparent oxygen barrier film (OB) plus a sheet of PE over the OB film. The stack silos were divided into two parts along the length: half was covered with PE film and half with OB plus PE film. During the filling of each silo, plastic net bags with fresh material were weighed and buried in the upper layer of each stack. When the feedout face reached a distance of 30 cm from the bags, these were removed from the silos for analysis. The fermentative characteristics, microorganisms' numbers and DM losses were affected by OB film. The results indicate that the OB film slightly reduced the occurrence of spoilage microorganisms and improved fermentation profile in corn silages at peripheral areas of the silos. All silos covered with OB film showed negligible visible mould. Four of the stacks had surface waste when were covered with PE film.

In Figure 4 are reported the contour map of temperatures of the silo face with two sealing system. During the feed-out phase the top temperatures at the working face exceeded 40°C in the silage covered with PE film, while in the silage sealed with OB film were similar than those recorded in central zone.

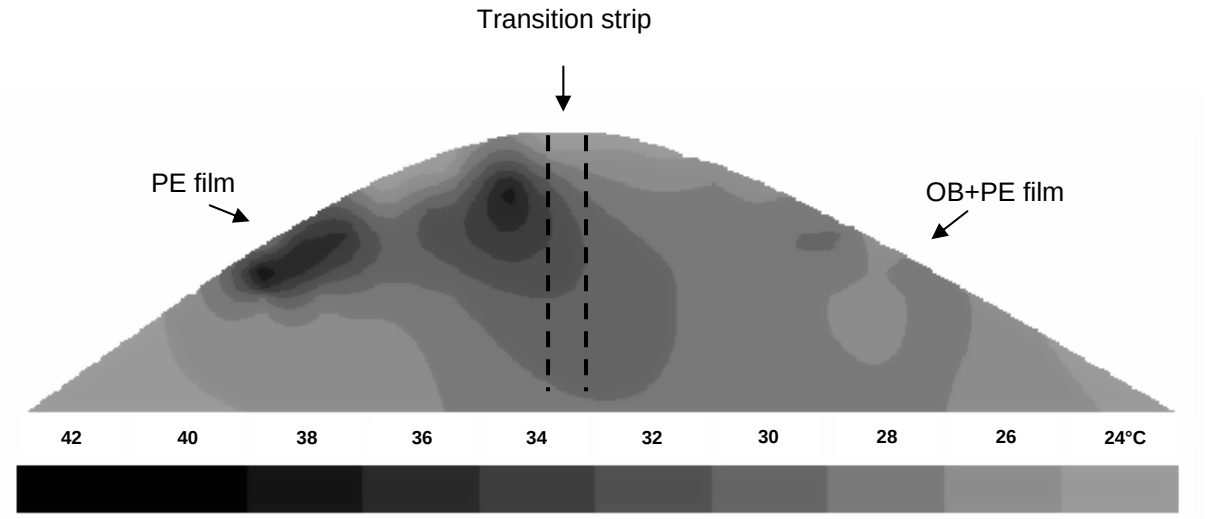

Figure 4. Contour map of temperatures of the silo face covered with polyethylene film (PE) or oxygen barrier film associated to PE (OB+PE)
Source: Basso et al. (unpublished data)

5. Use of bacterial and chemical additives on the top of the silos

Especially in warm climates whole-crop cereal silages such as corn, sorghum and wheat are susceptible to aerobic deterioration. This is because aerobic yeasts are most active at 20 to 30°C (Ashbell et al., 2002). Therefore, efforts are being made to find additives that would inhibit fungi and protect the silage near the surface.

Muck (1996) was the first to suggest that inoculation with *Lactobacillus buchneri*, that is an obligate heterolactic acid bacterium, might improve the aerobic stability of silages. Since that time, *Lactobacillus buchneri* has been used as a silage inoculant to enhance the aerobic stability in a variety of silages via the anaerobic degradation of lactic acid to acetic acid (Oude Elferink et al., 2001). In order to use additives to overcome the problem of aerobic deterioration of silages, it has been suggested that other types, such as sodium benzoate can be used (Kung et al., 2003).

A survey to verify the difference between polyethylene film (PE) and oxygen barrier film (OB) and an association with sodium benzoate (0.02%) or *Lactobacillus buchneri* (1 x 10^6 cfu/g) applied onto the top of the silos was carried out by Amaral et al. (2009). Fermentation quality and DM losses from silages are shown in Table 3. The pH values were lower in silages treated with additives (3.89) than control silages (4.14). The concentration of butyric acid was lower in silages covered with additives. The DM losses were decreased 18%

in silages treated with additives. The authors concluded that the use of *Lactobacillus buchneri* or sodium benzoate inhibited the aerobic deterioration from silos upper layer.

Table 3. Effects of sealing treatment on fermentation quality of corn silages

Item	Plastic film		Additives*		
	PE	OB	Control	LB	SB
DM, %	36.2	35.9	34.9[b]	36.3[a]	36.9[a]
Ash, % of DM	4.67	4.71	4.79	4.76	4.52
pH	3.97	3.96	4.14[a]	3.91[b]	3.86[b]
Acetic acid, % of DM	3.25	1.99	2.57	2.28	3.00
Butyric acid, % of DM	0.11	0.22	0.26[a]	0.11[b]	0.14[b]
DM losses, %	8.07	8.77	9.73[a]	7.69[b]	7.83[b]

PE = black-on-white polyethylene film (200 µm thick); OB = black-on-white coextruded polyethylene-polyamide film (125 µm thick); LB = *Lactobacillus buchneri* $1x10^6$ cfu/g forage[1]; SB = sodium benzoate 0.02% (fresh basis). $^*P<0.05$.

Source: Amaral et al. (2009)

6. Use of tires, gravel, soil, and sugarcane bagasse to weight down the plastic cover

To prevent deterioration in horizontal silos, the common practice is to use polyethylene sheeting held in place with used car tires. These materials have been widely used because of their low cost and the ready availability.

In a study reported by Ruppel (1993) there was a reduction in the temperature and improved protein availability of hay crop silage when the amount of tires per square meter increased. The losses of DM in the top surface of silage were also reduced as the amount of tires per square meter increased. Based on this research it is suggested that there should be 20 to 25 tires per 10 square meters (25 tires per 10 square foot approaches “tire to tire” placement). Ashbell & Weinberg (1992) studied several covering methods on the reduction of the top silage losses and inferred that higher tire density (30 tires per 10 square meters) and sand bags along the shoulders resulted in lower losses.

Borreani & Tabacco (2007) presented the results of a survey for silage sealing systems under some farm conditions. A farm bunker silo was covered with a single black-on-white sheet and the length of the bunker was divided into two equal parts: half was covered with

tires (25 kg/m^2) and half with gravel (200 kg/m^2) over the sheet. The silo was opened for summer consumption and had low feed-out rate (12 cm/d). The results showed that the difference in the sealing system affected the temperature in the peripheral area of the corn silage. The silage covered with tires reached the maximum temperature higher than 40°C.

It is important to emphasize that keep the plastic mostly weighed can be effective during storage and feed-out period. Borreani et al. (2008) reported that during feed-out, air can penetrate in the peripheral areas of a silo to up to 4 m from the feed-out face, especially when the sealing cover is not weighed down or is weighed only with tires, suggesting that, in these situations, daily removal rates should be higher than 30 cm/d to avoid extended aerobic spoilage.

The amount of soil placed on top of the polyethylene plastic also has an effect on silage quality. Bernardes et al. (2009a) studied the effectiveness of several sealing strategies that are used in Brazil on the reduction of the top losses. Lower losses were associated with decreased pH and ash content and lower counts of yeasts when corn silages were covered with soil (100 kg/m^2) over the black sheet. The temperature of the silage anchored with soil was lower during throughout the storage period (Figure 5).

The most of farmers are particularly resistant to cover horizontal silos with soil, particularly large ones, feeling that the labor and trouble of handling the soil used to weigh the plastic are not worth. Moreover, the use of land can also be a source of contaminants in silage, mainly when unloading the silo.

Thus, another alternative has been studied in order to reduce aerobic deterioration in the peripheral area of the corn silage. Three treatments were evaluated: 1) black polyethylene (PE) film 200 µm thick, 2) black PE plus sugarcane bagasse (10 kg/m^2) over the sheet, and 3) black PE plus soil (30 kg/m^2) over the sheet (Amaral et al., unpublished data). Changes in the silage temperatures in the top layer of treatments are given in Figure 5. The results showed that the difference in the covering system did not affect the temperatures during the storage period. However, after about 80d of fermentation the temperature started to rise in the control silage. This can be attributed to the effect of oxygen permeability of the film during a long storage period because the gas transmission rate is reduced by the presence of soil or sugarcane bagasse over the sheet. These results also suggest that the degree to which plastic is permitted to billow in the wind influences the amount of air drawn into the silo.

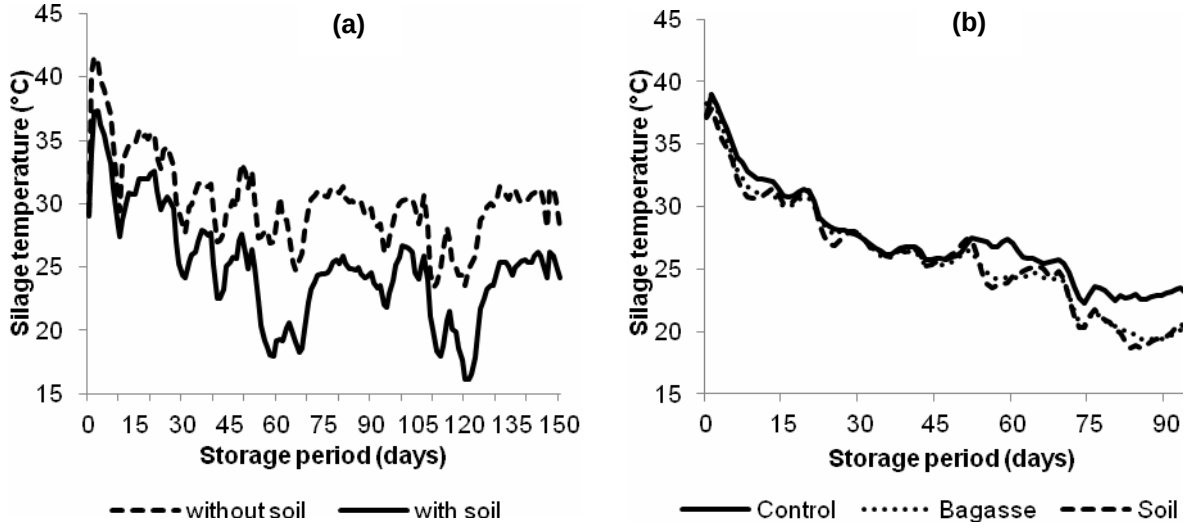

Figure 5. Effect of the covering system on silage temperature. (a) Bernardes et al. (2009a); (b) Amaral et al. (unpublished data)

7. Biofilms and organic covers

An environmental objective is to reduce the quantity of plastic used in agriculture, and there may be the opportunity for achieving this by reducing the use of the film for sealing silos. However, horizontal silos produce less plastic wastes than most other systems that use polyethylene film for air tightness. Savoie (1988) reported that round bale silage requires at least 5.5 kg of plastic/t DM. Stack silos uses about 1.3 of plastic/t DM, four times less than the round bale silage system.

Edible biofilms made from starch, protein or vegetable oil, have been proposed to protect the top surface of silage and eliminate the problem of plastic waste handling (Brusewitz et al., 1991). Currently, biofilms are not used commercially due to their relatively high cost and inadequate protection from air infiltration.

Considering the low cost of raw material and the beneficial impact on the environment, biodegradable coatings have become a possible alternative for sealing horizontal silos. The incorporation of beef fat in the heated coating formulation and the addition of a hydrophobic layer of zein were able to preserve the quality of silage for at least 4 months under laboratory assay (Denoncourt et al., 2004). Soy - and casein - based biodegradable coatings were evaluated for their ability to exclude oxygen and preserve corn silage. The results showed that biodegradable coatings were able to protect the quality of silage during 4 weeks but the

biodegradable coatings were not as good as plastic at preserving silage after 8 weeks of storage (Denoncourt et al., 2006).

In summary, both farmers and scientists have tried alternatives to plastic sheet such as grain, chopped straw, molasses-based products, lime, and edible films. To date, the alternatives have been better than no cover but considerably less effective than the plastic film.

8. Conclusions

The detrimental effect of air at silage near the surface is a key point to avoid losses of dry matter and of quality. Unfortunately, no alternative to the use of plastic in covering bunkers or stacks has proven commercially viable for silage producers. Given the widespread use of horizontal silos in Brazilian farms, it is vitally important that the film used possesses good oxygen barrier properties as well as good mechanical properties.

In horizontal silos, the plastic needs to be held tightly to the crop. This is usually accomplished with used tires, but many other means are used such as gravel, sugarcane bagasse or soil. Besides that, lining bunker walls with plastic improves silage quality along the walls.

The silos' sealing will continue evolving to meet future needs in a conservation of fresh forage, to minimize loss and cost, reduce environment contamination, and to provide a safe and efficient on-farm feeding system.

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Silage bacteria & toxins and ruminant health

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Introduction

If prerequisites for the desired lactic acid fermentation during silage making are not met, organisms other than lactic acid bacteria (LAB) may multiply and for shorter or longer time dominate the ensiling process. This eventually leads to the accumulation of harmful fermentation products (amines, toxins) and loss of energy as well as palatability. In addition badly-fermented or aerobically deteriorated silage may facilitate the survival or multiplication of undesired organisms that normally would be eliminated in well-fermented silage. To assess the risk if a certain undesired organism would be able to grow in silage of some kind, it is important to collect information of the environmental conditions that permit its growth. Lindgren (1991) listed minimal inhibitory concentrations (MIC) of some undissociated organic acids for some silage bacteria (Table 1). He states that the levels of undissociated organic acids in silages are more reliable parameters for microbial inhibition than the frequently used pH values. The antibacterial action of an organic acid is explained partly by its pH-decreasing action and partly by the growth-inhibiting effect of the undissociated acid on the microorganism in question (Baird-Parker 1980). The reason why Lindgren recommended to use undissociated and not total acids was because i) only the undissociated acid can pass through the cell wall, release its H-ion into the cell contents and thus reduce or inhibit growth by disturbing the micro-organism's metabolism and ii) MIC concentrations of the undissociated part of an organic acid is relatively constant within a pH range typical for

Table 1. Minimal inhibitory concentrations (MIC) of *undissociated* lactic acid (LA) against some silage associated organisms (Lindgren 1991). 10 mM lactic acid = 0.9 g/litre silage juice equivalent to 0.63 g/kg FM or 2.1 g/kg DM in a silage with 30% DM.

Organism	MIC, mM LA	Valid pH range
Enterobacteria	6-10 mM	4.2 – 5.4
E. coli	4-6 mM	4.2 – 5.4
Cl. tyrobutyricum	5-10 mM	4.8 – 5.4
Listeria monocytogenes	1-3 mM	4.5 – 5.1

silage (pH 4 – 5). The proportion of the undissociated part of an acid at a certain pH can be calculated as:

Proportion of undissociated acid (%) = $100 / (1 + 10^{pH-pKa})$

Figure 1 demonstrates that at a certain pH a much larger part of acetic or propionic acid is undissociated compared with lactic of formic acid.

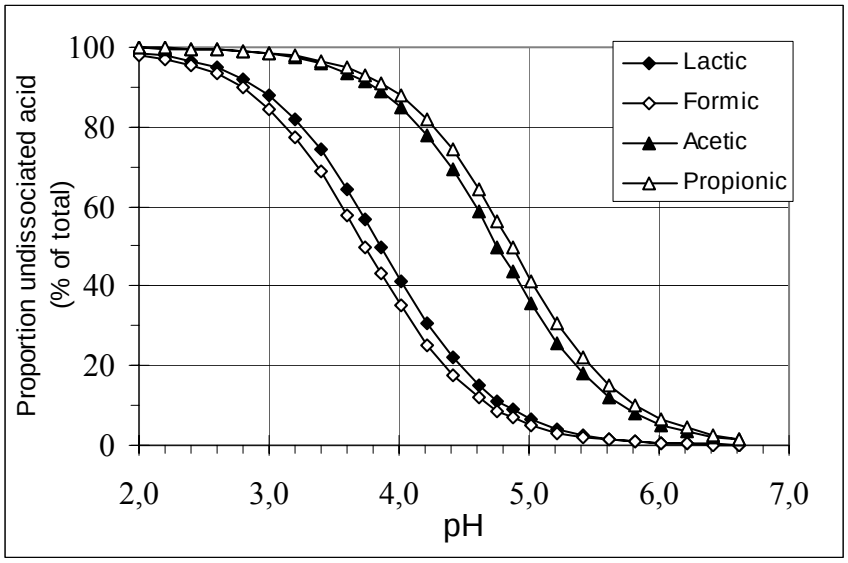

Figure 1. Proportion undissociated acid as % of total acid in the pH range between 2 and 7.

Well-fermented silages, in which fermentation is not restricted by intensive wilting (<50% DM) or addition of chemical additives, contain commonly approx. 50 -

100 mM of undissociated lactic acid. This gives the impression that we should be safe from any of the silage-associated organisms listed above. Still, we frequently find badly fermented silages, which contain concentrations of undissociated LA that are much higher than the MIC values listed in Tab.1. The reason why MIC values, elaborated in carefully controlled lab experiments, often appear to be too low for full-scale silages might be explained by the heterogeneous nature of farm-scale silages (Spoelstra 1982, Pauly 1999). Silage samples collected from a farm silo might contain excellent silage together with small chunks of badly fermented silage. The example in Table 2 demonstrates how we can get such a proliferation of clostridia (log 5.1/g) in silage that contains 18 g of undissociated lactic acid per kg, a concentration that should inhibit the growth of clostridia with large safety margins.

Table 2. Example of a silage sample split into 10 small sub-samples. The composition of the composite sample (Total) would reflect the results from the lab analyses.

											Total
Fractions of the sample (1-10):	**1**	2	3	4	5	6	7	8	9	10	**18**
Undissociated lactic acid, g/kg:	**1**	22	20	20	19	21	18	20	22	18	**18**
Clostridial spores, log cfu/g:	**6.1**	2.0	2.2	2.1	2.1	2.0	2.3	2.0	2.0	2.1	**5.1**

Sometimes differences in silage composition are indicated by a divergent colour or odour, like when air leaks into a silo. Then we can respond to that and avoid or select these zones when we collect our samples. However, if we cannot sense any difference in colour or smell, we have not a clue where in the silo these badly-fermented pockets of silage are and how large these niches might be. When inhibiting growth conditions for undesirable silage microorganisms are discussed, we should be aware that we need to add safety margins to the values gained from lab experiments. Safety margins should be chosen in relation to the expected heterogeneity in the examined silage. A heterogeneous silage like most wilted bale silages would probably require larger safety margins than shortly wilted, precision-chopped bunker silage.

As the example in Table 2 shows it is common in silages that fermentation products and bacterial counts appear to disagree with another, like when bacterial counts are high but their fermentation products low or vice versa. This is because the activity of silage bacteria changes over time while fermentation products accumulate. Prerequisites for the production of well-fermented silage are sometimes summarised with the term ´good ensiling practices'. For more detailed information on silage making see McDonald et al. (1991) and Al-Amoodi et al. (2003).

Clostridia in silage

Clostridium species

Clostridia are Gram-positive, usually motile, spore-forming bacteria, which have their natural habitat in soil and in fresh- or saltwater mud. They are even found in intestinal tracts of mammals and fish and the gills and viscera of shellfish. Most species require icroaerophilic or strictly anaerobic conditions for growth and multiplication. Due to the lack of specific enzymes found in most aerobic microorganisms (superoxide dismutase, catalase) oxygen is toxic to them. When growth conditions are unfavourable, i.e. in the presence of oxygen, clostridia transform quickly from the active viable form into the inactive spore form. Thick cell walls of spores make them very resistant to adverse environmental conditions such as disinfectants, irradiation, drying or heat (Madigan et al. 1997). As spores they can survive for decades in a kind of stand-by stage in which their metabolism is reduced to a minimum. Spores cannot multiply. Only when spores germinate and form viable cells, counts might increase and exotoxins might be released.

Toxin forming *Clostridium* species

Bergey's Manual (Cato et al., 1986) lists 84 *Clostridium* species. Several of them are known to infect farm animals and man and can cause severe damages due to their ability to produce powerful toxins. The following three clostridia species cause enteritis, paralysis or tissue necroses but are not associated with silage feeding and will therefore not be discussed further.

Cl. perfringens	enteritis, tissue necroses from puncture wounds
Cl. difficile	enteritis or enterotoxaemia
Cl. tetani	muscle paralysis and spasms from puncture wounds

Cl. botulinum is the only toxin-forming species that occasionally is connected with silage feeding, usually deteriorated or decayed silage. In many regions *Cl. botulinum* is closely associated with extensively reared cattle chewing bones of dead animals to obtain phosphorus or protein. In endemic areas, type-specific vaccines and antitoxins are used to reduce or treat cases of botulism. Seven or eight different strains are known (A to G), grouped after the type of toxin they produce (Wilkins 2007). Different animals are sensitive to different types, e.g. cattle are mainly affected by type A, B, C and D, horses mainly by type B and humans by type A, B, E and F (Iowa State Univ.: Botulism Fact Sheet). Pigs and cats are relatively resistant to botulism. Notermans et al. (1978) and Roberts (1988) said to have found evidence that *Cl. botulinum* can multiply in the gastrointestinal tract of cattle or man and may continue to produce toxin. Botulinum toxins (BT) are proteins or polypeptides and are among the most powerful neurotoxins known. They block the release of acetylcholine, which makes a muscle contract, and cause progressive paralysis of muscles. Notermans et al. (1979) estimated that 10 to 100 g of highly contaminated silage may contain sufficient BT to kill a cow. The same authors tested in total 11 strains of *Cl. botulinum* (type A, B, C and E) and found that only the proteolytic strains of type A and B were able to produce toxin with only grass as substrate. This supports the fact that an animal protein source (such as a dead animal) is not a prerequisite for BT production in silage. *Cl. botulinum* reacts more sensitive to low pH levels than the typical silage clostridia (see below) and will therefore not grow in well-fermented silage. Never the less this species may at times survive and multiply in pockets in the silage where pH and water activity are high enough to permit growth. That could be in pockets with aerobically deteriorated or soil contaminated silage or inside a small animal (intestines) that was killed during cutting. The carcass must be large enough to protect its inside from the penetrating fermentation acids. This could be the reason why *Cl. botulinum*-contaminated insects

Picture 1. Adult specimen of *Arion lusitanicus*, 7-10 cm in

Picture 2. Grass ensiled with slugs in laboratory silos.

or invertebrates in silage do not appear to pose a threat to ruminant health but larger animals like cats, rabbits or deer calves might do.

In 2007 we had problems with a massive multiplication of slugs on leys on the west coast of Sweden. The slug was a new, invasive variety (*Arion lusitanicus*) from central Europe (Picture 1) and because of its cannibalistic habits it was called 'murder slug' by media and public. During the same year six calves died in this area and dairy cows refused to eat the slug-infested silage. Soon speculations were raised on the possible involvement of *Cl. botulinum* and farmers demanded quick actions from advisers and researchers. The year after we got economic support to make a preservation experiment in laboratory silos with an untreated control (no slugs) and 3 levels of slugs (corresponding to 20, 40 and 80 slugs/m^2) mixed into a grass crop with a DM content of 33% and 53%. Quite opposite to what was expected, the results indicated an advantageous effect of the slugs on fermentation characteristics of the resulting silages. The slugs contained relatively high counts of LAB, which increased the formation of lactic acid. Counts of *Clostridium* spp. and enterobacteria were below detection level (<10^2 and <10 cfu/g FM, respectively). DNA sequences from *Cl. botulinum* type C (PCR technique) were found neither in slugs nor silages. But even if the fermentation quality was excellent, cattle might still find slug-infested silage not very palatable.

Fertilizing forage crops with poultry litter, which often contains decayed carcasses, or mixing poultry litter with forage crops to increase the crude protein content of the resulting silage is common practise in some countries (Otter et al. 2006, Sharpe et al. 2008). However, that is a very risky operation because if mixing is uneven, air is leaking into the silo or acidification in some part of the silage is insufficient, an outbreak of botulism, usually with high mortality, would be highly likely. In addition it should be considered that toxins formed in poultry carcasses before ensilage cannot be destroyed by fermentation acids in the silage.

Sometimes cattle are forced to eat decayed or soil-contaminated silage because no other forage is available to them, e.g. when low-rank cows do not dare to approach the feed source where high-rank cows linger. But that is, strictly speaking, not a silage but a management problem.

The dry matter (DM) content of the silage might play an important part too, because the pH of the silage increases with increasing DM content (Morgan et al. 1980, Pauly & Tham 2003). High DM silage might therefore be a risk factor

depending on if the water activity (a_w) is low enough to inhibit growth of *Cl. botulinum*. Notermans et al. (1979) state that *Cl. botulinum* type A and B needed for toxin production a minimum water activity (a_w) of 0.94 at pH 5.8 - 6.5 and at least 0.985 a_w at pH 5.3. In theory DM content and water activity of a silage crop should be highly correlated to each other (negatively) but according to our experience with grass-based silage crops this correlation varies too much between cuts and years to estimate a crop's water activity from its DM content. Other crops might respond more predictable and it might be easier to state a safe DM range, which does not permit *Cl. botulinum* to grow. But if we deduct from Notermans et al. (1979) data that silage with a pH below 5[1] should be safe to feed, we can conclude that that could be easily achieved if we do not wilt the forage crop above 40-45% DM, possibly add a LAB-based additive and apply what is generally called 'good ensiling practices'.

However, farm silage is often not very homogeneous and pockets with deteriorated silages might exist right next to excellent silage (Spoelstra 1990). It is common practice to discard visually deteriorated silage, but not all quality flaws in a silage are discovered and occasionally pieces of decayed silage unintentionally end up in the forage that is fed. When total mixed rations (TMR) are fed, all ingredients are thoroughly mixed and animals have no possibility to avoid spots of the silage that might contain BT. On the contrary, a chunk with BT-containing silage might be distributed in the entire TMR and might affect many cows. For the vet it might prove very difficult to find the feed (or part of feed) that caused the disease. Even when blood (serum) or contents of intestines are sampled it is frequently difficult to find any BT and verify the type of organism or toxin that caused the disease.

Common *Clostridium* species in silage

Less then 10 *Clostridium* species have been isolated from silage or cattle manure (Table 1). The 4 most common species in silage are *Cl. sporogenes, Cl. tyro-butyricum, Cl. butyricum* and *Cl. bifermentans.* All of them ferment water-soluble carbohydrates (saccharolytic activity) and some of them lactate or amino acids and peptides (proteolytic activity). None of them is known to produce any toxins. Pahlow et al. (2003) state that proteolytic clostridia, which are unable to ferment

[1] Roberts (1988) states that *Cl. botulimum* is unable to grow at a pH ≤4.6, but that pH, water activity and temperature act in combination.

carbohydrates, are *not* commonly found in silage. This is probably why *Cl. botulinum* and *Cl. perfringens* are only rarely isolated from silage.

Table 3. Number of isolates of *Clostridium* species recovered from silage or cattle manure (Ali-Yrkkö et al. 1978 and Bühler 1985; after Pahlow et al. 2003).

Species	Silage	Manure
Cl. sporogenes	13	69
Cl. tyrobutyricum	51	28
Cl. butyricum	12	8
Cl. bifermentans	14	5
Cl. acetobutyricum	9	
Cl. perfringens		1
Cl. paraputrificum	5	2

When spore-containing silages are fed, the concentration of spores increases on the way through the gastro-intestinal tract about tenfold (Stadhouders et al. 1985). This is why the spreading of manure (solid) or slurry (liquid) constitutes a potential contamination risk. Large forage plants, like corn or sorghum, which are harvested without wilting and with a high stubble height (approx. 20 cm), are usually not contaminated with soil. Temperate grasses and forage legumes, which are commonly wilted in the field and cut at a low stubble height (approx. 5-10 cm), form a much larger contamination risk. Our experiences with temperate grass crops indicated that spreading of manure (solid) had a detrimental effect on silage quality, because small pieces of manure always ended up in the harvested forage where spores in the manure germinate and multiply (Rammer 1996). Spreading of slurry (liquid) was acceptable, if it was applied on the stubble right after the cut (i.e. no soiling of plants). Slurry injection in the soil was acceptable if the soil was moist and soft, but a dry and hard soil caused splashing of slurry and contaminated the plants and eventually the silage crop (Pauly & Rodhe 2001).

The clostridial spores commonly found in silage do not pose any health risk for man or animal and they do not produce any toxins. Butyric acid and ammonia, typical products of clostridial fermentations and easily identified by their pungent smell, should not be a problem for cattle because they produce large amounts of it in their

rumen. Then why should clostridial fermentations be avoided? Many farmers have experienced reduced silage intakes and decreasing weight gains or milk yields when clostridial silages were fed (McDonald et al. 2002). The main reasons why farmers should try to avoid any clostridial fermentation in their silage are:

- Clostridial fermentations lead to the production of H_2 which means that a large part of the energy content in the fresh crop is lost (approx. 18% of gross energy according to McDonald et al. 1991, p.242).
- Clostridia are able to ferment the amino acids that arise from their own proteolytic activity and the action of plant proteases. Amines, carbon dioxide and isobutyrate are produced by decarboxylation of amino acids and ammonia and organic acids by deamination (Rooke & Hatfield 2003). The production of toxic amines[2] is believed to be one of the main causes for the low DM intakes of clostridial silages (van Os 1997, Neumark & Tadmor 1968). In addition, the detoxification of ammonia and probably even amines in the ruminant is an energy-demanding process (McDonald et al. 2002).
- Dairies, which produce certain types of hard cheese, can expect economic losses when cheeses slowly inflate during storage due to excessive gas production (CO_2, H_2) by clostridia (mainly *Cl. tyrobutyricum* and *Cl. butyricum*) (Bergère & Accolas 1985). Many European dairies motivate farmers to deliver spore-free milk by reducing the payment if spore counts in milk rise above a given threshold value (e.g. 700 spores/litre).

Hence the main objective during silage making should be to take measures that inhibit or reduce the multiplication of clostridia in silage. In well-fermented silages the spore level will, remain on the same level as in the fresh crop because if growth conditions for clostridia in silage are unfavourable (pH <4.6, water activity <0.94, nitrate >2 g/kg DM) spores are not likely to geminate. The most important measures to inhibit clostridia growth are:

- Avoid contamination of forage with soil, faeces, animals (vertebrates) or decayed silage.
- Wilting decreases water activity, which will reduce or inhibit clostridial activity. Under controlled conditions clostridia have problems to multiply above 30% DM in

[2] Amines: cadaverine, putrescine, tyramine, histamine.

grass silage (McDonald et al. 1991). Taking the heterogeneous nature of farm silage into account, a DM content of at least 40% should be a safe level for most practical situations (Jonsson et al. 1990).

- When 40% DM is not achieved, clostridia-inhibiting additives can be applied. Such additives contain nitrate or nitrite, benzoate or other clostridia-inhibiting compounds as active ingredients.
- Ingress of air (oxygen) into the silo favours yeast and mould over LAB growth and tends to increase counts of clostridial spores (Jonsson 1991). The latter appears to be a contradiction because clostridia are known to grow and multiply only in an anaerobic environment. However, yeasts and moulds can metabolize lactic acid with the help of oxygen and thus create anaerobic niches with a high pH, in which clostridia might thrive. With a short length of chop, thorough consolidation and a tight silo cover, the quantity of air leaking into the silo can be minimized. In addition, these measures will help to reduce problems with aerobic deterioration (heating) when silages are fed out.

Inoculation of forage with *Clostridium* strains

Farmers should always try to minimize the spore contamination of forage crops. However, for researchers the inoculation of the fresh crop with clostridial spores might be a useful tool to challenge the ensiling process, e.g. when examining the inhibiting effect of silage additives or when clostridial fermentations are studied. When testing silage additives, it is imperative that the untreated control is going to ferment badly otherwise the potential quality-improving effect of an additive cannot be examined. The challenge for the researcher is to choose the right level of deteriorating actions so that the controls are negatively affected without turning *all* treatments bad. In some studies clostridia-containing soil was used as a spore source, but that would not test the clostridial influence *per se* because soil contains a vast range of other microorganisms plus buffering minerals and organic compounds, which all might interfere with the ensiling process. The best choice would be to add an aqueous suspension with clostridia spores to the forage crop. The question would be which species or strains and which inoculation level should we choose? In Sweden we used during the last 20 years a pure *Cl. tyrobutyricum*-strain selected from blown hard cheese and applied it at a rate of approx. 10^3 viable spores per gram of fresh forage (Pauly et al. 2008). However, in other parts of the world other

Clostridium species and strains and other inoculation rates might be more relevant. By collecting samples from badly fermented silages or milk products and by identifying the species, the most common and competitive *Clostridium* species could be identified. To culture and store selected strains is not difficult and can be done with basic lab equipment. The spore suspensions will keep for years. It is however important to assess the viability of the spores in the suspension before the experiment is performed because viability might vary within a wide range. Viable spore counts per mL of suspension are determined by making a few tenfold serial dilutions and culturing aliquots of the dilutions on *Clostridium*-selective agar plates (e.g. reinforced clostridial agar) in anaerobic jars.

Bacillus spp. in silage

Like clostridia *Bacillus* spp. are Gram-positive, endospore-forming bacteria usually found in soil, dust, manure and bedding material of farm animals. They are distinguished from clostridia by their aerobic growth (catalase positive). Some bacilli, like *B. cereus*, are facultative and can grow in anaerobic environments too. Like clostridia, high temperatures encourage bacilli to germinate and grow (Gibson et al. 1958).

The two following *Bacillus* species are well-known, but are here only briefly mentioned because none of them is associated with silage or silage feeding. *B. anthracis* is a soil bacteria that can produce a very powerful toxin that causes anthrax in humans and animals, often mentioned when biological warfare is discussed. *B. thuringiensis* forms upon sporulation crystals of proteinaceous endotoxins, which when ingested are pathogenic to many insects. This species is used for the production of biological insecticides and insect-resistant genetically modified crops.

Table 4. *Bacillus* spp. found in deteriorating silages (after McDonald et al. 1991).

Aerobic bacilli	Facultative anaerobic bacilli
B. sphaericus	*B. cereus*
B. lentus	*B. licheniformis*
B. firmus	*B. polymyxa*

B. cereus is known to be very versatile. It can grow from 4-5° up to 50°C and has been demonstrated over a pH range of 4.9 to 9.3 (Jay et al. 2005). It can cause infections of the skin, the eye or of the intestinal tract of humans or farm animals

(U.S. Food and Drug Administration) as well as mastitis in cows (Schiefer et al. 1976). Upon intestinal infections it can produce 2 types of toxins, which cause either a diarrheal type or a vomiting (emetic) type of illness (Jay et al. 2005). In dairies this species is well-known for its ability to survive pasteurization (as spores), to attach to surfaces and to cause coagulation (clotting) of fresh milk and cream (te Giffel 1997).

Some facultative anaerobic bacilli might play a minor role in the ensiling process as they can produce lactate, acetate, butyrate, ethanol, 2,3-butanediol and glycerol (Pahlow et al. 2003). Woolford (1977) tested the activities of 3 bacilli (*B. coagulans, B. licheniformis, B. polymyxa*) in grass silage with 14% DM and 8% water-soluble carbohydrates in DM. He summarized that these bacilli were not suppressed by organic acids or a low pH and had the ability to compete with LAB. However, only *B. coagulans* was capable to produce lactic acid and contributed slightly to a reduction in silage pH, but LAB were more efficient than bacilli in reducing silage pH. The tested bacilli contributed little to the fermentation and LAB dominated the silage fermentation. Moran et al. (1993) inoculated big bale silage made from ryegrass (28% DM) with a mixture of *L. plantarum, Serratia rubidaea* (an enterobacteria) and *B. subtilis*. When bales were assessed for mould occurrence approx. 3.5 months later, mould scores were lower for bales treated with the inoculant mixture than with the *L. plantarum* inoculant or the untreated control.

Strictly aerobic bacilli can survive the anaerobic storage period as spores and might germinate when silos are opened and air penetrates into the silage. They might therefore play a role during the later stages of aerobic deterioration in silages together with facultative anaerobic bacilli. They appear not to initiate the deterioration process, this is usually done by lactic acid-assimilating yeasts (Lindgren et al. 1985, Jonsson 1989) or acetic acid bacteria (Spoelstra et al. 1988). However, they are often observed at later stages in the aerobic deterioration process when silage temperature exceeds 45°C (Lindgren 1991). In moist hay they might be involved together with actinomycetes in the initial microbial heating that later might escalate and eventually, by chemical processes, leads to the spontaneous combustion of hay (Scott & Mercer 1997).

In conclusion, *Bacillus* spp. play a minor role in silages but deserve some attention on dairy farms (udder hygiene at milking), because of their negative implications to the dairy industry. Some *Bacillus* strains might become valuable

inoculants, particularly for high-DM silages, in the future because of their ability to produce specific bacteriocins or antibiotics.

Enterobacteria in silage

Enterobacteria are rod-shaped, motile, Gram-negative and facultatively anaerobic bacteria belonging to the group of *Enterobacteriaceae*. They constitute the prominent part of the gut flora in the intestines of most warm-blooded animals and are found in manure, soil and on forage crops. Sometimes the term 'coliform bacteria' or 'coliforms' is used, but that is by definition a subgroup that ferments lactose, a trait not very useful for forage plants, which lack lactose (Pahlow et al. 2003). It is now common practice (as suggested by Seale et al. 1990) to culture enterobacteria on the less selective violet red bile *dextrose* agar (VRBD) instead of violet red bile agar (VRB) that contains only lactose as the main energy source.

Enterobacteria are generally not regarded to be pathogenic but like all other Gram-negative bacteria they contain an endotoxin (lipid A) in their outer membrane (Lindgren 1991). When enterobacteria are digested and dissolve, the endotoxin is released and absorbed through the mucous membranes of the gastrointestinal tract into the blood stream. Large quantities of endotoxin are present in the rumen. There is evidence that the endotoxin concentration increases when concentrates are added to a hay diet (Haubro Andersen 2003). Cattle should be naturally adapted to this continuous flow of endotoxin, but large variations in susceptibility to endotoxin are reported. Healthy cattle appear to have mechanisms that can deal with the endotoxin, but animals with infected organs (udder, uterus) or enteritis might be more susceptible/vulnerable. The toxicity of the endotoxin might therefore be determined primarily by the health status of the animal rather than by the properties of the endotoxin *per se* (Haubro Andersen 2003).

There is a general concern that pathogenic species or strains of enterobacteria, such as *Salmonella* spp., *Klebsiella aerogenes* or *E. coli* O157:H7, might survive the ensiling process and spread from farm animals to man or vice versa (Lindgren 1991, O'Kiely et al. 1999). The same might be relevant to antibiotic-resistant strains, but tolerance to antibiotics has no competitive advantage in silage and these bacteria would probably not persist longer in silage than other enterobacteria. However, silage is a very variable substrate with a wide range of acidity and water activity depending on forage crop, DM content and maintenance of

anaerobiosis in the silo. Single ensiling experiments can only give an indication on the survival of a particular organism under particular conditions prevailing in a particular type of silage. Lab experiments that determine an organisms tolerance to fermentation products, pH and water activity are probably more useful for assessing the chance of survival in different kinds of silages. With respect to the heterogeneous nature of most farm silages, a safety margin should be added to the limiting growth conditions, which are determined in well-controlled lab experiments.

Enterobacteria are a large and versatile group of bacteria, which can, depending on the species, ferment a wide range of carbohydrates and give rise to fermentations products such as lactate, acetate, formate, ethanol, acetoin[3] and 2,3-butanediol[4] (Rooke & Hatfield 2003). Enterobacteria are sometimes divided into butanediol-producers and mixed-acid-fermenters. The latter group produces mainly acetate, formate and ethanol from glucose. They are not known to form larger quantities of acids and their capacity to acidify silage is therefore limited. Spoelstra (1985) states that they are inhibited by a pH <4.5, but points out that their ability to reduce nitrate (NO_3) to nitrite (NO_2) and further to nitrous gases (NO, NO_2, N_2O) is important for the ensiling process because nitrite and NO and NO_2 are very effective in inhibiting the multiplication of clostridia. Nitrate and the final product of the nitrate reduction, ammonia, have no inhibiting effect on clostridia. Well-fermented silages, which are made of forage crops low in nitrate often contain small quantities of butyric acid, which is produced by clostridia in the beginning of the ensiling process before a low pH inhibits their activity. The addition of nitrate to a forage crop low in nitrate usually results in butyric acid-free silages (Weissbach et al. 1993). The addition of nitrate or nitrite is advisable if the forage is ensiled below a DM content of approx. 35% DM and if the crop really is low in nitrate, e.g. has received low dressings of nitrogen fertilizer. Commercial silage additives, which have been used in Scandinavia for decades, can add between 400 g and 900 g of $NaNO_2$ per metric tonne of fresh matter (FM). Care must be taken to avoid any nitrate overdose because cattle might respond with reduced intake, poor growth and bad fitness. If nitrite is absorbed into the bloodstream, hemoglobin is converted to methemoglobin, which looses its ability

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[3] Acetoin has a 4 C structure with a carbonyl group (=O) on the 2nd C and a hydroxyl group (-OH) on the 3rd C. It has a pleasant buttery odour and is used as flavouring agent in bakery products.

[4] 2,3-butanediol has a 4 C structure with one hydroxyl group (-OH) each on the 2nd and the 3rd C.

to transport oxygen. Negative health effects might be expected if the daily nitrate intake exceeds 10-20 g/100 kg body weight (Undersander et al.).

Apart from their ability to reduce nitrate, high numbers of enterobacteria are not desired in silage because:

a) enterobacteria compete with LAB for fermentable carbohydrates during the initial stages of the ensiling process; if the content of soluble carbohydrates is low, LAB might be inhibited by the lack of fermentable substrate;
b) enterobacteria have, like clostridia, proteolytic activity, meaning they degrade protein into amino acids and peptides (Spoelstra 1983);
c) enterobacteria degrade nitrate and deaminate amino acids, which will produce ammonia that increases silage pH (Spoelstra 1987).

The number of enterobacteria found on temperate forage crops at cutting might range between 10^3 – 10^6 cfu/g FM (Pahlow et al. 2003). During the initial phase of ensilage numbers often increase up to 10^8– 10^{10} cfu/g FM (Rammer 1996), but then drop quickly after few days below detection level (Heron et al. 1993, Pahlow et al. 2003). The enterobacteria that dominate on fresh temperate grasses were often *Erwinia herbicola* (identical to *Enterobacter agglomerans*) and *Rhanella aquatilis*. After ensiling their numbers dropped fast and *Hafnia alvei* and *E. coli* dominated for a short time before they disappeared altogether (Heron et al. 1993). The rise and fall of enterobacteria in silage is very closely connected with the development of LAB. LAB numbers in the fresh crop are on average 100 times lower than enterobacteria counts (Pahlow et al. 2003), but when there is no shortage in substrate and good ensiling practices are applied, LAB will outcompete enterobacteria within a matter of days due to their better adaptation to anoxic and acidic environments. In corn silage, which has a low buffering capacity and contains plenty of fermentable substrate, the pH drop is faster than in most grass-based silages and enterobacteria are detectable only during the first day after ensilage (Pahlow et al. 2003). It is therefore not common to find enterobacteria 2-3 months after ensiling when silos are opened for feeding. However, enterobacteria might persist on spots in the silo that are contaminated with chunks of soil or manure or where air leaks in. But such silage is usually dark and smells bad and no conscientious farm worker would feed it.

Listeria in silage

Listeria monocytogenes is the causative organism of listeriosis, a disease, which affects both a wide range of animals and man (zoonosis). Six different listeria species are known but only *L. monocytogenes* is known to cause listeriosis. Typical manifestations of listeriosis are septicaemia and/or affection of the central nervous system (meningitis), abortions and occasionally mastitis (Waak 2002). Among farm animals sheep and goats appear to be particularly susceptible to listeriosis (Low & Renton 1985, Wiedmann et al. 1994).

Human listeriosis is a relatively rare food-borne disease, approx. 4-8 cases occur each year per 1 million persons (FDA, USDA & FSIS 2001). A distinction is made between infections limited to mild, flu-like symptoms (listerial gastroenteritis) and those that are severe and life-threatening (listeriosis). Although the number of human listeriosis cases is low, the mortality might be as high as 20-40% (McLauchlin 1997). Ill persons with a compromised immune defence, pregnant women, newborns and old people are particularly susceptible. The type of food that is most closely associated with outbreaks of human listeriosis is meat spreads (pâté) and Deli meats, smoked seafood, milk products (particularly unpasteurized soft cheese) and other refrigerated ready-to-eat foods (FDA, USDA & FSIS 2001). The ability of *L. monocytogenes* to grow at low temperatures makes it particular important in relation to food poisoning (Table 5).

L. monocytogenes is a rod-shaped, Gram-positive bacterium, which is spread world-wide and can be found in low numbers (<30/g) in sewage water, soil, herbage and faeces (Fenlon 1989, Weis & Seeliger 1975). Since the ’60 listeriosis has been associated with silage-feeding, in

Table 5. Growth limits for *L. monocytogenes* according to ICMSF (1996).

Growth factors	Minimum	Optimum	Maximum
Temperature (°C)	-0.4	37	45
Water activity	0.92	0.97	-
pH	4.4	7.0	9.4

particular poor quality silage (Seeliger 1961, Gray & Killinger 1966). With an imperfect aerobic and a facultative anaerobic metabolism *L. monocytogenes* is

stimulated by micro-aerophilic conditions as when air leaks into a silo (Fenlon et al. 1989). Farm silos and big bales are often not completely gas-tight. If the silage is not properly consolidated and sealed or if the plastic cover is damaged, the ingress of air will stimulate facultative anaerobic organisms like yeasts. Lactate-assimilating yeasts are able to metabolize lactic acid in the presence of oxygen. That consumes the oxygen, increases silage pH and eventually facilitates the growth of undesired organisms like listeria or clostridia (Lindgren et al. 1985). Listeria counts were noticed to be particularly high in the aerobically deteriorated parts of the silage and when the deteriorated parts were removed, the dose of *L. monocytogenes* in silage could be significantly reduced (Fenlon et al. 1989). Infection of animals or man is dependant on the number of ingested *L. monocytogenes* and on the immunological status of the individual (Gray & Killinger 1966). Because listeria counts in the fresh herbage are usually too low to cause infections in healthy animals, the most important task at silage making would be to avoid the multiplication of listeria in silage. Husu et al. (1990) examined the occurrence of *Listeria* species in 68 grass and 225 silage samples (average DM 21%) collected from 80 Finish dairy farms. *L. monocytogenes* was found in 38% of the grass samples, 16% of the silage samples and at least at one occasion on 34% of all farms. No signs of clinical listeriosis were detected in any of the herds probably because listeria counts were low (counts not determined) and animals in good health. Ensiling experiments with *L.* monocytogenes-inoculated forage (10^6-10^7 cfu/g FM) at 21%, 43% and 54% DM showed that the most important environmental factors for the fast elimination of listeria was a) a pH below approx. 4.5 and b) a storage time longer than 30 days (Pauly & Tham 2003). Decreasing water activity reduces the ability of listeria to grow at low pH (Fenlon 1989). The reduction of water activity by wilting from 0.99 (21% DM) to 0.95 (54% DM), which cohered with a pH increase from 4.9 to 5.9, could not reliably eliminate all listeria within a storage period of 90 days. However, the application of an efficient LAB inoculant increased the content of lactic acid and eliminated all listeria within 30 days (Pauly & Tham 2003). To get a reliable pH reduction from a LAB inoculant, the fresh forage must contain at least 15-25 g water soluble carbohydrates per kg FM (Pettersson 1988, Pahlow 1990). At DM contents above approx. 45% only osmotolerant LAB should come into use and few if any inoculants are efficient at DM contents above 55%.

The most important measures to avoid multiplication or survival of listeria in silage are:

a) prevent ingress of air into the silo, which implies that the silage should be well consolidated and the integrity of the silo cover must be maintained;
b) if there is a risk for listeria contamination, an intensive lactic acid fermentation should be stimulated, e.g. by a short wilt up to approx. 30-40% DM and the application of an effective LAB inoculant.

In conclusion, when good ensiling practices are applied and the silage is properly sealed and stored for at least 2-3 months, the risk that some listeria might survive the ensiling process should be very scarce. To eliminate all listeria during the storage period in the silo is important because surviving listeria might be able to multiply when silos are opened for feeding and air can penetrate the silage.

Conclusions

Many of the pathogenic bacteria mentioned above can be restricted or inhibited in silage by what is generally known as ‘good ensiling practices’. The most important measures are:

- Contamination with soil, manure, animals (vertebrates) or decayed forage is reduced to a minimum,
- the forage is wilted to a level that guarantees sufficient supply of water-soluble carbohydrates for an unrestricted lactic acid fermentation (Weissbach 1996),
- the forage is chopped, consolidated and sealed in such a way that ingress of air (oxygen) during storage is reduced to a minimum,
- the silo is not opened for at least 2 months after ensilage to outcompete or reduce numbers of undesired bacteria,
- the removal rate from the silo is at least 2.0 m per week to reduce the risk of aerobic deterioration during feed-out.

The problem with silage making is often that technical aspects and logistics receive a lot of attention while microbiological aspects appear to be invisible because they are much more difficult to grasp, e.g. the assessment of contamination risk or how well the silo is consolidated and sealed. It is human that we tend to repeat mistakes from previous years when all our attention is focused on to fill the silo at the shortest time possible. With respect to the consequences of what a silo filled with badly-fermented silage means for the coming feeding period, more farmers should consider to engage a silage specialist, who helps to plan and perform the ensiling work at site (i.e. on farm). Small changes in the planning can many times work

miracles, e.g. to ensure that there are enough people left (often late at night) when the silo has to be sealed. Three aspects are particularly important when it comes to question on how the survival and multiplication of undesired silage bacteria can be avoided:

a) The maintenance of anaerobiosis in the silo, because ingress of oxygen eventually creates niches or zones of aerobically degraded silage that facilitate the survival of many undesired bacteria.
b) Efforts should be made to increase the level of homogeneity in the silage during silo filling, e.g. by trying to reduce or level out DM variations after wilting or by avoiding contamination with soil or manure particles.
c) If the undesired bacteria cannot be eliminated reliably with good ensiling practises, the application of an effective additive should be considered. It is only meaningful to apply an additive if the selected additive has a documented activity against the organism in question and if the additive can develop that activity at the targeted DM content.

With these suggestions in mind the risk for the development of deleterious bacteria in silage should be very slim.

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