



PROCEEDINGS OF THE II INTERNATIONAL SYMPOSIUM ON FORAGE QUALITY AND CONSERVATION



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Potential of Energy Production from Conserved Forages

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The world is at a critical time relative to energy supply. Fossil fuels have been the primary sources of energy in our lifetime and for more than 100 years before us. However, there are considerable concerns about how sustainable those sources will be for the future. One, we know that the supplies of crude oil, natural gas and coal are finite. These resources may not be exhausted in our lifetimes, but inexpensive crude oil is already coming to an end, raising the costs of food and other necessities of life in addition to the costs of the goods and services that make life comfortable. Two, the demand for energy is going to continue to climb. The global human population is nearly 7 billion and expected to reach 9.5 billion by 2050 (U. S. Census Bureau, 2011). Beyond the increase in population is the reasonable desire by people in developing nations to live like people in developed countries where energy consumption per capita is much higher. Finally, use of fossil fuels is a major contributor to global warming as we return carbon that has been sequestered in the earth for millions of years back into the air as carbon dioxide. Logically, we need to seek solutions to our increasing demand for energy that minimize our carbon footprint.

Given these issues, plants, in general, including forages have a potential role in meeting that demand for energy because they fix carbon through photosynthesis, producing carbohydrates that can become an inexhaustible source of potential energy. Perennial forages are attractive for various reasons. One, both the monetary and energy cost of planting is spread over multiple years. Two, we already have the equipment for harvesting, storing and transporting this source of biomass. Three, legume forages or legume-grass mixtures need no or little nitrogen fertilizer, a major energy input for most crops. Four, forages can be grown on marginal agricultural lands and so do not compete with land that could be best utilized for direct human food production. Finally, perennial forages stabilize and enhance soil systems, minimizing erosion and building up soil organic matter, sequestering carbon.

In the United States, a major survey indicated that biomass from agricultural land and forestlands could sustainably supply more than one-third of the country's current petroleum demand (Perlack et al., 2005). To accomplish that, their assessment estimated that perennial forages would account for more than a quarter of the biomass, and crop residues, which could

be harvested, stored and processed similarly to perennial forages, would represent one-third of the biomass. Consequently, there is a significant potential for forages and crop residues to contribute to the energy supply in the U.S. When one considers that the U.S. population has the highest per capita energy consumption, other countries may be able to produce an even larger fraction of their energy needs from forages.

The potential for forages to be sources of biomass for energy production in other countries will depend on many factors. A key will be the arable land base relative to the population and the amount of land needed for food production. Countries that are unable to meet the food demands of their human population are unlikely to have significant arable land resources that can be devoted biomass production. Countries that are net exporters of food are potentially good candidates for biomass production. However, even in countries with a surplus of food, the potential for energy production from biomass will depend upon the cost of producing energy from biomass compared with the cost of energy from more traditional sources, any incentives provided by governments for alternative energy sources, various political and social pressures to move toward alternative energy sources, and finally the infrastructure and technologies to accomplish efficient conversion of biomass to energy.

Our roles as scientists and engineers are to provide the information and technologies to permit biomass to be converted to energy. This includes increasing yields, efficiently harvesting crops, minimizing losses in storage, investigating potentials for pretreating crops in storage, developing systems for efficient transport to bioprocessing facilities, and discovering new technologies for the bioprocessing plant to improve the efficiency of energy production and maximize the value and utilization of the byproducts.

In this paper, I will briefly survey some of the opportunities for using forages as a source for energy. The most likely means of producing energy from forages are ethanol production (enzymatic breakdown of structural carbohydrates to simple sugars followed by fermentation of the sugars by yeasts or bacteria to ethanol), anaerobic digestion (fermentation of proteins, carbohydrates and lipids by various groups of bacteria to methane and carbon dioxide to methane) and combustion (generating electricity from turbines run by steam). Other possibilities will be discussed as well.

Species

A wide variety of forage species are being investigated as sources of biomass. In the U.S., C4 grasses have been the principal species studied because of their ability to produce high yields from one or possibly two cuttings per year. The emphasis has generally focused

on grass species with modest fertilizer requirements. The most common species studied have been switchgrass (*Panicum virgatum*), various *Miscanthus* species, maize (*Zea mays*), forage sorghum (*Sorghum bicolor* subsp. *bicolor*) and sudan grass (*Sorghum bicolor* subsp. *drummondii*). The principal C3 grass investigated has been reed canarygrass (*Phalaris arundinacea*).

The C4 grasses are also the primary focus in other countries. Switchgrass and *Miscanthus* species are commonly being investigated for their potential. In Brazil, sugarcane (*Saccharum* spp.) is already a major source of sugar for ethanol production. It is a potential biomass source in the U.S. Elephant grass (*Pennisetum purpureum*) has been studied to a lesser extent.

Why have the legumes and C3 grasses been largely ignored? I think it is because the emphasis has been on obtaining low-cost, high-yield forage from one cutting per year. With many C3 grasses and legumes, multiple cuttings per year are necessary, and the total yields are lower than those of the C4 species that are being proposed which require only one cutting.

Another factor is nitrogen content. Nitrogen fertilizer is a major energy input to crop production. In fact, analyses of the net energy produced by ethanol production from maize grain sometimes show a negative balance, and fertilizer N is a major contributor to that (Farrell et al., 2006). Many of the C4 grasses being studied for biofuels have modest N fertilization requirements compared with other grass species. For example, optimum yields (~11 Mg/ha) in switchgrass were observed at fertilizer rates of 120 kg N/ha (Vogel et al., 2002). A rate of 100 kg N/ha did not improve the yield (28-38 Mg/ha, varying by year) of *Miscanthus giganteus* over that at 50 kg/ha (Danalatos et al., 2007). In addition, the nitrogen content of the C4 grasses is more than sufficient for the nitrogen needed by the yeast or bacteria used to produce ethanol. For example, the only additional nitrogen used in a proposed lignocellulosic ethanol production system from maize stover would be to grow the *Zymomonas mobilis* inoculum for the fermentation (Humbird et al., 2011). So excessive plant N is of little value for biofuel production. Legumes would have an advantage of requiring no N fertilization. However, the N contents of legumes are typically double or more than that of the grasses being proposed.

Is this focus on high yield, low N requirement grasses justified? Possibly. However, it is essential when selecting a prospective forage for biofuel production to find the lowest cost biomass that meets the requirements of the production system. Sugarcane is an ideal grass for ethanol production because of the high yield of biomass with high sugar content and low energy input. Under tropical conditions, energy in:energy out can reach 1:8 when used to

produce ethanol (U. S. Department of Energy, 2011). Even under more temperate climates, energy in:out may be 1:3. With switchgrass or *Miscanthus* spp., we are looking at high yields, but these will only be economically feasible if the structural carbohydrates (cellulose and hemicellulose) can be enzymatically broken into sugars at reasonable cost. At our center, we have observed that switchgrass is much less amenable to enzymatic breakdown of structural carbohydrates than reed canarygrass (Digman et al., 2010). This suggests reed canarygrass might provide a higher yield of ethanol per hectare than switchgrass in some environments. So if our target is ethanol production, our focus needs to be the yield of readily available carbohydrates rather than total yield. If the target is methane production from anaerobic digestion of forages, methane yield may be more important than biomass yield. Similarly with pyrolysis, biodiesel, etc., yield of the target fuel per hectare is more important than yield of crop.

Another factor that affects the choice of forage is the value and utility of the byproducts of the process. In the U.S., maize grain is currently the main source of ethanol. However, the expansion of ethanol production from maize currently depends on selling the distiller's grains as livestock feed, and this byproduct will inevitably displace other concentrates in livestock rations (Farrell et al., 2006). If forages will be grown for ethanol production for example, what will be the potential uses of the byproducts and will they provide significant revenue to make the whole process profitable?

A final issue relative to crop selection is how a forage fits into a crop rotation. Productivity of crops when continuously grown in the same field declines with time. Continuous cultivation of a crop may reduce soil fertility and increase disease and insect pressures. The inclusion of a legume forage like alfalfa (*Medicago sativa*) into a rotation provides substantial N credits to succeeding crops, greatly reducing fertilizer N needed for two or more years after the alfalfa is replaced.

Harvesting

It would appear that the technology for harvesting and handling forages is mature. Mowing equipment has been growing faster in capacity than the technology of cutting. Various means are used to speed drying: conditioning rolls on the mower, rakes, tedders, and mergers. Equipment for baling dry hay in various size packages has been available for decades: small rectangular bales, large rectangular bales, large round bales. Bales are readily transportable between farms today and can just as easily be transported between farms and a bioprocessing plant. Precision chop forage harvesters are the dominant means of chopping

forages for ensiling and are growing in capacity as well. On large farms, chopping is often into trucks for rapid transport to the silo. Similarly trucks can readily move ensiled forage. Consequently the maturity of the harvesting and handling systems are an advantage forages have over crop residues such as maize stover as biomass feedstocks.

This maturity, however, does not mean that there is no need or opportunity for innovation in harvesting systems for forages being used as biomass feedstocks. Current harvesting machines are designed for traditional forage crops being cut at more immature stages relevant to meeting the nutritional needs of livestock. In contrast, forage for biomass is geared toward harvesting mature forage with thicker, more lignified stems that will be more abrasive and require more power to cut and process (U. S. Department of Energy, 2011). Mowing equipment may need to contend more frequently with lodged forage. Current harvesting equipment may be challenged to handle the high yields (>30 Mg/ha) of some of the *Miscanthus* species and may require specialized harvesting equipment. Creating stable, dry bales from some of the high yield species may be difficult.

Beyond improvements to current harvesting systems, there are opportunities for novel harvesting approaches. For example at the University of Wisconsin-Madison, a prototype forage harvester that strips leaves for direct ensiling and cuts stems to dry has been designed and tested (Shinners et al., 2007). The goal has been to create a high protein leaf fraction for use as an animal feed and a low protein fraction more suitable for biomass uses than the whole plant. It has been tested on switchgrass, reed canarygrass and alfalfa. Research on grasses has not shown as much potential in creating two divergent fractions as in alfalfa. Alfalfa stems wilt in several hours to typical ensiling conditions [35% dry matter (DM)], and the leaves when ensiled without wilting (~25% DM) have generally ensiled and preserved well (Muck et al., 2010). Consequently such a harvesting system appears to have potential in legumes for producing two crop streams, the leaf fraction for livestock and the stems for biofuels.

Storage/Pretreatment

There are two primary means by which forages can be stored – as dry hay or by ensiling. Dried forage has the advantage of being stable for long periods with low losses (<5%) if the forage is kept dry (e.g., Shinners et al., 2010). However if bales are stored outside without cover in humid climates, significant losses can occur and result in bales of variable quality. Biofuel production schemes benefit from consistent feedstock quality. So good storage environments for dry hay will most likely be required.

In humid environments, both tropical and temperate, production of dry hay may be difficult without rainfall damage during field drying. In these environments, ensiling of forages may be a better choice to reduce harvesting and storage losses. Normally we expect dry matter losses of 5 to 15% from ensiling. However, these losses are increased in a farm setting by the slow removal of silage from a silo, bag or pile, normally taking months to empty a silo while feeding livestock. Losses should be much lower when a silo is opened and rapidly emptied for transport to a biofuels facility. When bag silos have been emptied in a day, losses of 5% or less are possible (e.g., Shinnars et al., 2010).

In the normal ensiling process, preservation is caused by a combination of the exclusion of oxygen and the natural fermentation of sugars by lactic acid bacteria to lactic acid and other products, lowering pH. The lack of oxygen prevents aerobic microorganisms from growing whereas the low pH is the principal mechanism inhibiting the growth of detrimental anaerobic microorganisms. These mechanisms will work whether the forage is destined for livestock or biomass uses.

When forage is to be a biomass feedstock, ensiling is an opportunity for pretreating the crop during storage, potentially increasing the availability of structural carbohydrates for ethanol or other biofuel production. Various typical standard silage additives have been tested on different forages and crop residues. Homofermentative lactic acid bacteria, the most common silage additives, can improve silage fermentation. However, these do not appear to substantially improve potential ethanol production (e.g., Table 1). A heterofermentative lactic acid bacteria like *Lactobacillus buchneri* may help aerobic stability of the ensiled forage by increasing acetic acid and decreasing yeasts and molds. This would be beneficial to minimize losses while the ensiled forage is transported from the farm to the biofuels production facility, but there could be negative consequences for ethanol production by yeast if acetic acid concentrations are high.

Table 1. Acid detergent lignin and cellulose concentrations (g/kg DM) and estimate of the sugar available for ethanol (SSC, relative fluorescence units/g DM) of ensiled maize stovers (Muck et al., 2008).

Treatment	Acid Detergent Lignin	Cellulose	SSC
Control	33	321	36000
Inoculant	34	322	37800
Enzyme	34	294	34200

Enzyme+Inoculant	36	291	38400
LSD at P<0.05	2.3	6.3	2380

Cell wall degrading enzymes were tried several decades ago for ensiling to increase sugar content from structural carbohydrates. These additives are not in most markets due to high cost relative to inoculants. However, they may have a role in partially degrading cell walls during storage. Several studies have looked at these (e.g., Table 1). Some of the current enzymes from major enzyme manufacturers reduce the amount of fiber in forages during silage storage, but the effect on potential ethanol production has been more modest. An alternate enzymatic approach may be to add enzyme-producing fungi to a moist crop, keeping the crop aerobic until enzyme activity is sufficient and then ensile the crop, allowing the enzymes to work during storage and killing the fungi by the anaerobic conditions (Tengerdy et al., 1996).

Less standard approaches have been investigated such as the addition of acids (e.g., H_2SO_4) and bases (NaOH, $Ca(OH)_2$), typically at a rate of 50 g/kg DM. Both acids and bases can be effective at breaking linkages in plant cell walls, particularly in the hemicellulose fraction. With these treatments, natural fermentation is inhibited by lowering or raising pH, respectively, out of the region of microbial activity so that there is good preservation of dry matter provided an anaerobic environment is maintained during the storage period. Both acids and bases have increased the ethanol potential of forages and crop residues (e.g., Table 2) and should reduce the degree of pretreatment needed at the biofuels plant. Research is still needed to determine optimum levels with various crops, develop safe methods for application at the farm level, and possibly develop techniques to recover product at the plant.

Table 2. Cellulose in grasses converted to ethanol by simultaneous saccharification/fermentation after on-farm treatment of the grasses with sulfuric acid or calcium hydroxide at 50 g/kg DM and anaerobic storage (Digman et al., 2010).

Treatment	Cellulose Conversion (% of total)			
	Reed Canarygrass		Switchgrass	
	Acid	Lime	Acid	Lime
Treated	56*	20*	25*	27*
Untreated	21	17	13	14

* Treated is significantly different ($P<0.05$) from the untreated.

Transportation/Logistics

A major issue that is often ignored is that of moving biomass – from the field, to and from storage, to the processing plant and handling at the plant. After processing, products and byproducts are leaving the plant. The magnitude of the problem is illustrated in the analysis of Humbird et al. (2011), regarding the production of ethanol from maize stover. They analyzed the costs and design for a plant processing 2000 Mg dry stover/day and producing 330 L ethanol/Mg dry stover. This would require collecting approximately 10% of the stover in an 80-km radius. Twelve trucks per hour, 24 h/d, 6 d/wk, would be needed to supply the plant. The transportation in this example is somewhat magnified with maize stover because of its low density compared with most forages. Even so, the movement of forage biomass to a processing plant will be substantially more intense and more likely to have an effect on roads and infrastructure than the movement of hay from one farmer to another.

It is still to be determined what may be the most efficient way to move forage biomass from the field to the plant. Large rectangular bales of dry or wrapped moist forage would be easily transportable, but there are more limited options for pretreatment at the farm plus once the forage arrives at the plant considerable labor would be needed to break open bales and grind the forage. Chopped forage has the advantage of high harvest rates and ease of handling at the processing plant, but the lower density of chopped forage may increase transportation costs. An alternative to these standard options may be pelletization of forage at the farm (Sokhansanj et al., 2009). This would increase density of product shipped and would reduce grinding at the plant, but increase the cost of the feedstock at the plant gate. Beyond the form of the forage and its effects on transportation, there are various means of transport: truck, train, barge, etc. Lastly, the size of the plant may affect the most efficient solution. As plant size increases the area required to supply that plant increases and the average distance feedstock must transported increases. Greater distances may favor one means of handling and transport over another.

Ethanol

Ethanol is an attractive target for producing a liquid fuel from biomass. Ethanol can be substituted into gasoline (petrol) at low levels (e.g., 10% is common in the U.S.) for cars or trucks without any special provisions. Engines are available that run on ethanol or 85% ethanol/gasoline mixtures.

In the U.S., ethanol is largely produced from maize grain with amylases breaking down starch to glucose for fermentation by yeasts. In Brazil, sugarcane is the major source of

ethanol. In both cases, ethanol production competes for these crops with other uses so that ethanol production costs can swing widely dependent on the demand in other food sectors.

In the longer term, ethanol production is expected to move to ligno-cellulosic sources such as forages. The steps to accomplish this will be different from those to produce ethanol from maize grain or sugarcane. Recently the National Renewable Energy Laboratory (Humbird et al., 2011) has published a report on their vision of a feasible (technically and economically) system for ethanol production from maize stover. One would expect a similar process for other forages. The steps are as follows:

- Milling of feedstock to a mean size of 4-6 mm.
- Pretreatment with dilute sulfuric acid at high temperature and pressure for a short time (18 mg sulfuric acid/g dry matter (DM), 158°C, 5.5 atm, 5 min).
- Simultaneous enzymatic hydrolysis of structural carbohydrates and fermentation of sugars, using enzymes prepared at the plant and *Zymomonas mobilis* to ferment both 5- and 6-carbon sugars to ethanol.
- Enzyme mixture prepared by growing a fungus (like *Trichoderma reesei*) aerobically on glucose.
- The fermentation mixture would be distilled to collect the ethanol and the remaining mixture would be separated into liquid and solid fractions.
- The liquid would go through anaerobic and aerobic digestion to produce methane and be cleaned sufficiently so that the water could be recycled.
- Solids would be dried and used for combustion to produce high-pressure steam for electricity production and process heat for pretreatment and distillation.

By their analyses and assumptions, ethanol could be produced for a minimum selling price of \$0.57 per liter assuming they could purchase feedstock at \$64.50/Mg DM. This selling price estimate is substantially lower than similar estimates less than 5 years ago (\$0.93/liter), but still higher than market surveys for the cost of producing ethanol from maize grain and sugarcane, \$0.40 and \$0.30/liter, respectively. With continued advancement of technologies, I think it is reasonable to assume that the costs of producing ethanol from forages will come down and become economically viable. There is still research to be done to develop low cost systems to produce ethanol, both issues at the farm and plant. Certainly other mechanisms for pretreating and fermenting forages are under investigation.

A concern is that only 30% of the carbon that enters the ethanol plant will leave in ethanol. Most of the rest leaves as carbon dioxide. Energy recovery is much greater, with 72% of the carbohydrate energy from the crop being captured as ethanol (Humbird et al., 2011). However, petroleum inputs for producing ethanol from switchgrass (0.1 MJ petroleum/MJ ethanol) were calculated to be similar for the best scenarios for maize, and net greenhouse gas production (12 g CO₂/MJ ethanol) for producing ethanol from switchgrass was approximately 20% of that for maize (Farrell et al., 2006). In addition the net energy of producing ethanol from switchgrass (23 MJ/L) was found to be more than twice that of the best analysis for producing ethanol from maize (9 MJ/L).

Anaerobic digestion

Particularly in Europe there has been a growing interest in fermenting silages in anaerobic digesters to produce methane. Anaerobic digestion has been used for decades in treating waste streams (e.g., sewage sludges and manures), producing methane. The methane has been used for heating or electric generation. In some cases, it has just been flared where waste treatment was the main goal and there was no immediate use for the methane.

Methane production is a multistep process carried out by a variety of anaerobic bacteria. Sometimes it is described more simply as a two-step process whereby various bacteria breakdown and ferment proteins, carbohydrates and lipids to acetic acid, hydrogen, carbon dioxide, ammonia, and hydrogen sulfide. Then methanogens convert the acetic acid, hydrogen and carbon dioxide to methane. The gas produced by the digester will be a mix of methane, carbon dioxide and traces of other gases, typically 50 to 70% methane. Typically in an industrial process the process is carried out in a single, stirred tank with retention times of 20 to 30 days. Farm-scale digesters may be plug-flow covered trenches with manure entering on one end and the digested waste leaving on the other end.

With anaerobic digestion of forages and other crops, cattle or other livestock are being bypassed. In a basic scheme, forages would be harvested and ensiled to preserve the crop until digested. The silages would be added to the digester on a daily basis, possibly with water, other organic products or wastes. The waste exiting the digester would be stored and spread back to cropland. The methane produced by the digester could be used for heating, electric generation, or cleaned of carbon dioxide and compressed for addition to natural gas pipelines.

The energy return from methane production could be substantial. (Berglund and Borjesson, 2006) calculated energy balances for methane production from various sources.

With forages, energy inputs would be 40% of energy output or the energy from methane is approximately 2.5 times greater than the energy invested in the process.

Methane yields of up to 0.50 m³/kg volatile solids (VS) have been reported. Assuming a more modest yield of 0.30 m³/kg VS and a crop yield of 10 Mg/ha with an ash content of 50 g/kg DM, methane production would be 2850 m³/ha or approximately 101 GJ/ha.

While anaerobic digestion is a relatively mature technology in waste management, considerable research is needed to optimize this technology for ensiled forages. Research is in the relatively early stages to investigate silage additives and ensiling conditions on methane potential as well as pretreatments such as NaOH to increase methane yield (e.g., (Pakarinen et al., 2009)). There are also variations being investigated such as also producing hydrogen (Pakarinen et al., 2009) or using liquid-solid separation, digesting only the liquid fractions and using the solids for combustion (Buhle et al., 2010; Richter et al., 2011a, b).

Combustion

Combustion has already been mentioned in relation to ethanol and methane production for utilization of byproducts. Dry forages could be substituted for coal or co-fired with coal for electric generation. In various places, co-firing has been tested at pilot and full-scale. The lower energy density, variability in energy content and the higher ash content of forages compared with coal suggest that such enterprises will be limited. However, there are plants generating electricity from combustion of various biomass sources, largely wastes from the paper, lumber and sugarcane industries (Farrell and Gopal, 2008).

Recently, a life-cycle assessment was made for various renewable energy sources for transportation (Campbell et al., 2009). Ethanol and combustion for electricity produced from either maize or switchgrass were compared. Switchgrass produced more net mileage and more greenhouse gas offsets than corn. Further, the analysis showed that electricity production from switchgrass on average resulted in 81% more net mileage and 108% more net greenhouse gas offsets than if the switchgrass was converted to ethanol.

This analysis suggests that combustion to produce electricity may be a promising route for producing energy from forages, but there are challenges. Efficiencies for the conversion of biomass to electricity are currently in the range of 20 to 25% (Farrell and Gopal, 2008). To improve those efficiencies, research is needed in regard to handling and preprocessing the biomass as well as in reactor design. Also strategies are needed to deal with the variability in the biomass feedstock and its effect on generating plant performance.

Other Possibilities

Other technologies for converting forages to fuel or electricity are not as advanced as ethanol or methane. Pyrolysis, the heating of organic substances under pressure in anaerobic conditions, is capable of producing various biogases, bio-oils and chars. The products vary by crop and treatment conditions. Forages are being studied [e.g., switchgrass (He et al., 2009); elephant grass (Strezov et al., 2008)]. However, considerable research is still needed to find which products are the best targets of pyrolysis of forages and the optimum conditions for making those products. Only then will it be possible really analyze if the process could be economically viable.

Fermentation of forages by *Clostridium acetobutylicum* to n-butanol is now receiving attention (e.g., Pfromm et al., 2010). Butanol could be converted to gasoline, making it more attractive than ethanol. However, the energy yield of butanol from corn or switchgrass is approximately half that of ethanol. This makes butanol production from forages an unlikely target biofuel in the near term without major advances.

Sustainability

Today it appears that we are on the doorstep of seeing forages utilized for energy production – ethanol, methane, electricity. Assuming we solve the technical challenges, there are still issues to having a stable, sustainable and profitable energy production enterprise from forages. First, we need to have a forage production system for biofuels that is not in competition with food production. In the U.S., we have seen maize prices climb dramatically with the added demand for maize to produce ethanol. This is not in the best interest of average citizens as they see more of their income spent on food. As maize prices increase, the economic viability of ethanol production from maize declines. Forages do have an advantage over grains because they can be grown on marginal soils that are unsuitable for other crops. This could potentially minimize competition if we bring marginal lands into forage production.

Second, forage production should be tied to a good crop rotation scheme. Crop rotations have long been recognized for maintaining soil fertility, increasing yields, and minimizing plant diseases and insect pressures. For each environment, we need to study what mix of crops holds the best potential. We may have a situation where multiple energy crops could be grown in rotation, including grasses, legumes and grains.

Third, even if we can technically produce biofuels from forages, we need the infrastructure to make the production system work. Farmers need to be paid a fair value for

their crops for them to have an incentive to raise those crops. Roads and transportation systems need to be in place to accommodate the movement of forages to the plant and biofuels and byproducts from the plant.

Finally, we need to look at potential byproducts of biofuel production for both economic and environmental reasons. Currently in the U.S., distiller's grains are a significant income stream in ethanol production from maize, keeping a valuable feed from merely being disposed in landfills or spread on fields. Schemes to separate leaves and stems or liquid and solid fractions of forages may maximize the economic value of both fractions. We need to look at novel products that could be derived from those fractions beyond biofuels. For example, several groups are investigating lactic acid production from various forage fractions. Perhaps there are high value proteins or other compounds that could be derived from forages in addition to energy that would support the profitability of the whole enterprise.

We live at an exciting time when we have the possibility of generating all of our energy needs from renewable sources. Forages will most certainly play a role in that, and we have the challenge to help make that happen.

Acknowledgements

The author wishes to acknowledge the helpful reviews of M. F. Digman and Z. G. Weinberg in the preparation of this manuscript.

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Potential use of native microorganisms strains of forage for silage production

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1. Introduction

Ensilage is the name given to the process of conserving forage plants or other products through fermentation. This process is characterized by fermentation, mainly by lactic acid bacteria (LAB) from carbohydrates present in the original material that primarily produce lactic acid as well as other acids that lower pH and conserve the ensiled forage.

The quality of this fermentation is influenced by the type and quantity of the acids formed, which, in turn, depend on the conditions under which the silage was produced, and especially by the microorganism population present throughout the process. Microorganisms are beneficial when they produce acids and lower pH. However, they can also be harmful when they break down proteins, produce compounds that are toxic for animals or even man, or just compete with beneficial microorganisms for substrates.

Silage fermentation may occur naturally due to the epiphytic microorganisms present in the plant to be ensiled or added microorganisms to improve the silage process and quality. Microorganisms added at the beginning of the fermentation process are called inoculants or culture starters.

Inoculant adequacy for individual forage types is measured by fermentation improvement. Although all inoculants share some general features, individual forage characteristics and silage conditions may require specific inoculants to improve the

fermentation. The purpose of the present work is to discuss bacteria selection for silage inoculation with an emphasis on native tropical forage bacteria strains.

2. Selection of silage inoculants

Bacterial inoculants are microbial additives used in the fermenting process to improve the quality of the final product. Several biotechnological processes related to fermented beverages, meat, yogurt, and probiotics among others are concerned with selecting the most efficient microorganisms. In general, these industrial processes are easy to control; however, the same cannot be said for silage. The main problems in silage production are difficulty in controlling fermentation on a large scale, ease of contamination and impossibility of interfering in the process during fermentation.

Lactic acid bacteria (LAB) stand out in microorganism selection for silage inoculation. These bacteria can be homofermentative or heterofermentative. Both bacteria types are potentially beneficial to the silage process. Homofermentive bacteria primarily decrease pH levels, inhibit undesirable microorganism growth and improve fermentation quality (Cai et al., 1999). Heterofermentive bacteria, on the other hand, produce lactic, acetic and/or propionic acids (Axellson, 1998), which are more effective in inhibiting fungal growth and subsequently improving stability of silage exposed to the air (Driehuis et al., 2001; Filya, 2003).

There is a wide variety of commercial silage inoculants. The species most commonly used belong to the genera *Lactobacillus* (*L. plantarum*, *L. acidophilus*, *L. buchneri*), *Enterococcus* (*E. faecium*), and *Pediococcus* (*P. acidilactici*), as well as propionic acid (*Propionibacterium acidipropionici*) bacteria. Many studies describe advantages of these silage inoculants (Kleinschmit et al., 2006; Zopollatto et al., 2009). Despite the epiphytic LAB population being able to transform soluble carbohydrates into organic acids and lower the pH, this population is generally small and produces

lower quality silage. Although studies generally demonstrate that the use of inoculants provide some benefits, results are contradictory when different forage cultures are evaluated under diverse conditions. Researchers have come to the following conclusion: the positive effect of inoculants on the fermentation process or silage quality depends on several factors related to forage culture and ensiling conditions.

The following should be considered during selection of silage bacterial strains: microorganism origin, characterization and identification; definition of pre-selection characteristics; microorganism evaluation on a small (laboratory) and large (farm) scale as well as evaluation of different cultures.

2.1 Effect of microorganism origin on selection

The origin of microorganism strains considered for selection may be different. According to Muck (2008), a determining factor in success of silage microbial inoculants is plant-microorganism compatibility. Forage crops have diverse chemical compositions and can store different types and concentrations of carbohydrates. These factors associated with the environment where a plant grows interfere in microorganisms' adaptation to a culture. Thus, it is believed that when a microorganism is selected to act on a specific forage plant from which it was selected, its adaptation to the species will improve performance during fermentation. Hill (1989) isolated three *Lactobacillus plantarum* strains in corn (*Zea mays* L.), alfalfa (*Medicago sativa* L.) and sorghum (*Sorghum* sp) that were applied to their respective plant species during ensiling. This author found the dominant strain to be native to the silage plant in question.

Native plant strains are not necessarily more efficient than strains isolated from other environments. Strains selected from other environments or strains selected from

different forage cultures can also perform well in the silage process of a specific forage culture. Ávila et al. (2010) evaluated characteristics of sugarcane (*Saccharum* spp.) silage inoculated with strains isolated from sugarcane itself and commercial strains isolated from other environments (Table 1). Results showed that strains isolated from sugarcane did not always present the best results. The lower ethanol levels were found across silages inoculated with strains isolated from sugarcane (*L. brevis* UFLA-65-SIL; *L. buchneri* UFLA-72-SIL) and commercial strains isolated from other environments (*L. plantarum* Biomax 5[®]; *L. buchneri* Silo MaxLalsilCana[®]).

Strains with “target” characteristics are commonly used in the selection process. In this case, strains with recognized activity against silage deteriorating microorganisms such as coliforms, endospore-forming bacteria (*Clostridium* and *Bacillus* genera), *Listeria* spp., yeast and filamentous fungi could be included. It is important to verify if these strains could survive under ensiling conditions, in other words in an environment with reduced concentration of oxygen and low pH. Saaristo et al. (2007) had positive results regarding inoculation with strains from non-silage fermentation processes (barley gain, pickled cabbage, meat inoculums). These authors concluded that these strains were effective in improving fermentation.

Table 1. Lactic acid, acetic acid, propionic acid, ethanol concentrations and lactic: acetic acid ratio in sugarcane silages treated with inoculants

Silage	Lactic acid	Acetic acid	Lactic:acetic acid	Propionic acid	Ethanol
	(% of DM)				
Control, without inoculant	1.73 b	1.74 c	1.00 b	0.58 c	6.14 a
<i>L. plantarum</i> UFLA-1-SIL strain	3.02 a	1.75 c	1.76 a	0.16 d	3.44 b
<i>L. paracasei</i> UFLA-67-SIL strain	3.80 a	1.83 c	2.07 a	0.17 d	3.67 b
<i>L. brevis</i> UFLA-65-SIL strain	2.10 a	4.97 a	0.43 b	0.12 d	1.15 e
<i>L. buchneri</i> UFLA-72-SIL strain	3.06 a	4.07 a	0.75 b	1.34 a	2.10 d
<i>L. buchneri</i> Pioneer 11A44TM	3.17 a	4.30 a	0.73 b	0.71 b	2.85 c
<i>L. plantarum</i> Biomax 5 [®]	2.58 a	3.17 b	0.79 b	0.16 d	2.05 d
<i>L. buchneri</i> Silo MaxLalsilCana [®]	3.03 a	4.55 a	0.66 b	0.16 d	2.15 d

Average	2.81	3.30	1.03	0.44	2.94
Coefficient of variation (%)	18.9	12.6	20.9	16.3	10.9
Contrasts					
Control vs others ²	**	**	*	**	**
<i>L. buchneri</i> vs <i>L. plantarum</i> ³	ns	**	**	**	*
<i>L. brevis</i> vs <i>L. buchneri</i> ⁴	*	*	ns	**	**
<i>L. buchneri</i> vs SiloMaxLalsilCana ^{®5}	ns	ns	ns	**	ns

Application rate of 10⁵ ufc/g of forage.

^{a,b} Means followed by the same letter in a column do not significantly differ from each other by the Scott-Knott test (P>0.05) .

²Contrast between silage not inoculated vs inoculated; ³Silage inoculated with *L. buchneri* vs *L. plantarum*; ⁴Silage inoculated with *L. brevis* vs *L. buchneri*; ⁵Silage inoculated with *L. buchneri* isolated from sugar cane vs commercial inoculants containing this same species.

ns: not significant; *(P<0.05); **(P<0.01).

It is important to stress that the effect does not depend solely on the specie. Although microorganisms from the same specie present similar characteristics, there are variations across strains. Ávila et al. (2010) and Saarisalo et al. (2007) found that inoculation with different strains that were, although belonging to the same species, also resulted in silage with different characteristics, which shows that studies should consider strains and not just species.

Several characteristics of silage inoculants has been described such as: rapid growth and the ability to compete with natural plant microbiology; intense production of lactic acid to quickly reduce pH; tolerance to acidic conditions; no pathogenic effect and survival throughout the silage fermentation process (McDonald et al., 1991; Saarisalo et al., 2007). A strain rarely presents all of the above characteristics. Consequently, the choosed forage to be ensiled should be analyzed. The forage fermentation process should be known as should the microorganisms involved. In this way, possible silage problems can be identified and specific microorganisms can be selected.

2.2 Criteria for inoculant selection

- Substrate use and metabolite production

Different forage plants have different substrates available for bacteria (inoculants) to use. In temperate grasses, water soluble carbohydrates mainly consist of fructan, sucrose, glucose, and fructose (McDonald et al., 1991). The main reserve carbohydrate in tropical forage is starch, with the exception of sugarcane which mainly accumulates sucrose (Van Soest, 1994). Some LAB are capable of using starch; however, most use simple carbohydrates in their metabolism.

The silage fermentation is a very dynamic process. A wide variety of microorganisms are present during the fermentation. These microorganisms have varying abilities to use different substrates and that diversity may make the fermentation process more complicated. On the other hand, the ability of inoculants to use carbohydrates can be an advantage in the competition against other microorganisms, mainly when limited amounts of soluble carbohydrates are available (Saarisalo et al., 2007).

The bacteria's ability to use a specific carbohydrate depends on the presence of enzymes capable of breaking down the carbohydrate structure. Species and strains within the same species perform differently. Consequently, strains should be evaluated concerning growth rate, carbohydrate use and the quantity and quality of the metabolic products (acids) formed. The main or most abundant fermentation products are lactic acid, acetic acid and propionic acid, which are also the most important ones for silage conservation. Decreased pH is also important; however, this characteristic should not be used singularly, since it is generally related to lactic acid production. For this reason, heterofermentative strains that are important for acetic acid production and less able to lower pH should never be selected since they have an inverse relationship.

Figure 1 illustrates principle components analysis of metabolites produced during fermentation of LAB strains in sugarcane juice. Note that each group is

correlated with the production of one of that evaluated metabolites (acetic acid, propionic acid, ethanol, methanol or lactic acid). Bacteria most highly correlated with acetic acid are in different quadrants than those correlated with lactic or propionic acids. This can be explained in function of homo- or heterofermentative metabolism and demonstrates that selection should be directed toward specific acid production (Figure 1).

- Antimicrobial Activity

Many species or microorganism groups are considered undesirable during the ensiling process. These microorganisms can undergo proteolysis which reduces the nutritional value of silage such as enterobacteria and bacteria of the *Clostridium* genus; perform secondary fermentation, use the lactic acid produced and consequently hinder conservation such as clostridia and yeast; as well as produce toxic compounds or reduce silage acceptability to animals such as ammonia nitrogen, butyric acid, biogenic amines, bacterial toxins (enterobacteria and *Bacillus*) and mycotoxins produced by filamentous fungi. Many microorganisms (fungus, *Bacillus*, *Listeria*) are directly involved in the aerobic deterioration of silage. Furthermore, some microorganisms may be present that are considered pathogenic to animals or man, such as *Listeria monocytogenes* species or *Clostridium* genus. LAB may potentially inhibit these microorganisms by producing acids or bacteriocins. The latter are proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strains.

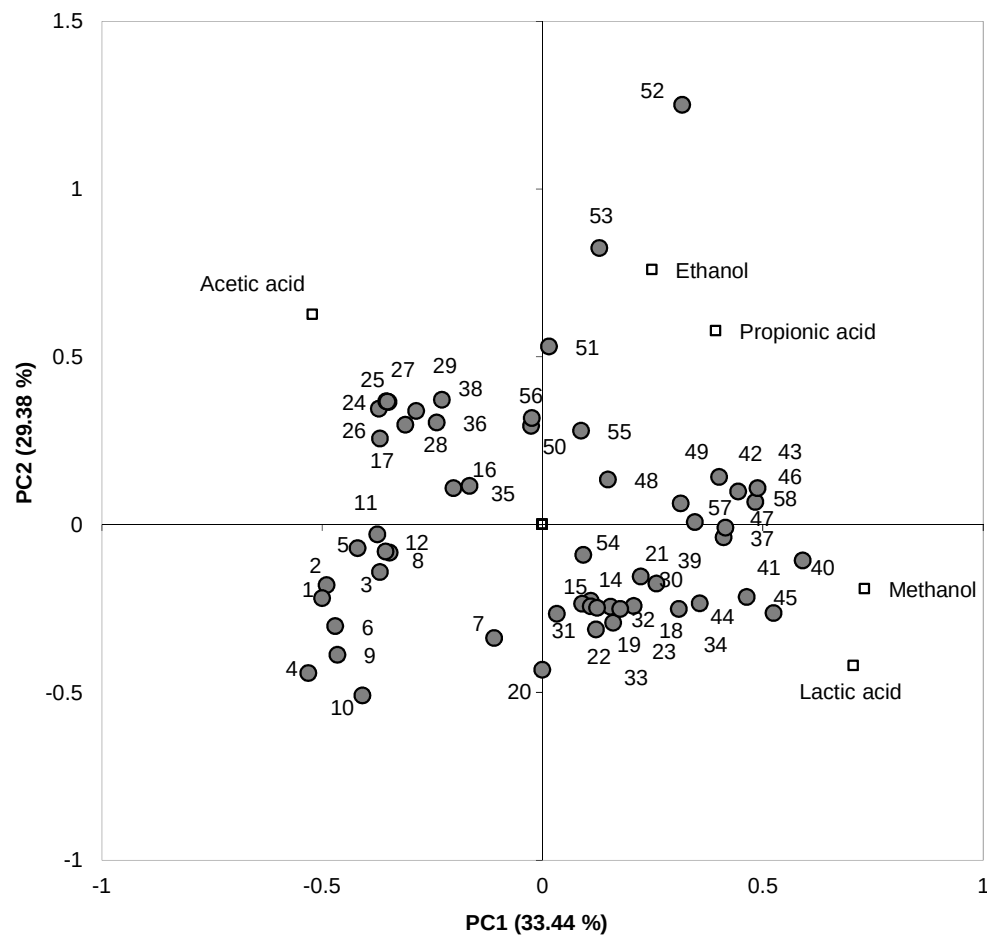


Figure 1. Principle components analysis of metabolites produced during fermentation by LAB strains in sugarcane juice (Ávila, 2010; unpublished data).

Studies have shown bacteriocins' potential to control growth of pathogenic microorganisms in food products. Bacteriocin producing *Lactobacillus* have emerged as protective cultures in fermented meat, olive and dairy food products (Hammes and Vogel, 1994; Leroy and De Vuyst, 2004). The ability to inhibit is inherent to each strain; however, evaluation of this capacity is interesting in a selection process.

- Biogenic amine production

LAB strains may be able to produce biogenic amines. This production is linked to protein breakdown during ensiling and is formed by enzymatic decarboxylation of free amino acids and transamination of aldehydes and ketones (Nishino et al., 2007).

The relationship of LAB with biogenic amine production may occur in two ways: LAB may be able to produce biogenic amines or they may inhibit microorganisms that produce these compounds. Either way, it is an important characteristic that should be evaluated when selecting bacterial strains for silage. Nishino et al. (2007) evaluated two strains of *Lactobacillus buchneri* and one of *L. casei* in different silages. The effects were variable depending on the strain and ensiled material. The *L. casei* strain inhibited biogenic amines in two silages, and the two *L. buchneri* strains inhibited biogenic amines in only one silage type.

Due to the complexity of the ensiling process, it is difficult to determine which microorganisms are actually involved in biogenic amine production, since many microorganism genus such as *Bacillus*, *Klebsiella*, *Escherichia*, *Pseudomonas* and LAB (*Lactobacillus*, *Pediococcus* and *Streptococcus*) can produce these substances (Santos, 1996).

- *Survival of bacterial strains during ensiling and tolerance to adverse conditions*

In order for strains to survive during the silage fermentation process, they should be able to tolerate conditions that prevail in the silo, such as high acidity and a low concentration of O₂. All LAB species grow anaerobically; however, most are not sensitive to oxygen and can grow in its presence (Madigan et al., 2010). LAB are generally resistant to acidic conditions, and lactobacilli are more resistant than other lactic acid bacteria. Regarding temperature, most LAB are mesophilic. In other words, they grow in moderate temperatures of about 35°C.

In general, ensiling conditions are conducive to LAB growth; however, many times these bacteria are exposed to high temperatures. This exposure may occur when inoculants are mixed with water in application tanks that are exposed to high

temperatures (40 a 45°C). Furthermore, temperature increases may also occur throughout the fermentation process, which may reduce inoculant viability and efficiency in the silo. Due to Brazil's tropical climate, room temperature and that inside the silos can reach values above 45°C, which negatively affects viability of many inoculant strains. A bacterial strain's ability to resist a given stress factor may be key to inoculant success. Selection of strains tolerant to high temperatures is extremely important, as verified by Mulrooney and Kung Jr. (2008) and Cai et al. (1999), since the number of viable strains was greater when silage temperature remained within that for superior strain growth.

Viability of inoculant strains should be verified in order to determine if an inoculant strain can dominate the natural microflora and act throughout the entire fermentation process. On the other hand, verification is somewhat difficult. The most common method to determine strain viability in an environment is plating on culture media. Nevertheless, to know if an isolated strain is the same one that was inoculated, it needs to be identified, which can be time consuming. In this case, molecular techniques would be more interesting. Qualitative molecular techniques, in other words those that only identify species, cannot determine the microbial population present. Consequently, quantitative or real-time polymerase chain reaction (PCR) would be a better option. This technique can quantify a specific microbial species and associate results with qualitative data regarding fermentation, pH, production of organic acids, microbes, etc. (Schmidt et al., 2008).

Conditions concerning growth, lyophilization and storage are also important during large scale microorganisms' production. Freezing and lyophilization are commonly used in industrial inoculant production. During the lyophilization, freezing is detrimental to the cells. Strain viability after lyophilization depends on a

microorganism's initial growth conditions. Factors like culture growth phase, temperature, microorganism concentration and protective additives may change microorganism viability; however, these conditions vary greatly from microorganism to microorganism (Morgan et al., 2006). As a result, several factors may influence cell viability after lyophilization, and these factors should be taken into account. Many studies have focused on understanding or improving resistance to bacterial strains. Cryoprotectants like glycerol, lactose, sucrose, trehalose, ascorbate and glutamate are usually added to protect cells from stress damage. Nevertheless, some microorganisms cannot tolerate lyophilization (Morgan et al., 2006). All these factors are very important for inoculant efficiency. Therefore, strain tolerance under different conditions should be evaluated.

- Other characteristics evaluated

Inoculants have been reported to improve animal performance even when pH and acid production were not altered (Contreras-Gouveia, 2011). Consequently, these effects may be due to silage microorganisms in the rumen. According to Weinberg et al. (2004), inoculants may have a probiotic effect on ruminant performance, although the mechanism responsible for this is still not clear. LAB interaction with rumen microorganisms may improve rumen activity and fiber degradability. Another possibility is that LAB produces bacteriocins in silage, which might inhibit harmful microorganisms in both silage and rumen.

Little is known about how inoculants act during rumen fermentation. However, some studies indicate that inoculation with bacteria improve not only forage fermentation but also animal performance, as indicated by increased milk production,

weight gain and/or feed intake (Contreras-Gouveia et al., 2011; Kung Jr. and Muck, 1997; Kung Jr. et al., 2003).

Other LAB characteristics that have already been explored by the food industry could also be studied for silage. LAB strains may be able to grow in the presence of phenolic compounds or use these substances which are secondary plant metabolites able to bind proteins. This quality is important for waste use in animal feed as well as fermentation during ensiling and in the rumen (Lopez-Guzman et al., 2009). An example would be inoculants used jointly with crude glycerin, which is a biodiesel waste product. According to Axellson (1998), some *Lactobacillus brevis* and *L. buchneri* strains can use glycerol as an electron acceptor in co-fermentation with glucose. The end products of this co-fermentation are lactate, acetate, CO₂, and 1,3-propanediol. Dias Júnior et al. (2010) observed that glycerin used jointly with an inoculant containing a *L. buchneri* strain isolated from sugarcane silage resulted in sugarcane silage with less DM loss. This reduction was not observed when glycerin was used alone.

Some studies have shown that LAB can bind aflatoxins, which are one of the main mycotoxin groups produced in silage. This reduces its bioavailability or permits biotransformation into less toxic compounds (Niderkorn et al., 2007; Hernandez-Mendoza et al., 2009).

3. Results obtained with selection of bacterial strains for sugarcane and corn silage

The strain selection process for silage inoculation contains three main steps: pre-selection in laboratory, selection in lab silos and selection in farm silos. Pre-selection should be based on aforementioned characteristics, observations of the cultures to be ensiled, and fermentation problems. Studies conducted at the Federal

University of Lavras (UFLA) have focused on selection of microorganisms isolated from its own silage to be used primarily to ensile sugarcane and more recently corn.

To proceed with selection, microorganisms should first be isolated and characterized. The strains isolated from sugarcane silage have been identified as belonging to the *Lactobacillus* genus. The primary species found were *Lactobacillus plantarum*, *L. buchneri*, *L. brevis* (Ávila et al., 2009) and *L. paracasei* (Ávila et al., 2010). For pre-selection, strains are identified at least at a genus level, so that only bacteria from the LAB group are included. Pre-selection characteristics are different for each forage culture. In the present study, metabolite production and capacity for strain growth were evaluated in forage-like substrates, being sugarcane juice and corn extract.

Metabolite production is a leading characteristic in pre-selecting strains for ensiling sugarcane and corn. In a study to evaluate metabolite production of 58 strains isolated from sugarcane silage, metabolite production in sugarcane juice varied greatly across strains (Ávila, 2011, unpublished data). Production of lactic, acetic and propionic acids as well as methanol and ethanol respectively varied 0.88 to 8.5; 0.89 to 6.12; 0 to 0.46; 0 to 3.83 and 0 to 0.05 g/L. These differences are related to the strain's natural metabolism. Heterofermentative strains were generally the ones that produced the greatest amount of acetic acid, although some homofermentative strains also showed increased production of this acid compared to heterofermentative strains. This indicates that a greater concentration of acetic acid may also be related to other factors.

Results were submitted to principle components analysis (Figure 1). Strains were grouped according to the metabolites produced. The greatest producing strains of each evaluated acid were grouped into different quadrants. Strains correlated with propionic acid were also correlated with ethanol, and those correlated with lactic acid were also correlated with methanol. Some isolated strains were not correlated with any

desirable metabolites for silage and were consequently discarded from the strain selection process. Higher lactic acid production is desired to efficiently reduce silage pH. Furthermore, acetic and propionic acids have been proven to be effective in controlling fungi. Regarding metabolism, greater lactic acid producing strains are not the greatest producers of acetic and propionic acids.

Based on these results, the best producing strains of each metabolite of interest were chosen and a new experiment was conducted in PVC silos to evaluate silage characteristics. Figure 1 presents the best producing strains of acetic acid (numbers 17, 24, 25, 27 and 35), lactic acid (numbers 17, 32, 33 and 34), and propionic acid (numbers 41, 42, 46, 51 and 55). In the silo experiment, no differences were observed among LAB counts, yeast counts and silage pH values (Table 2). On the other hand, DM loss widely varied (Figure 2) throughout the fermentation process and among aerobic stability parameters (Table 3) (Carvalho, 2011 unpublished data).

DM loss during sugarcane ensiling is a great concern. Consequently, an inoculants' ability to reduce DM loss is one of the most important characteristics evaluated. In the present study, DM losses in silage generally increased with fermentation and varied greatly depending on the inoculant (Figure 2). There were no significant interactions among inoculant factors and fermentation time; however, there were differences among strains with average values varying from 11.38 to 22.90% (Table 3). It is interesting to correlate DM loss during ensiling with metabolite production in inoculated silage strains. All strains selected for greater lactic acid production did not improve (strain 33) or increase DM losses (19, 32 and 34) in silage compared to the control (Table 3). Among silage with less DM loss, two are better producers of acetic acid (17 and 24) and two are better producers of propionic acid (51

and 55). Results proved that this pre-selection methodology was efficient in sugar cane silage.

Table 2. Lactic acid bacteria (LAB) and yeast populations, pH values 4 d of air exposure in sugarcane silage inoculated with different LAB strains.

Variable	Duration of fermentation (days)			
	12	30	61	126
LAB (log ufc/g silage)	8.64 a	6.69 b	6.97 b	6.51 b
Yeast (log ufc/g silage)	5.77 ab	6.04 a	5.28 bc	5.00 c
pH	3.59 b	3.63 b	3.62 b	3.98 a

Means followed by the same letter in a row do not differ among themselves according to the Tukey test ($P > 0.05$).

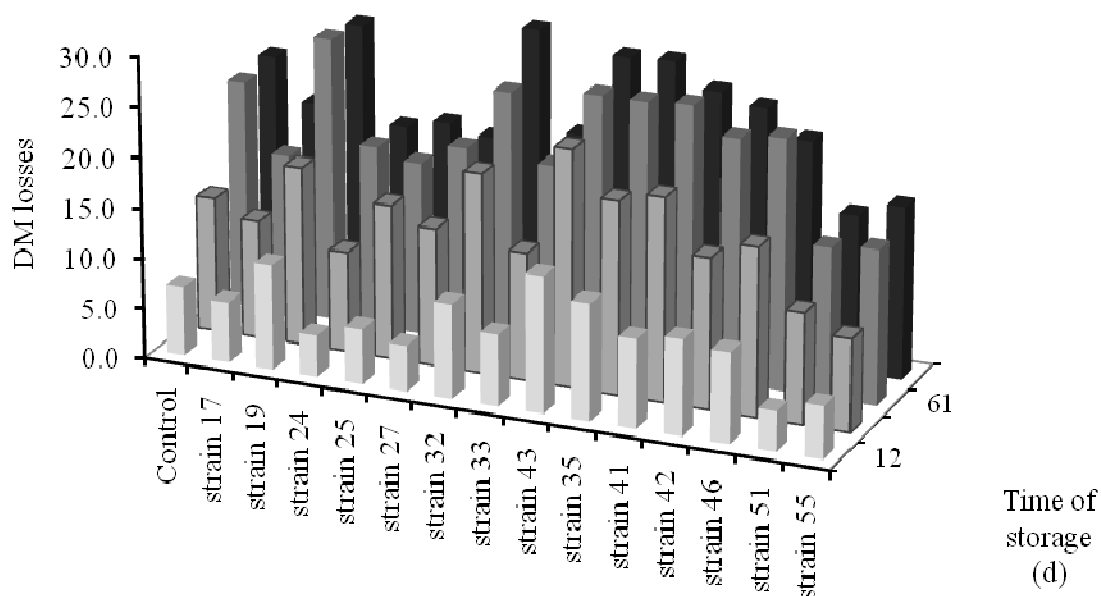


Figure 2: DM loss in sugarcane silage without inoculants (control) and inoculated with 14 different lactic acid bacteria strains (Carvalho, 2011 - unpublished data)

Regarding aerobic stability, time (h) to lose stability was statistically different for the control (14.2 h) and silage inoculated with strain 55 (30.2 h). In other words, the inoculated silage was twice as stable. Silage temperatures after opening the silo were evaluated. Temperature of silage inoculated with strain 55 was always lower than the control. This strain stood out for decreasing DM loss and increasing aerobic stability, and was considered to have high potential for ensiling sugarcane.

Even though an inoculant may not influence the general population of some microbial group; it may act on some species and thereby modify fermentation quality.

Often, this can also explain why no differences among LAB populations are detected while differences in silage quality are noted. Total LAB population counts did not indicate which species survived. As can be seen, different species present very different characteristics and consequently act differently in silage.

Table 3. Total DM loss throughout 126 days of fermentation and aerobic exposure of sugarcane silage inoculated with LAB strains.

Silage*	Dry matter loss (%)	Maximum temperature (°C)	Time (h) to reach maximum temperature	Time (h) to lose stability (26°C)
Control	16.42c	47	22.0	14.2b
Strain 17	13.07d	46.3	28.8	18.0ab
Strain 19	20.93a	45.2	26.0	17ab
Strain 24	12.50d	45.0	33.3	18.2ab
Strain 25	14.19c	43.8	27.5	16.0b
Strain 27	13.89c	47.2	28.0	18.3ab
Strain 32	21.05a	43.5	42.2	20.2ab
Strain 33	14.57c	46.0	27.2	17.8ab
Strain 34	22.90a	43.0	48.2	21.0ab
Strain 35	21.38a	41.2	35.7	22.0ab
Strain 41	20.45a	43.8	30.3	19.8ab
Strain 42	18.40b	44.2	28.5	17.5ab
Strain 46	18.23b	43.2	27.5	17.8ab
Strain 51	11.38d	41.8	58.0	21.5ab
Strain 55	11.75d	43.8	54.0	30.2b

Averages followed by the same letter in a column do not differ statistically according to Scott-Knott test

*strain names represent codes used in figure 1(PCA)

Another experiment was conducted to evaluate pre-selection and performance of some of these strains in corn silage. For this, some strains previously evaluated for sugarcane silage and other strains also isolated from this culture were grown in corn extract agar. After 48 h fermentation, samples were removed from the medium for metabolite analysis using high efficiency liquid chromatography (HELIC), and results were submitted to principle components analysis (PCA) (Figure 3). In this experiment, a test was included to evaluate the ability of strains to inhibit growth of deteriorating and pathogenic microorganism (*Escherichia coli* – ATCC 11229; *Clostridium perfringens* – ATCC 3624; *Bacillus cereus* – ATCC 11778 e *Listeria monocytogenes* – ATCC 19117) (Oliveira, 2011 - unpublished data).

Metabolite production in corn extract varied greatly among the strains evaluated. Strains were also grouped differently in this medium. Those that correlated with greater acetic acid also correlated highly with propionic acid. On the other hand, similar to growth in sugarcane agar, strains that correlated with lactic acid did not correlate with propionic and acetic acids (Figure 3). Most strains tested for growth in sugarcane agar (36 strains) were also tested in corn extract medium. Results varied between the two mediums. In other words, the greatest producers of a certain acid in sugarcane extract were not good at producing the same acid in corn extract. This indicates that selection should also differ. Selection should reflect a strain's capacity to use the substrates present in the different media (sugarcane or corn extract). Just as these strains act differently in the two mediums, they should act differently in silage.

Strains were ranked based on metabolite production, and nine strains were selected for study in PVC silos (codes in Figure 3). Strains associated with increased production of lactic acid (6; 39; 44 and 62); acetic acid (4; 9 and 60) and propionic acid (4; 9; 43; 44; 60; 62 and 65), and those that produced lower ethanol levels (6, 44 and 65) were chosen. Some of these strains also presented antimicrobial activity at *in vitro* tests against *Listeria monocytogenes* (ATCC 19117) (4; 6; 43 and 44); *Clostridium perfringens* (ATCC 3624) (6); *Escherichia coli* (ATCC 11229) (39; 43; 44 and 62) and *Bacillus cereus* (ATCC 11778) (6).

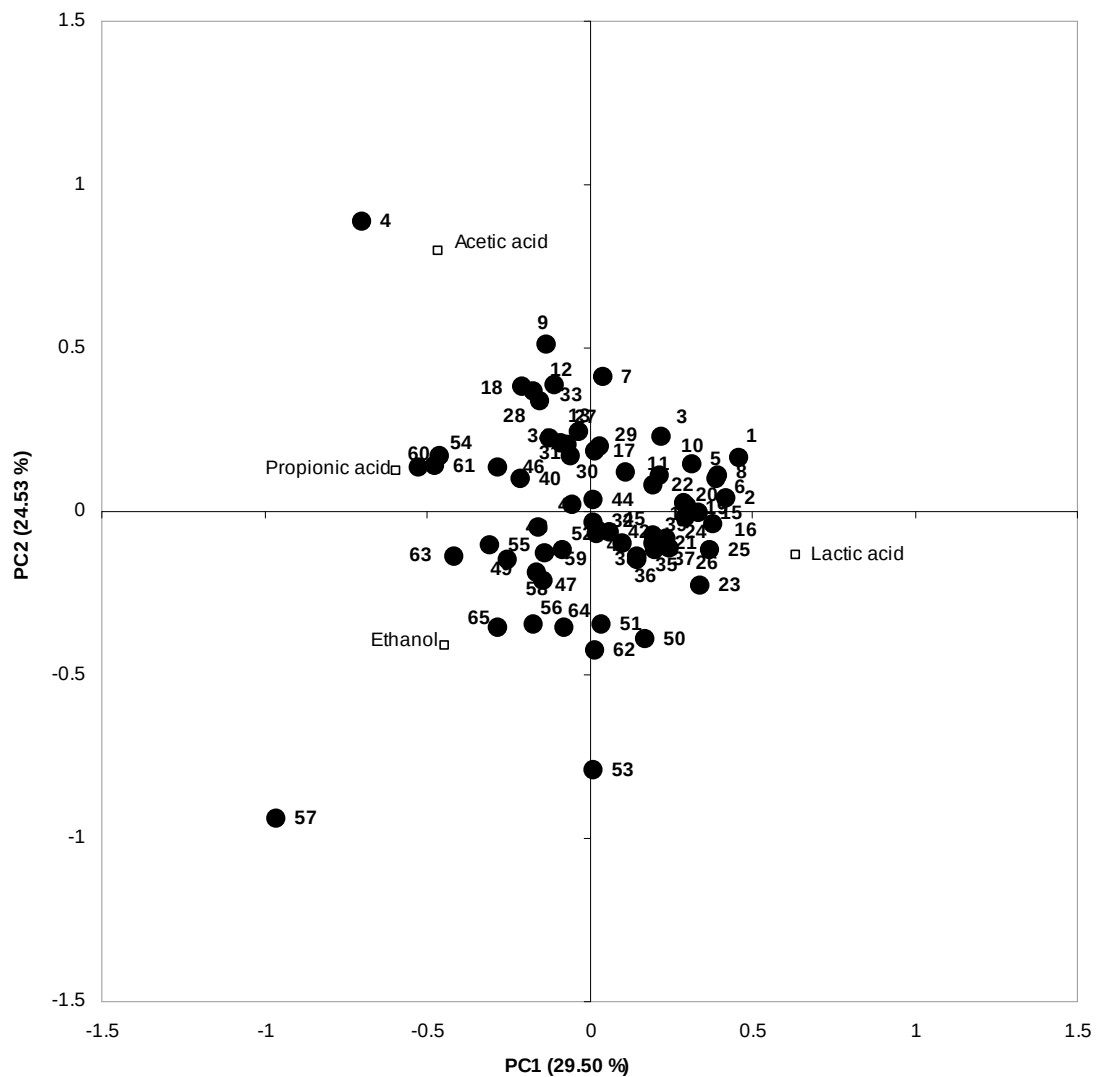


Figure 3. Principle components analysis of metabolites produced during fermentation of LAB strains in corn extract culture medium (Oliveira et al., 2011; data not published).

Results for silage in experimental silos are presented in Table 4. Yeast, mold and total enterobacteria counts were not different across treatments at 60 days of fermentation. However, there were differences among LAB population and pH values. Two strains with high LAB counts also presented high pH values (strains 9 and 60). This is most likely related to their metabolism, since these strains were not classified as the best lactic acid producers but rather as good producers of acetic and propionic acid. On the other hand, strain 9 provided silage with the greatest aerobic stability, a lower maximum temperature (31.0°C) and a longer time period to reach this temperature (149.17 hours). The later is also related to metabolism. In other words, this strain is a good producer of acetic and propionic acids. These strains should be further studied for

silage inoculation. Based on available data, they have high potential for use at corn ensiling.

The strain that provided the greatest stability in sugar cane silage (coded in Figure 1 and Table 3 as strain 55) was also tested in corn silage (coded as strain 65 in Table 4). In corn silage, this strain did not show the best results for aerobic stability, proving once again that selection should be specific.

Table 4. Populations of lactic acid bacteria (LAB), yeast, filamentous fungi (FF), enterobacteria and pH values of corn silage without inoculants and inoculated with different LAB strains.

Treatment*	LAB	Yeast	FF	Enterobacteria	pH	Aerobic Stability	
						Tmax ^a	Time ^b
						(°C)	(hours)
Control	7.25c	4.65	4.09	5.30	4.06b	37.83b	54.67c
Strain 4	7.96b	4.2	3.23	5.04	4.04b	37.83b	54.50c
Strain 6	7.06c	3.40	3.76	4.45	4.02b	39.00b	41.17d
Strain 9	8.72a	4.88	4.37	5.17	4.16a	31.00a	149.17a
Strain 59	7.36c	3.97	2.15	4.45	4.05b	37.17b	71.33b
Strain 43	7.59c	4.94	1.92	5.14	4.03b	38.67b	48.5d
Strain 44	6.87c	4.35	3.22	4.87	4.09b	38.83b	34.33d
Strain 60	8.34b	2.18	2.84	3.87	4.14a	38.67b	58.00c
Strain 62	7.38c	4.40	2.32	5.0	4.03b	39.83b	37.33d
Strain 65	8.12b	4.38	4.12	4.47	4.06b	37.83b	71.83b
<i>P</i>	0.000	0.2	0.33	0.53	0.0002	<0.001	<0.001
CV(%)	3.76	25.27	42.79	19.46	0.74	3.51	15.35

^aTmax.: maximum temperature observed during 10 days of air exposure

^btime: time to reach maximum temperature

Means followed by the same letter in a column do not differ statistically by the Scott-Knott test at a 5% significance level.

*strain codes are same as those presented in Figure 1(PCA)

3. Final Considerations

Preliminary results of strain selection for both corn and sugarcane silage show that strains isolated from silage have great potential for use as inoculants. The wide variety of strains, their characteristics and performance in silage also became clear. In addition, it is important to highlight that use of a specific inoculant should not be generalized across cultures, since as discussed above, performance varies. Furthermore, when using different selection criteria, the most important one for the silage in question

should be observed first eg. reduced DM loss in sugarcane silage. In this way, selection should be directed for the different cultures and based on various characteristics related to microbiological and chemical composition of silage.

In conclusion, these are only preliminary results from a study that may be considered to still be in an initial phase, but one that should be continued and expanded. To do so, the study of the fermentation process of different cultures is of extreme importance. Tropical cultures should be emphasized since published data is lacking in this area.

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Agricultural management of sugarcane fields for silage production

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1- INTRODUCTION

Sugarcane is considered to be the best bioeconomic forage option for feeding dairy and beef cattle (NUSSIO et al., 2003; RESENDE et al., 2005; SIQUEIRA et al., 2008). However, exceptions do exist, especially in a country the size of Brazil. Many consider the advantages of sugarcane to be countless such as roughage in ruminant feeds. On the other hand, there are several indications that it should not be used as feed for animals with proven genetic potential, since it has inadequate protein and mineral levels as well as low quality fiber. Although concerns regarding the nutritional limitations of sugarcane are real, they should be considered from a different angle.

Producers often overlook aspects related to variety choice, agricultural management, forage harvesting and chopping; consequently, this forage resource is often unsuccessful. Furthermore, general manner of use is another problem. Sugarcane is harvested on a daily basis for animal feed. Logistics can make this task difficult or impossible; consequently, silage has become an alternative.

In order to compare sugarcane with other forages, the diets need to be adjusted for a fair comparison. In studies in which an inadequate change in dietary bulk occurred, the corresponding authors believe that theoretical and practical validations were missing.

This review was prepared to discuss important aspects of agricultural management of sugarcane for silage production.

2- Sugarcane Silage

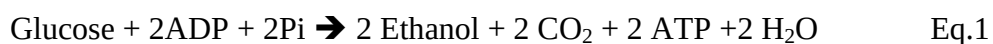
Sugarcane is rich in soluble sugars, about 23% (PEDROSO et al., 2005), with low buffering capacity 7 e.mg HCl/100 g dry matter (SIQUEIRA et al., 2007a) and adequate dry matter content, ranging from 25 to 35% (BERNARDES et al., 2007, SCHMIDT et al., 2007

and SIQUEIRA et al., 2007b). These characteristics give sugarcane high fermentative capacity.

According to previously cited data, sugarcane could theoretically have intrinsic characteristics that are adequate for ensiling and much superior to other cultures, such as corn, sorghum and tropical grasses. These conditions along with silage management of particle size, compaction and sealing are favorable for development of desired microorganisms, such as *Lactobacillus* and *Pediococcus* genera from homofermentative bacteria. Acid production begins during the anaerobic phase of the ensiling process due to high substrate availability. Together with low buffering capacity, acid production reduces pH quickly. EVANGELISTA et al. (2009) and SIQUEIRA et al. (2011a) observed decreased pH with values below 4 at three days fermentation, which is desirable in the ensiling process of any roughage.

However, sugarcane has an epiphytic microflora rich in yeasts that can reach 1×10^6 ufc/g fresh forage (ROTH et al. 2010, ÁVILA et al., 2010, SIQUEIRA et al., 2011a). Most yeast species need oxygen for growth, because the respiratory pathway presents greater energetic yields. However, some yeast species grow under anaerobic conditions and can maintain large populations under these circumstances due to sugar fermentation (WALKER, 1998). During the sugarcane ensiling process, pH drops quickly and yeast dominate the fermentation process, because they are not inhibited by the reduced pH of the food and are able to grow in pH levels ranging from 2 to 8. This characteristic allows yeast to inhabit different environmental niches compared to bacteria (McDONALD et al., 1991; WALKER, 1998), besides the fact that ethanol, a byproduct of yeast fermentation, is toxic to many microorganisms.

According to McDONALD et al. (1991), CO₂ loss during yeast fermentation is responsible for a 48.9% loss in mass (Equation 1).



Gas loss and consequently recovery of ensiled dry matter are primary concerns in sugarcane silage. The decision to ensile this forage instead of daily harvesting should consider losses inherent to the process, since they inflate the cost of effectively stored dry matter for use.

Despite lower gas loss, effluents are still produced, which can be considered another source of loss. Considering the DM content of an effluent, 6% as suggested by SCHMIDT (2006), effluent loss normally observed in sugarcane silage (15 – 40 kg/t ensiled forage) does not represent more than 1% dry matter.

One of the consequences of dry matter loss is a proportional increase in fibrous constituents, and consequently reduced *in vitro* dry matter digestibility of silage, compared to sugarcane at the time of ensiling (Table 1).

Note that on average for each increase in neutral detergent fiber (NDF), *in vitro* dry matter digestibility decreased 0.83 units. In general, NDF in silage without additives increased 16%, which corresponds to 13.3% digestibility (Table 1).

Sugarcane silage loss should be measured quantitatively and qualitatively. Siqueira et al. (2007b) stated that simplistic evaluation of bromatological characteristics without correcting for quantitative losses may underestimate the true extent of qualitative losses. Seeking a more comprehensive quantification, these authors suggested recovery of digestible dry matter as a variable that comprises quantitative and qualitative concepts of loss during ensiling.

Table 1. Variations in neutral detergent fiber (NDF) and *in vitro* dry matter digestibility (IVDMD) in sugarcane at ensiling and at opening

Source	Fresh forage		Silage		Variation	
	NDF	IVDMD	NDF	IVDMD	NDF	IVDMD
PEDROSO et al. (2005)	49.6	62.9	72.9	45.5	+23.3	-17.4
BALIEIRO NETO et al. (2007)	55.5	66.5	63.3	62.1	+7.8	-4.4
SIQUEIRA et al. (2007b)	52.1	52.6	75.3	35.1	+23.2	-17.5
FERREIRA et al. (2007)	55.3	58.1	69.7	46.5	+14.4	-11.6
SCHMIDT et al. (2007)	55.5	51.3	66.0	41.9	+10.5	-9.4
SANTOS et al. (2008)	52.9	59.0	67.1	48.7	+14.2	-10.3
SOUSA et al. (2008)	48.7	63.6	68.1	50.4	+19.4	-13.2
CAVALI (2006)	44.0	66.9	62.9	48.4	+18.9	-18.5
MALDONADO (2007)	43.9	58.7	55.4	51.9	+11.5	-6.8

Several studies have been conducted regarding the effect of additives on storage loss. The most promising were calcium oxide (BALIEIRO NETO et al., 2007; SANTOS et al., 2008; AMARAL et al., 2009; ROTH et al., 2010, SIQUEIRA et al., 2011b); *Lactobacillus buchneri* (PEDROSO et al., 2007; SIQUEIRA et al., 2007a,b; ÁVILA et al., 2009), sodium benzoate (PEDROSO et al., 2007; SIQUEIRA et al, 2007a,b); urea (PEDROSO et al., 2007;

FERREIRA et al., 2007; LOPES & EVANGELISTA, 2010), calcium carbonate (SANTOS et al., 2008) and moisture sequestrates (BERNARDES et al., 2007; LOPES & EVANGELISTA, 2010).

After a brief background of issues related to sugarcane silage, issues regarding agronomic management will be addressed.

3- AGRICULTURAL ASPECTS

3-1. VARIETY CHOICE

When starting a livestock project that will use sugarcane as roughage, the first decision is variety choice. Culture type has a greater impact on sugarcane than it does on corn and sorghum, because sugarcane is a semi-perennial plant. A leading criterion in determining the feasibility of sugarcane use is field longevity. NUSSIO & PONCHIO (2004) demonstrated through simulations that increased field longevity, about 5 years on average, profoundly reduced the cost per ton of harvested dry matter.

MIGUEL et al. (2011) evaluated different contractual arrangements of sugarcane production and observed that average cost of forming 1 ha for sugar and/or ethanol production in northern São Paulo was U\$ 1,766. This high implementation cost needs to be diluted in subsequent cuts. Thus, the choice of suitable varieties is fundamental, since reformulation only occurs after at least 6 years, otherwise, the main advantage of low cost per ton will not be reached.

An example of longevity can be seen in Torres & Costa (2001). These authors managed sugarcane for 10 years while maintaining high production. In the tenth year, they corrected the soil and used organic fertilizer, and again succeeded in raising productivity of the varieties under study. In agricultural ventures, goals for field use are normally set for six years. If these goals are met, the strategy of variety choice is considered a success. In practical terms, greater longevity is desirable but difficult to achieve.

Another fundamental point in variety selection is productivity. SIQUEIRA et al. (2008) argued that the main reason for using sugarcane in animal feeds is its high mass per area unit. Sugarcane productivity (t/ha) should be 2.5 to 3.0 times that obtained with corn for it to be viable in animal feed systems. As a result, varieties with low productivity should be excluded from the production system. In the sugar-alcohol industry, there is a wide range of varieties available that are adapted to diverse edaphoclimatic conditions. It is understandable that productivity of a variety varies greatly depending on environmental conditions. In this

way, responsive varieties are more efficient under favorable environmental conditions. On the other hand, more rustic varieties should be used in restrictive environments.

The use of sugarcane in animal feed is questioned because of its low concentration of protein (nitrogen) and minerals. Due to its low crude protein content, sugarcane is frequently reported in the literature as inadequate for use in animals of medium to low production. Conversely, SIQUEIRA et al. (2011c) state in a review on sugarcane in ruminant feed that the greatest competitive advantage of sugarcane is its low nitrogen content. These authors show that among forage types normally used in animal feeds, sugarcane is the most efficient in assimilating biomass depending on the amount of nitrogen applied. According to these authors, this quality, which has always been considered a disadvantage and has often even been recognized as a nutritional limitation, is the main advantage for its use in sugarcane. In the review, they also suggest that the reader estimate the quantity of nitrogen fertilizer needed to produce 30-50 t dry matter sugarcane with a hypothetical value of 10% protein.

It is worth mentioning that sugarcane's low demand for N, related to potential mass production, is due to the system's high efficiency in CO₂ fixation, in other words, participation of the ribulose-1,5 phosphate carboxylase (RUDP) system associated with phosphoenolpyruvate carboxylase (PEP). The greater photosynthetic efficiency of plants that develop a C₄ cycle, like sugarcane, facilitate adaptation to high temperatures (35 to 38°C) associated with greater water and nitrogen use efficiency (LONG, 1999; MOSER et al., 2004). The C₄ carbon fixation pathway gives plants greater adaptation to environmental factors typical of tropical regions, like central Brazil, where high temperatures and light are recorded.

The real limitation to sugarcane use in animal feed is low digestibility of fibrous fractions. This is caused by the low NDF degradation rate, which according to SILVEIRA et al. (2009) is less than 2%/h, and the high undegradable fractions (NDFu), which are more than 50% NDF (MACEDO et al., 2011). In corn silage, the degradation rate of fibrous fractions is 5%/h and undegradable fractions are less than 30% (PIRES et al., 2010). Increased fibrous fractions lead to rumen fill and consequently reduced voluntary intake.

CORRÊA et al. (2003) noted reduced milk production in Holstein cows fed sugarcane (31.9 kg milk/day) compared to cows fed corn silage (34.4 kg milk/day). These authors attributed this decrease to lower dry matter consumption (1.5 DM/cow/day), possibly caused by the lower NDF digestibility in sugarcane (23.1%) compared to corn silage (42%). Another interesting observation was a reduction in dry matter intake with an extended evaluation period, which was not observed when animals were fed corn silage. This suggests a reduction

in passage rate and an increase in total retention time, as observed by MAGALHÃES et al. (2006) in a study to evaluate substitution of corn silage with sugarcane.

In order to determine the genetic variability of sugarcane varieties, CARVALHO et al. (2010) evaluated the effects of fibrous fraction components on digestibility of dry matter and fiber. They found stem NDF to have lower digestibility (11.9%) than leaf (23.9%). Nevertheless, stem NDF content is lower (38.2%) than that of leaves (68.7%). These authors did not consider residues that probably have higher NDF values than leaves. Another interesting observation in this study was that when varieties were harvested at the beginning of the season, the reduced NDF/POL (sucrose value) ratio was vital in increasing dry matter digestibility. However, when sucrose accumulation is higher (September), NDF digestibility has a pronounced effect on dry matter digestibility. Lastly, NDF digestibility was found to be related to the lignin/NDF ratio and not with concentration of lignin, NDF, POL or NDF/POL. According to the authors, this demonstrates that fiber digestibility is related to the quality and not quantity of this fiber.

The fibrous part of sugarcane clearly limits its inclusion in ruminant feed. The search for alternatives to increase the passage rate of sugarcane should be pursued. An alternative is to improve fiber digestibility through genetic breeding programs; however, this cannot be obtained at the expense of production level. Another question to consider is how much digestibility can be improved without overturning the sugarcane field since operational difficulties related to sugarcane harvesting are another obstacle to its use.

As previously mentioned, the main criterion used in variety selection is productivity. An important parameter to consider is yield of digestible dry matter per hectare, since it considers both quantitative and qualitative aspects. A joint analysis of data obtained by ANDRADE et al. (2003) and BONOMO et al. (2009) found that the relationship between digestible matter production/ha is more related to dry matter production than digestibility. Studies of sugarcane cultivars that consider production and nutritional composition, like the two mentioned above, are rare. Variety selection aiming to improve quality cannot be realized at the expense of productivity characteristics. It should be stressed that there are varieties on the market that meet the quantitative and qualitative requirements for use in animal feed.

Few articles evaluate the effect of variety on silage. Schmidt (2006) evaluated the effect of two varieties, IAC86-2480 and IAC87-3184, and found that the latter presented silage with lower gas production and higher dry matter recovery, regardless of the presence or absence of additives and harvesting time. Although variety IAC86-2480 presented higher

losses at the opening of the silos, it also presented greater levels of soluble carbohydrates and digestibility. Thus, a joint analysis of quantitative parameters is needed to direct sugarcane for silage, which is represented in the present study by loss and quality after opening the silo.

RODRIGUES et al. (2008) observed large variations in dry matter recovery, from 54.29 to 98.7% (Table 2).

Table 2. Variables evaluated, mean, standard deviation, minimum and maximum values of 50 sugarcane varieties

Variable ¹	Mean	Stand. Deviation	Min	Max
DMs	32.39	1.98	25.66	37.54
DMo	24.99	2.72	17.28	33.68
VDM	7.40	2.46	-0.67	12.43
GAS	17.15	3.04	9.02	23.18
DMR	74.04	6.53	54.29	98.70
pH	3.15	1.22	2.80	3.45
BRIX	21.49	1.42	16.94	24.00
POL	19.99	1.68	14.76	22.77
RS	0.45	0.09	0.33	0.87
PUR	92.88	2.68	80.72	96.64

1-DMs- dry matter in silage, DMo- dry matter at opening, VDM- variation of dry matter, GAS- losses by gas, DMR- dry matter recovery, BRIX- total soluble solid content, POL- Sucrose content, RS- reducing sugars (glucose + fructose) and PUR- purity of juice.

Source: RODRIGUES et al., 2008

The authors' objective was to identify correlations among chemical composition, sugar level, and dry matter loss in silage. However, dry matter recovery was not significantly related to sugar content or type. The authors argue that issues related to epiphytic microflora may have led to the lack of correlations. SILVA et al. (2008) observed a direct effect of soluble carbohydrate content on ethanol content and dry matter loss. These authors estimated the level of soluble carbohydrates to be 12.4% DM for no ethanol production.

However, it should be stressed that the search for varieties or management techniques to reduce the level of soluble carbohydrates at the time of ensiling in order to reduce loss does not seem to make much sense, since the energetic source of sugarcane is

soluble carbohydrates. In this sense, the stoichiometric balance of variety choice needs to be calculated with two goals. One aims to reduce loss without affecting the energy level, which can be obtained via soluble sugars. The other aims to reduce the contribution of compounds produced during fermentation, like ethanol.

3.2. HARVESTING TIME

The sugarcane and alcohol sector constantly uses the concept of physiological maturity related to variety. In this way, sugarcane harvesting is always realized when the stalk nears maximum sugar accumulation. Physiological ripeners are often used to increase sugar concentration. The livestock sector rarely uses physiological ripeners and tends to harvest indiscriminately.

FERNANDES et al. (2003) evaluated 10 sugarcane varieties (five early and five intermediate/late) to be used in ruminant feed. These varieties were harvested at 426, 487 and 549 days after planting. Results showed an interaction between Brix (total sugars) and harvesting age. Intermediate/late varieties have higher concentrations of Brix when harvested late. Although no significant differences were found, it is clear that delaying the harvesting period increased the concentration of total sugars in intermediate/late varieties, increasing from 18.0 (426 days) to 22.1 (549 days). On the other hand, early varieties kept close Brix levels, 18.6 and 19.7 at 426 and 549 days, respectively. Based on the varieties studied, the authors concluded that varieties with an intermediate production cycle have better nutritional value than early varieties with lower NDF and ADF values, less total rumen repletion, and greater percentages of TDN and Brix. Nevertheless, these results should be interpreted with caution, since they involve the sugarcane plant (first cut). These authors believe that in subsequent cuts a greater amount of interactions should exist, which justifies use of early varieties at the beginning of the dry period (April and May) and intermediate and late varieties for the remaining dry months.

Comparing ensiled sugarcane at 12 and 24 months regrowth, SANTOS et al. (2006) found the strategy of allowing sugarcane to go from one year to another without harvesting should be omitted in comparisons with first year sugarcane silage. These authors observed significantly greater values of fibrous fractions, NDF and ADF, in both fresh and ensiled sugarcane in response to adoption of this practice.

There is usually an increase in digestibility due to the increased participation of soluble carbohydrates in sugarcane with prolonged maturity. SCHMIDT et al. (2007)

observed this effect in varieties IAC86-2480 and IAC87-3184 when analyzed before ensiling; however, after ensiling this trend was inverted. The authors suggest that this result was due to greater intensity of dry matter loss in silage of plants harvested at 18 months.

A classic study, developed to evaluate the effect of harvesting time on sugarcane silage quality realized by KUNG Jr and STANLEY (1982), shows the increase in nutritive value of the fresh forage and the decrease in *in vivo* dry matter digestibility of silage in response to increased harvesting age. According to the authors, this fact is related to ethanol production during the fermentation process, which resulted in decreased soluble sugar content.

3.3. ROW SPACING

Reduced spacing between rows tends to raise sugarcane productivity (MURARO et al., 2011; GALVANI et al., 1997; BASILE FILHO, 1992). A pioneer study performed by AGUIRRE Jr. & ARRUDA¹ (1954) and cited by BASILE FILHO (1992) evaluated three row spacing (1.00, 1.30, 1.60 and 1.90 m) over five consecutive years, in other words a complete production cycle of a plot. They concluded that smaller spacing produced the greatest number of tillers per area, but did not affect mean stem weight; consequently, productivity increased. On the other hand, BASILE FILHO (1992) observed sugarcane cultivated at 1.0 m spacing compared to 1.45 m reduced tillering per linear meter. However, due to the larger number of linear meters, when the evaluation was realized per area unit, a greater number of tillers were observed as well as greater productivity.

The abovementioned studies were directed toward the sugar-alcohol industry. On the other hand, MURARO et al. (2011) evaluated the effects of reducing row spacing of sugarcane cultures used in animal feeds. Row spacing of 0.90 and 1.30 m were compared using RB72-454 variety, which is a common variety for ruminant feed. Assessment was carried out in the first year crop (cane plant). Sugarcane harvested at 420 days presented an increase from 48.12 to 56.67 t DM/h (17.7%) with reduced spacing. Increased productivity may be attributed to the increased number of tillers per area, since reduced weight of tillers (14.7%) was offset by an increase in number of tillers (35%), since the number of tillers per linear meter was not reduced. Analysis of the bromatological composition of sugarcane produced by adopting these treatments did not present any significant differences in any of the

¹ AGUIRRE JÚNIOR, J. M.; ARRUDA, H.C. Study of sugar cane varieties and spacing. In: CONGRESSO PANAMERICANO DE AGRONOMIA, 2. 1954, Piracicaba. Resumos. Piracicaba, 1954, p.49

parameters analyzed, which shows that productivity gains did not affect quality (MURARO et al., 2009).

Another possibility of exploring reduced spacing is by maintaining the productivity achieved by decreasing the number of seedlings per linear meter and consequently maintaining the number of seedlings per hectare. SIQUEIRA et al. (2011c) suggested that this would be an interesting strategy to facilitate the harvesting using forage harvesters projected to harvest crops like corn and sorghum. In MURARO et al. (2011) and other reviews, the same number of seedlings (13-15 buds) were used in each linear meter. Thus in order to facilitate harvesting, these authors suggested proportionally reducing the number of seedlings to about 10 buds/linear m in planting with 0.9 m row spacing. The practice of reducing row spacing is not much used in the sugar-alcohol sector, due to the increase in furrows and difficulties in mechanical operations because the machine needs to traffic over the planted rows. However, the authors of this text only suggestively believe that the intensity of managing plots for animal feed is less intensive and cause less damage to stumps. Note that with 0.9 m spacing it is possible for equipment to move with two lines between the wheels, but this form of management requires more skilled operators than that required for only one line.

3.4. HARVESTING AND RESIDUE

Almost all of the aerial part (leaves, stalks and husk) of sugarcane is removed during harvesting. When harvesting is manual, the husk and tip, as defined by RIPOLI & RIPOLI (2001) as the apical part of sugarcane stalks formed by internodes in formation covered by sheaths of leaves, can be removed to possibly improve the nutritional value of the forage.

Manual harvesting is performed by operators using machetes or pruning shears to segregate stem from its connection to the root system. Cuts should be made at the stem base, seeking to gather as much forage as possible. This harvesting form is carried out throughout the period of use.

Mechanical harvesting began with the use of harvesting machines for cultures like corn and sorghum. However, forage density per linear meter of sugarcane is nearly four times greater than that of other cultures. This fact reduces the useful life of machinery and sugarcane fields as well as increases forage loss.

Even with this scenario, NUSSIO et al. (2006) showed that mechanical harvesting significantly reduced production cost of roughage. When harvesting is performed with a forage harvester, losses are between 8 and 10 t fresh matter/ha, due to the “stem base” residue

left by the machine (RODRIGUES et al., 2008; 2009; SCHOGOR et al., 2009). Besides this considerable loss, the productive sector has always questioned the fate of the residue obtained. The first recommendation, based on concepts of manual harvesting, was to remove this post harvest residue using pruning shears until it is level to the ground. This practice makes it almost impossible to harvest mechanically, since it would overtax the operation with increased labor. In properties that use sugarcane for large herds, hydraulic rotary mowers have been used to lower this residue. The theory is that if the post-harvest residue remains in an area, aerial shooting will occur and production and future harvesting cycles will be compromised.

Some studies have been conducted to better understand these issues. RODRIGUES et al. (2008) observed increased production when post-harvesting residue was not removed. They attributed this effect to a possible contribution of carbohydrates in stimulating plant shooting. SCHOGOR (2008) observed that after mechanical harvesting without lowering residue, aerial tillers are emitted, but only survive up to 90 days. Also, harvesting type, being manual, mechanical or mechanical with lowering, did not alter the number of basal tillers or production.

Another study to evaluate the effect of residue height on tillering and sugarcane productivity was realized by SILVA et al. (2009). Three cutting heights were analyzed (0, 10 and 20 cm, above soil level). Heights 10 and 20 cm presented the greatest tillering, with this effect being attributed to reserve energy accumulated in the stalk base, especially under unfavorable climatic conditions. However, this increased tillering did not result in increased productivity, considering as mentioned above that lowering this residue would be another production cost. In this way, considering the inability of harvesters to work with sugarcane near the soil surface, a lack of productivity differences in the previous cut may be a good result.

3.5. Burning

The time that sugarcane is physiologically mature, in other words harvesting time, is during the dry period of the year. This is one of the features that make sugarcane interesting for use in animal feed; however, the risk of accidental or deliberate fire during this time of year is high. This scenario raises the issue of what can be done if the sugarcane field is burned.

Research considering accidental burning of sugarcane fields used for animal feed is deficient. BARBIERI & SILA (2008) claim that burning temperature and time directly influence the intensity of the process of exuding juice from the stems. This is an extremely important aspect, because with juice exudation, yeast growth before ensiling is stimulated due to increased substrate availability for microorganism growth. ROTH et al. (2010) noted this fact while performing yeast counts (Figure 1) in a sugarcane field after burning.

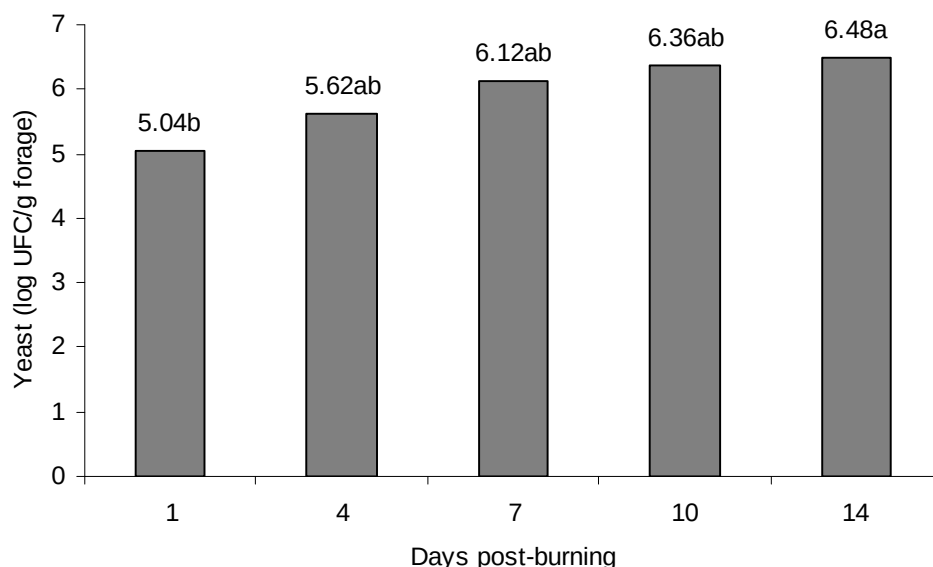


Figure 1. Yeast population count in sugarcane related to time after burning.

Source: Roth et al. (2010)

In addition to juice exudation, another consequence of sugarcane burning is altered sugar composition. ROTH (2009) noted that the Brix concentration was not changed over time after burning, but changes in the composition of total soluble solids, reducing sugar content, and reduced sucrose content over time after burning (Figure 2). The author attributes this fact to the action of invertases that are able to convert sucrose into glucose and fructose (reducing sugars). The activity of this enzyme could have been stimulated by the high temperatures that occurred during the burning of the sugarcane.

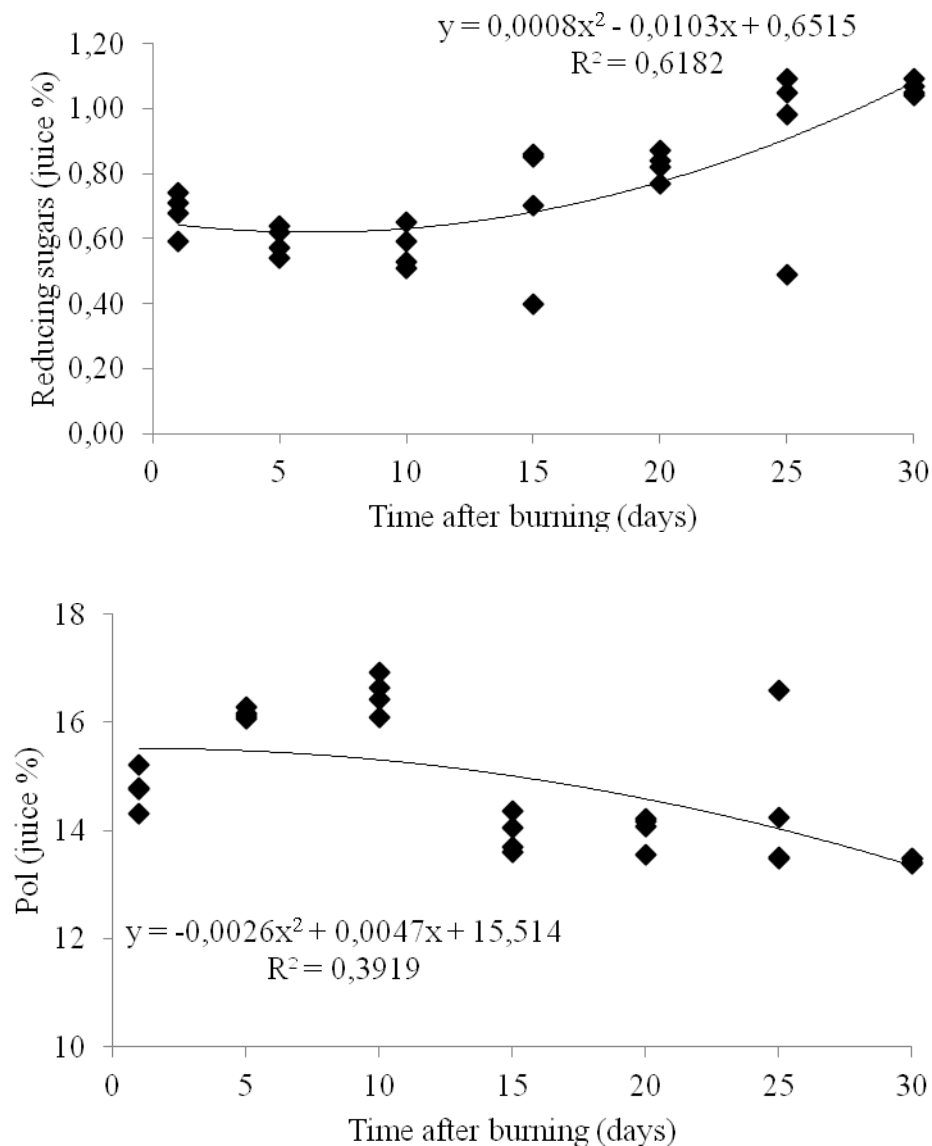


Figure 2. Juice levels of reducing sugar (fructose + glucose) (A) and sucrose (B) in sugarcane harvested after different times following burning

Adapted from Roth (2009)

The effect of high temperatures during burning were cited by BERNARDES et al. (2007) as destructive to the wax layer that surrounds the cell wall, which causes cracks in the stem and subsequent exudation of cell content (sugars), increasing chances of microbial contamination. Temperature increases and sugarcane storage for long times can cause sucrose to break down into glucose and fructose, which can facilitate alcoholic fermentation by yeast. The authors reported higher yeast counts and ethanol production in burned sugarcane compared to fresh sugarcane.

SIQUEIRA et al. (2011d) raised the following question: “My sugarcane burned. What can I do and how long do I have to take action?” After extensive review, the authors suggested ensiling sugarcane after burning, if the producer needs to use this forage resource for an extended period of time. Based on available information, the window to cut sugarcane for beef cattle feed is 10 days after burning. For dairy cows, studies confirming this hypothesis need to be performed.

4. FINAL CONSIDERATIONS

Most research on agricultural management is performed to evaluate its effect on sugarcane destined for the sugar-alcohol sector. Studies seeking to quantify its effects on silage and animal nutrition are rare.

Variety choice should be based primarily on productivity and secondly on the search for improved quality of the fibrous portion, aiming to maximize production of digestible organic matter per area.

Use of the concept of early, average and late varieties need to be adopted by producers so that forage is always used at its best nutritional value.

Reduced spacing appears to be an interesting alternative to either increase productivity or facilitate sugarcane harvesting.

After mechanical harvesting post harvesting residue stimulates tillering and does not compromise productivity of the next cycle.

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Use of winter cultures for forage conservation

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Introduction

Forage production is seasonal throughout most of Brazil. Most of the country lies within the tropical zone, which favors development of C₄ plants. Furthermore, the climate in the south and parts of the southeast and central-west regions can be used for forage production of C₃ plants in the winter, since rainfall is sufficient for plant growth at this time of the year.

In countries with more severe summer climate than Brazil, it is difficult to cultivate forage without irrigation, which favors use of winter crops as conserved forage for shortages. According to Novak et al. (2011), grains at an immature developmental stage (silage or hay) can be harvested as a response to a cyclical demand for forage. Harvest can also be voluntarily integrated into a forage system as a means of overcoming seasonal shortages and consequently provide a forage resource in areas where irrigation is not possible or desired.

In Europe, winter grains have gone beyond the status of “sporadic” forage crops to that of a “base” forage crop. In the first case, the grain ensures forage supply during times of drought in the summer. In the latter, the crop ensures all or part of the forage stock under different situations, making this a true strategy in forage planning (LEMAIRE et al., 2006).

Use of winter cultures for forage production can be an important tool in improving animal production in Brazil. This strategy is interesting from an economical point of view. It is also greatly sustainable in some areas of the South, where pastureland or underused winter areas can be destined for silage production. In this way, the risks of seasonal culture loss (winter corn, grains or silage) can be minimized. Also, competition with summer cultures for silage production would be reduced, which would free up useful areas to produce grains for human consumption.

In this context, better use of qualitative and strategic aspects of silage production of winter crops will be discussed to better use these systems in animal production.

Nutritional potential of winter grain silage

The nutritional value of grasses grown in temperate climates (C_3 metabolism) is different from that of tropical grasses (C_4 metabolism). Temperate grass has more abundant cell content and thinner cell walls which make it easier for herbivores to digest and absorb nutrients. Furthermore, greater cell content positively affects the silage fermentation process, since a higher concentration of soluble carbohydrates favors lactic acid production. On the other hand, some negative aspects associated with this type of grass may jeopardize the fermentation process. The main disadvantage of this type of grass is its low dry matter (DM) content (Table 1).

Table 1. Chemical-bromatological composition and fermentation characteristics of winter grain silage.

Parameters (%)	Berto & Mühlbach (1997)	Emile et al. (2007)	Wallsten et al. (2010)	Oliveira et al. (2010)
	Oat	Wheat	Barley	Triticale
DM, %	15.30	33.50	41.60	29.20
CP, % DM	17.70	8.10	10.00	11.40
NDF, % DM	46.70	53.20	41.10	61.30
ADF, % DM	34.80	---	26.30	37.80
pH	4.60	3.91	4.50	4.30
N-NH ₃ (% total N)	11.80	9.90	6.70	13.80
Acetic acid, % DM	3.60	2.98	0.80	1.93
Propionic acid, % DM	---	---	0.10	0.20
Butyric acid, % DM	0.18	0.09	0.00	0.13
Lactic acid, % DM	8.4	3.83	2.90	6.90
IVDMD, % DM	---	60.80	67.50	---

Berto & Mühlbach (1997) (Table 1) observed lower dry matter values in oat than those desired for good fermentation in the silo (desired values are between 30-35% - McDonald et al., 1991). Consequently, one can infer that this low dry matter content together with the greater protein values observed may have caused the elevated pH level (4.60) and probably increased proteolysis as well, which can be verified by the higher ammonia nitrogen rates (11.80% total N) present in the silage.

A similar effect was observed by Oliveira et al. (2010) with triticale silage. These authors observed elevated pH and ammonia nitrogen values with less than desired dry matter content. Lower dry matter content at the time of ensiling may also trigger the onset of clostridia activity in the silage material, favoring proteolysis and consequently greater ammonia nitrogen levels in the final product.

On the other hand, Emile et al. (2007) and Wallsten et al. (2010), working respectively with wheat and barley, presented more consistent results on the quality of silage fermentation when ensiled with more adequate dry matter levels. From a nutritional point of view, these forages present important nutrient and fiber levels for herbivores, making wheat and barley interesting options for forage conservation to overcome shortages in a forage planning strategy.

In order to improve the nutritional value of winter grain silage, intercropping these grains with legumes has been suggested. In Europe, mixing winter grains and legumes for silage production has raised producers' interest and motivated research for the ideal mix for this purpose.

Intercropping winter grains and legumes can provide advantages in agriculture (savings of nitrogen fertilizers) and animal production (better forage nutritional value), thereby strengthening the appeal of sustainable production systems, primarily European ones.

According to the Official Journal of the European Union (2002), development of legumes individually or by intercropping with grasses brings some important environmental advantages for the European Union. Some of which are: greater biodiversity, better crop rotation, better nitrogen balance, maintenance of the soil structure, healthy reaction to a tendency toward grain monoculture, and overall, decreased use of production factors such as nitrogen fertilizers, pesticides and fuel.

With this in mind, some studies have set out to verify the quality of forage produced by intercropping (Table 2).

Table 2. Chemical-bromatological composition and fermentation characteristics of winter grain silage intercropped with legumes.

Parameters	Dias, (2007)		Bumbieris Jr, (2009)		Huuskonen et al. (2010)	
	TSP	TSOPV	TSP	TSOPV	BSV	WSV
DM (%)	25.28	24.46	23.44	22.57	26.80	31.50
CP (% DM)	10.27	9.50	11.63	13.06	10.60	13.30
NDF (% DM)	56.85	56.90	58.52	56.39	54.00	54.70
ADF (% DM)	36.35	36.02	37.48	36.11	---	---

pH	3.44	3.80	3.83	4.20	3.98	3.85
N-NH ₃ (% total N)	12.80	13.70	11.40	14.80	7.10	8.30
Acetic acid (% DM)	1.90	2.95	2.79	4.70	---	---
Propionic acid (% DM)	0.03	0.03	0.13	0.13	---	---
Butyric acid (% DM)	0.06	0.06	0.08	< 0.05	---	---
Lactic acid (% DM)	10.55	2.86	8.24	7.28	4.50	6.30
IVDMD (% DM)	60.94	61.03	59.70	60.28	68.5	57.80

TSP=Triticale Silage + Forage Peas; TSOPV= Triticale Silage + Oats + Forage Peas + Vetch; BSV= Barley Silage + Vetch; WSV= Wheat Silage + Vetch.

Dias (2007) and Bumbieris Jr (2009), working with triticale silage intercropped with oat and legumes, showed that insertion of legumes improves forage nutrient content. Although the ammonia nitrogen content was high, it was still within acceptable limits (15% total N), since legumes normally present higher nitrogen content than grass as well as some inorganic ions.

Furthermore, Huuskonen & Tokola (2010), working with barley (BSV) and wheat (WSV) silage intercropped with vetch, obtained very satisfying results regarding fermentation quality and bromatological composition.

In Brazil, research of intercropping winter crops with legumes for silage is still limited; however, some efforts show promising results. Fontaneli et al. (1991), in a study on the effect of intercropping grasses with winter legumes, observed that oat stood out among the grasses and vetch presented the best phytomass yield among the legumes studied. Tomm et al. (2003), in a study of hibernating grasses intercropped with forage peas, observed that the best grass adapted to intercropping with this specie was triticale. The greater stature and vigor of this plant allow it more access to light. Also, the fewer layers of triticale resulted in less loss of pea plants by rotting, resulting in greater pea and triticale production (triticale grain production nearly 2,300 kg/ha).

Of the different ways to utilize these temperate species, forage quality is an attractive factor in deciding use, because it has a direct effect on animal performance.

Wilted Winter Grain Silage

Wilting winter grains before ensiling increases forage dry matter content. Consequently, this technique is widely used to help assure appropriate conservation conditions for ensiled forage.

Furthermore, wilting is considered a management strategy to reduce environmental pollution (LORENZ et al., 2010; SCHMIDT et al., 2011). Cell rupture and cytoplasm leakage during forage storage create silage effluent, which can lead to significant feed losses. The drier the crop, the less silage effluent is produced. Thus, partial water removal from plants has been proposed as a method to reduce silage effluent (SCHMIDT et al., 2011). These authors also reported that effluent production is primarily influenced by the moisture content of the forage taken to the silo, and secondly by particle size, compacting, additives, silo type and plant characteristics at the time of ensiling.

McEniry et al. (2007), in a study on wilting perennial ryegrass (*Lolium perenne*) after 0, 24 and 48 hours, observed that silage effluent was only produced in non-wilted silage with a value of 39 g/kg ensiled forage. Weinberg et al. (2010) reported that silage porosity and its susceptibility to air penetration depend on the degree of silo compaction, which is directly influenced by culture DM content and particle size.

McEniry et al. (2008) observed that the silage process became progressively more restrictive as DM content increased in the ensiled forage, with lower concentrations of fermentation products and increased pH values (Figure 1).

Previous reports relate the importance of wilting on silage, primarily by reducing effluent production and restricting fermentation. However, decreased moisture benefit silage as long as levels are at least 30% DM (WOOLFORD, 1984) and associated with a quick drying rate (DAWSON et al., 1999).

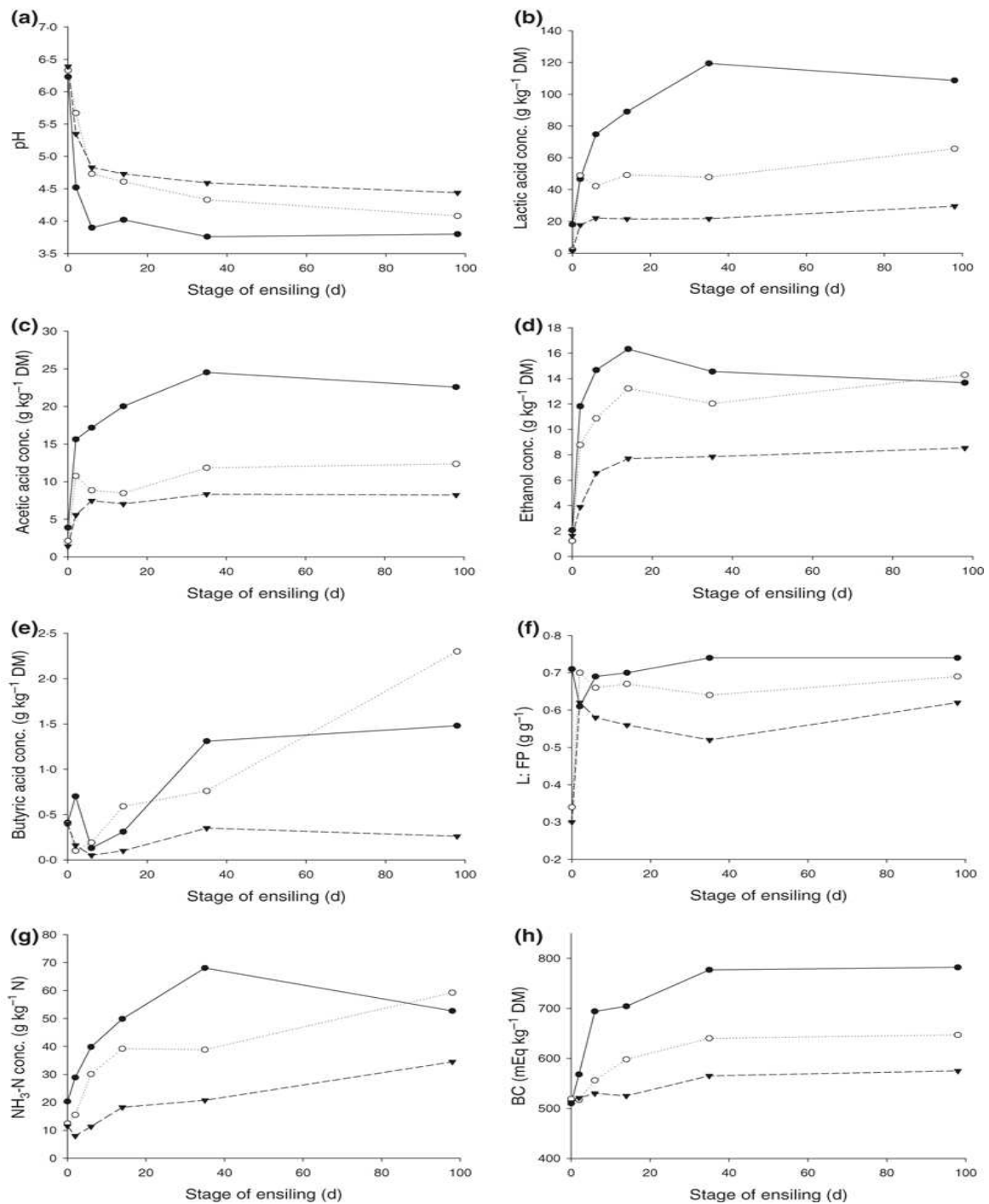


Figure1. Effects of wilting duration and stage of ensiling on (a) pH, (b) lactic acid, (c) acetic acid, (d) ethanol, (e) butyric acid, (f) ratio of lactic acid (L) to total fermentation products (FP), (g) N-amoniacal concentration and (h) buffering capacity (BC) of silage during fermentation at 0h(-●-), 24h (-○-), and 48h (-▼-) of wilting.
Source: McEniry et al. (2008).

Maturation stage X winter grain silage quality

It is well known that forage chemical composition is influenced by plant developmental stage. Consequently, different maturation stages are recommended for silage.

Beck et al. (2009) reported that DM production increased as wheat (*Triticum aestivum* L.) matured, producing between 2,781 kg/ha DM (21.5% DM) and 6,261 kg/ha DM (46.7% DM) respectively for flowering and dough stages. Working with oat (*Avena sativa* L.) silage, David et al. (2010) obtained productivity values from 3,547 kg/ha DM at flowering to 9,170 kg/ha DM at soft dough stage.

Crude protein (CP) decreased as winter grains matured (FONTANELI & FONTANELI, 2009; COBLENTZ & WALGENBACH, 2010). Beck et al. (2009) also observed decreased CP in wheat silage respectively for flowering and dough stages (from 15.2 to 8.9%). Fontaneli & Fontaneli (2009) evaluated the nutritional composition of wheat, oat and rye silage based on CP at three maturity stages. These authors observed average CP values around 17.7, 13.0 and 10.2% respectively for boot, milk and dough development stages. Coblenz & Walgenbach (2010) observed that reductions in digestible CP levels as plants matured were offset by an increase in non-fibrous digestible carbohydrate levels.

Lopes et al. (2008), in a study of triticale (*X Triticosecale Wittmack*) silage at different harvesting ages (83, 90, 97, 104, 111 and 118 days), observed that as plants matured the neutral detergent fiber (NDF) also increased until flowering. Then, fiber fraction levels decreased (53.9, 65.9, 57.7, 54.9, 52.5 and 48.2% DM). This element may become diluted by the greater starch levels produced as the grain matures. Coblenz & Walgenbach (2010) observed that NDF concentrations presented a quadratic response in wheat, triticale and oat cultures at different harvesting times. On the other hand, Beck et al. (2009) and David et al. (2010) did not observe any reduction in NDF as plants matured.

Coblenz & Walgenbach (2010) observed a linear effect in concentrations of total digestible nutrients (TDN) with longer cutting intervals. David et al. (2010), in a study on oat ensiled with 26.6 and 31.9% DM, found TDN to respectively increase from 53.7 to 56.3% DM. However, Beck et al. (2009) did not observe any changes in TDN as wheat matured.

Wallsten & Martinsson (2009) observed that as barley (*Hordeum vulgare* L.) matured, NDF and DM digestibility reduced and starch accumulated. These authors also reported that higher stages of maturity decreased milk production, fat, protein and feed efficiency in dairy cows. Beck et al. (2009) did not detect any effect of maturity stage of wheat silage on beef cattle performance. On the other hand, Wallsten et al. (2010) in an evaluation of wheat and barley silages reported that as plants matured, digestibility of organic material decreased (68.3 to 63.3% DM and 69.8 to 66.1% DM) as did NDF (74.6 to 60.7% DM and 69.8 to 59.6% DM) respectively for flowering and dough stages.

Coblentz & Walgenbach (2010) suggested a wide window for harvesting according to the nearly constant energy density at different harvesting times. However, Ashbell & Weinberg (2000) recommended using late cultivars for silage production in hopes of extending the harvesting window since the drying rate in hot climates is very quick which hinders control of the correct silage stage.

Reports on the maturity stage of winter grains in the literature are inconsistent. These discrepancies can be justified by differences in plants, nutrient translocation, soil types, hydric availability, growth stage, cultivars, fertilization program, and the distinct drying rates between hot and temperate regions.

Winter grain silage X corn silage

According to Jobim et al. (2007), forage quality refers to the nutritional value of the forage mass interacting with the animal's consumption and potential performance. In this way, forage quality effects consumption and energy density, which determine animal productivity.

It is important to remember that winter grain silage is less productive than that of tropical grasses. Fontaneli et al. (2009) evaluated productivity of 14 genotypes of six winter grain species. They observed that rye (cv. BRS Serrano) presented the greatest DM yield in cutting for silage (9.721 kg/ha), with productivity of the other winter grains varying from 3.641 for barley (cv. BRS 195) to 7.027 for rye (cv. BR1) (Table 3). These authors reported that variations in winter silage yields is caused by interactions among the genetic potential of the cultivars, management practices, climatic conditions and the maturity stage at the harvesting time

Table 3. Cutting height, dry matter content and production (kg/ha) of winter grains.

Winter Grain	Cutting height (cm)	DM (%)	kg/ha DM
White Oat UPF 18	111b	29.5ef	6,159bc
Black Oat IPFA 99009	117b	28.5fg	6,455bc
Black Oat Agro Zebu	112b	25.7g	5,419bcde
Rye BR 1	136a	37.8ab	7,027b
Rye BRS Serrano	142a	39.1a	9,721a
Barley BRS 195	57f	31.7def	3,641e
Barley BRS 224	73de	30.2def	4,696cde

Barley BRS 225	66ef	32.5cde	3,962de
Triticale BRS 148	99c	33.0cd	5,375bcde
Triticale BRS 203	96c	32.8cd	4,738cde
Triticale Embrapa 53	93c	35.2bc	5,590bcd
Wheat BRS Figueira	68ef	36.9ab	5,022cde
Wheat BRS Umbu	77de	38.1ab	5,091cde
Wheat BRS 277	80d	38.4ab	5,175cde
Average	94.7	33.5	5,577

Averages followed by the same letter in a column are not significantly different ($P>0.05$) according to the Tukey test.

Source: Adapted from Fontaneli et al. (2009)

Winter grains produced lower energy silage compared to corn silage for several reasons, being anatomical, morphological and physical-chemical in nature (FONTANELI & FONTANELI, 2009). However, Coblenz & Walgenbach (2010) observed greater concentrations of TDN in wheat cultivars compared to oat and triticale (69.3 compared to 67.8%), which demonstrates the potential of this crop for silage.

Assessing nutrient intake and cattle performance of perennial ryegrass and a 40:60 ratio of corn and wheat silage, Keady et al. (2007) concluded that perennial ryegrass plus corn provided better carcass weight due to better use of metabolizable energy. However, these authors also reported that perennial ryegrass plus wheat did not alter DM intake (DMI) or digestible energy intake (DEI) compared to the corn silage mix.

In test animals receiving silage of perennial ryegrass (17.4% DM), corn (31.5% DM) and wheat (40.4% DM) in addition to 3 kg concentrate/day, Walsh et al. (2008a) did not observe any differences in DMI (7.02 vs. 7.31 kg DM/day), DEI (1.2 vs. 1.15 kg/day) and carcass weight (335 vs 329 kg) respectively for corn and wheat silage.

Walsh et al. (2008b) evaluated nutrient intake, digestibility and performance in beef cattle fed wheat and barley silage ensiled at two cutting heights (0.12 and 0.29 m) compared to corn silage. They did not detect any influence of diet on DMI, *in vivo* digestibility, DEI and final carcass weight. Therefore, the authors concluded that wheat and barley silage with high grain levels (> 50% DM) provide the same level of performance as those obtained with corn silage. Similarly, Addah et al. (2011) found no significant differences in weight gain of animals fed corn or barley silage (1.26 and 1.43 kg/day, respectively).

On the other hand, Oltjen & Bolsen (1980) found different weight gains for animals fed corn or oat silage with respective gains of 1.15 and 0.5 kg/animal. Coombs et al. (1997)

observed similar behavior in animals fed corn or wheat silage with respective gains of 1.07 and 0.77 kg/animal/day.

As can be seen, there are varying reports in the literature regarding winter grain silage and animal performance. These different responses may be attributed to differences in the nature of the diets, roughage: concentrate ratio and the genetic potential of the animals. The roughage ratio of a diet can equally influence animal performance and product characteristics through its effect on DMI differences in rumen fermentation and digestion kinetics (Galyean & Defoor, 2003).

Additives for producing winter grain silage

In Brazil the most common silage additive is bacteria. However, there are several other types of additives such as nutrient additives [absorbent (grains or not), non-protein nitrogen (NPN), urea and ammonia], enzymes, as well as organic and inorganic acids. Their main function is to increase the nutritional value of silage, and/or improve fermentation by reducing losses during storage and/or silage use.

Additive response will depend on the type of forage being treated as well as the treatment itself. Corn silage has little need for additives to improve fermentation. It is easily ensiled since values of DM, soluble carbohydrates and buffering capacity are all within the desired range. On the other hand, nutrient additives, like urea and ammonia, are beneficial, because they increase the CP level of the ensiled material (COOMBS et al., 1997). When use of NPN is compared for animal performance, the literature shows mixed results. Coombs et al. (1997) observed that corn and wheat silage with added N ammonia (3.5g/kg fresh basis) resulted in weight gain similar to that of silage with no additives, but with protein levels corrected at feeding.

Winter grain silage often has problems in the fermentation process, since DM content at the moment of ensiling can be below desired levels (DIAS, 2007; BUMBIERIS JR, 2009; HUUSKONEN et al., 2010), which facilitates fermentation of undesirable substances. Consequently, use of additives that increase DM content (absorbent) and increase the amount of desirable bacteria may help improve the conservation process in this feed.

Positive results of these additives can be seen in the works of Coan et al. (2000 and 2001); Evangelista et al. (2002) and Lima et al. (2002).

Chemical composition and subsequently the fermentation characteristics of corn and winter grains are very different. Corn has a low level of protein and minerals, but high concentrations of fermentable carbohydrates. On the other hand, winter grains vary in

proteins, but when harvested at an adequate maturity stage they generally have high concentrations of this nutrient and are good sources of minerals, although they have a low concentration of fermentable carbohydrates.

Higher DM levels of material that is wilted before ensiling have sometimes proven to be prejudicial to the fermentation process (McEniry et al., 2008) as well as labor intensive. Thus, use of absorbent additives, especially nutrient additives (corn grits, wheat bran, citrus pulp) may be a more practical strategy. In addition to increasing DM content, these additives also increase the concentration of nutrients of high nutritional value in the ensiled material.

Corn, sorghum, other grains (wheat, oat and triticale) and molasses may be added to forage at the time of ensiling. Addition of these grains has several advantages. Two of which are increased silage energetic value and decreased need for supplementary concentrates. If silage were the primary or the only feed offered it will become a more complete food. Another interesting aspect is that the grain added to the silage will already have been stored and will not need any preservation treatment. These additives (grains) are not intended to be used as a source of soluble carbohydrates, since starch (the main carbohydrate in grains) is not readily fermented in the silo. The main purpose is to increase DM levels and nutritional value. Molasses, unlike grains, provides fermentable carbohydrates; therefore, its addition may improve fermentation of some forage crops.

Microbial inoculants are added to forage to increase the number of desirable bacteria at the time of ensiling. A number of different inoculants are currently available and many studies have evaluated their efficiency, although research results are varied. The main microorganisms used in additives are *Lactobacillus plantarum*, *L. acidophilus*, *Pediococcus acidilactici*, *P. pentosaceus*, and *Enterococcus faecium* among others.

The population of epiphytic bacteria in corn has proven to effectively promote good fermentation and conservation of these materials. However, it may not be enough for winter crops. Inoculation may reduce storage losses, since most experiments with added bacteria inoculants increased the rate at which pH decreased and increase of lactic acid concentration in the silage (ADDAAH et al., 2011).

According to Addah et al. (2011), inoculation with a combination of *L. plantarum*, *E. faecium* and *P. acidilactici* did not alter DM losses, but during ensiling losses were greater for barley silage than for corn silage. Barley silage was more stable than that of corn after two days of aerobic exposure. Inoculation of barley and corn silage did not affect DM intake, average daily gain (ADG) or feed efficiency compared to animals fed non-inoculated silage. Nevertheless, animals fed barley silage in general had higher DM intake, greater ADG and

higher feed efficiency compared to animals fed corn silage. Inoculation was more effective in improving aerobic stability and fermentation in barley than in corn.

Hristov & McAllister (2002) did not observe any effect of microbial additives on the degradation rate of DM in barley silage. These authors concluded that more consistent results for barley and grasses depend on the microbial composition of the inoculant. However, it is known that multiple factors interfere with response to microbial inoculants in silage.

The decision to use an additive should take into consideration the forage species, DM content of the forage, animal category and the potential productivity of the animals to be fed. Addition of grains to winter silage can reduce production of effluents. Although no additive can substitute good silage practices, it can improve fermentation and nutritional value.

Use of winter grain silage as a strategy to feed cattle

Use of winter grains to feed dairy cattle has become increasingly popular in regions that favor their cultivation. Seeking to maximize use of these grains as a source of roughage, the potential of forage conservation in the form of silage has increasingly grown.

Use of these grains as a strategy has been accomplished by integrating crop and livestock production for grazing use. More recently, pasture management begins before harvesting for conservation in the form of silage. Dias (2007) observed that after grazing triticale, grain production reached 78% compared to non-grazed areas (5,300 kg grains); consequently, the use of this grain for grazing and following harvest is recommended.

With this in mind, silage production after recovering a culture under grazing is possible, as suggested by Meinerz (2009). This author managed winter grain areas (oat, triticale, rye, wheat and barley) in the Santa Maria region of Rio Grande do Sul by cutting before harvesting for silage. After recovering the crops, plants were ensiled. This study concluded that winter grains may be used in this region for a dual purpose. Of the genotypes evaluated, triticale cv. BRS 148 and rye cv. BR1 were the earliest for silage production, and rye BR1 and oat cv. UPF 18 provided the best yields of pre-ensiled mass. Also, wheat cv. Umbu presented the greatest participation of grains in the ensiled mass, providing greater nutritional quality to the silage.

According to Novak et al. (2011), winter grain silage intercropped with legumes can be used to feed dairy cows in late lactation, dry cows and heifers with no adverse effects. These authors observed that lactating cows fed triticale silage and triticale + legumes silage

respectively produced 19 and 21 liters/animal/day. Inclusion of these silages for cows with greater production potential should be evaluated and possibly be partially substituted for corn silage.

Concerning feed for Braford heifers weaned early, Eifert et al. (2004) used triticale silage as a strategic source of roughage associated with 35, 45, 55 and 65% concentrate. These authors observed weight gains of 0.270, 0.536, 0.728 and 0.784 kg/animal/day respectively for the four concentrate levels studied. These results are encouraging, since the cost of producing winter grains is normally less expensive than corn or sorghum cultures. Another positive point to the use of these silages is that areas set aside for fall and winter crops were used so that these areas remain being used over the whole year, which even decreased risks involving any unpredictable climatic factors (such as frost) that could affect sorghum and corn cultures.

Development of dual-purpose cultivars (grazing, grain and/or silage) has been crucial in advancing the use of winter grain silage within animal production systems. Such use is often strategic over climatic factors and has managed to be successful when correctly used as an adequate forage resource for the region.

Further research should be performed in order to validate the use of these grains for silage as a strategy to feed ruminants and adapting the best genotypes for each region. In the central – south region of Brazil, winter grains have the potential to move from the status of a secondary crop to that of a staple crop. The benefits of this strategy have already been observed in producers who are currently using it.

Considerations

In Brazil, use and research of winter grain silage are still sparse. The southern region, which favors these silages, widely uses integration of pastureland and crops. Winter grazing of these species is widespread and is preferable to conservation methods, like hay and silage, which are more expensive.

Use of intercropping winter grains and legumes for silage as well as wilting and additives may be considered strategies to improve the fermentation quality of these silages.

Research to validate the use of these silages as feed in Brazil may effectively motivate the strategic use of forage planning, thereby promoting greater use by producers.

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OCCURRENCE OF MYCOTOXINS IN SILAGE

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INTRODUCTION

Mycotoxins are a large, diverse group of naturally occurring toxic metabolites of fungi. Currently, more than 300 mycotoxins have been identified. A number of mycotoxins have been implicated in diseases in animals and humans. Mycotoxins can be found in a wide variety of crops all around the world, including crops that are commonly fed as silage, such as maize, wheat and grasses. Some mycotoxins, such as trichothecenes, zearalenone and roquefortin C occur frequently in silage crops, whereas others are only sporadically found. Mycotoxins in silage are of dual concern. Firstly, they can have adverse effects on animal health and cause production losses. Secondly, they may jeopardize the safety of food products of animal origin. Of the major mycotoxins in silage crops, the second concern holds true for aflatoxin only, as is described later in this paper.

This paper summarizes scientific knowledge about the major mycotoxins occurring in silages, under which conditions they are formed and how they can be prevented. The metabolism of mycotoxins in ruminants and their carry-over into milk are briefly described. Toxic effects of mycotoxins in animals and man, analytical methods for detection of mycotoxins and legislative aspects are not described in this paper. Information about these topics can be found elsewhere (1-5).

MAJOR CLASSES OF TOXINOGENIC MOULDS AND MYCOTOXINS

The major feed crops that are conserved by ensiling are grasses, maize, lucerne (alfalfa), small grain cereals (wheat, triticale, rye, barley and other) and sorghum. Moulds and mycotoxins that are of relevance for silage produced from these crops are listed in Table 1. A distinction is made between mycotoxins that are formed before ensiling and those that are formed after ensiling. It is important to make this distinction because

different types of moulds, different types of mycotoxins and different types of agricultural factors influencing mycotoxin levels are involved. Mycotoxins that are formed before ensiling are associated with moulds that infect a crop during its growth in the field or by endophytic moulds that live as symbionts in for instance grasses or cereals (field-derived mycotoxins). These mycotoxins include trichothecenes, zearalenone, fumonisins and aflatoxins. Mycotoxins that are formed after ensiling are associated with moulds that develop in silage during storage or feeding-out (ensilage-derived mycotoxins), usually as a result of poor silage management practices. These mycotoxins include mycotoxins formed by *Penicillium roqueforti* and *P. paneum* and the diverse group of mycotoxins formed by *Aspergillus fumigatus*.

Field-derived mycotoxins

The major toxinogenic moulds capable of producing field-derived mycotoxins are *Fusarium* species, *A. flavus* and *A. parasiticus* and endophytic *Claviceps* and *Neotyphodium* species. Important factors influencing mould growth are moisture (water activity (a_w)), temperature and availability of nutrients and oxygen. Moulds are generally tolerant to low a_w . In contrast to most bacteria, many mould species are capable of growth at a_w values between 0.80 and 0.90. Mechanical damage or insect attack of plants or grain kernels plays an important role in mould infestation and subsequent mycotoxin contamination because these events cause disruption of the protective plant cell wall. This creates entry points for infective moulds. In addition, it causes release of nutrients from the plant endosperm that can be used by moulds for growth. The ability of a mould species to produce mycotoxins is genetically determined. However, the expression of these genes and the production of mycotoxins are influenced by environmental factors. Usually production of a mycotoxin occurs only over limited ranges of a_w and temperature.

Fusarium mycotoxins (trichothecenes, zearalenone and fumonisins)

Fusarium species are pathogens of various feed crops, including maize, wheat, barley and grasses. These moulds are occurring world-wide, but seem to be particularly prevalent in temperate climates. Infection of plants by *Fusarium* can take place via

kernels, leafs, the stalk or infected seeds. Soil and decaying plant residues in the field are the main sources of *Fusarium* spores and conidia. A high level of mechanical or insect damage of the plant increases the risk of infection and is often associated with higher mycotoxin levels. Weather conditions strongly influence development of *Fusarium* mycotoxins. Examples of plant diseases associated with *Fusarium* infection include ear rot and stalk rot in maize and ear blight in wheat. The predominant species causing these diseases are *F. graminearum* and *F. culmorum*. These species are capable of producing zearalenone and different types of trichothecenes, including deoxynivalenol (DON; synonym for vomitoxin), nivalenol, diacetoxyscirpenol and T-2 and HT-2 toxin. DON is the most commonly occurring trichothecene. DON and zearalenone are often co-occurring in contaminated crops. An important and often unrecognized feature of DON and zearalenone contamination of maize and wheat is that these mycotoxins occur not only in the grains and kernels but also in the green parts of the plant, *i.e.* the leafs and stalk. This is of significance because these crops often are fed as whole crop silage. The limited information that is available on this topic indicates that DON and zearalenone levels in leafs and stalk of maize can be even higher than in the cob (6). Fumonisin are formed by *F. verticillioides* (syn., *F. moniliforme*) and *F. proliferatum*, species associated with pink or white ear rot disease in maize. Fumonisin are found exclusively in maize.

It is generally assumed that DON, zearalenone and other *Fusarium* mycotoxins are not produced in silage. *Fusarium* species do not survive the acidic and anaerobic conditions of silage and usually have a low prevalence in comparison with *Aspergillus*, *Penicillium* and *Monascus* species. However, few studies report development of *Fusarium* mycotoxins in silage. An example is a study conducted in Italy in which zearalenone concentrations in highly aerobically deteriorated peripheral areas of maize silage were detected that were up to 40 times higher than in non-deteriorated central areas of the silage. The concentration in the central areas was similar to the concentration of the forage at ensiling (7).

Aflatoxins

Aflatoxins are produced by *A. flavus* and, to a lesser extent, *A. parasiticus*. Aflatoxins are highly toxic and carcinogenic to man and animals. Aflatoxin B₁ is the most

prevalent and most toxic form. Aflatoxin B₁ is transformed in the liver of cattle into aflatoxin M₁, the form in which it is (partially) excreted into milk. With respect of the risks of mycotoxins in feed in relation to safety of food products to consumers aflatoxin M₁ is the only mycotoxin of concern. This relates to its significant feed-to-milk carry-over rate and high toxicity. Though *Aspergillus* is generally classified as a mould associated with mycotoxin production during storage of commodities, it can infect crops in the field under favourable conditions, especially in subtropical and warm temperate climates. *A. flavus* and *A. parasiticus* are associated with aflatoxin production in a number of crops, including maize, sunflower, peanut and several tree nuts. Maize plants can become infected by *Aspergillus* conidia from the environment, usually soil or insects. A high level of insect damage increases the risk of infection. If conditions are favourable the mould colonizes the cobs and penetrates into the kernels. Aflatoxin development in the kernels occurs within narrow ranges of moisture content and temperature. Drought stress generally increases aflatoxin development in maize.

Alkaloid mycotoxins

Alkaloid mycotoxins are produced by *Claviceps purpurea* in rye and barley and some grasses and by endophytic *Neotyphodium* moulds in perennial grasses. *Claviceps purpurea* infects the plant when flowering. It produces a resting structure about the size of grain kernels, called sclerotia or ergots, which allow the mould to survive adverse conditions. These ergots contain high concentrations of alkaloids (e.g. clavines and lysergic acid amide). Several grasses, such as perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*), harbor endophytic *Neotyphodium* species capable of producing alkaloid mycotoxins (e.g. lolitrem B and ergovaline). Benefits for the plant from this symbiosis include increase in drought tolerance and resistance to insects. Endophytic *Neotyphodium* are highly prevalent in 'wild' grass populations in natural or extensively managed pastures in the US, Australia, New Zealand and Europe. The prevalence of mycotoxin-producing endophytes in intensively managed pastures is generally low. Grass cultivars selected for grazing or silage production often do not contain these types of endophytes.

Ensilage-derived mycotoxins

The majority of mould species are aerobic micro-organisms. Therefore moulds do not develop in well-preserved, anaerobic silage. However, in practice silages are not completely anaerobic. Firstly, because silage covering materials are generally not fully airtight. Secondly, because of unintended damages to the silage covering during storage (for instance caused by rodents, birds). Moreover, exposure to air becomes inevitable after the silo is opened for feeding. Growth of moulds and development of mycotoxins in silage are associated with the duration and extent of air infiltration. The extent of infiltration of air into the silage mass is mainly dependent on the porosity and density of the silage and the rate of silage removal after opening. The occurrence of moulds in silage is usually highest in surface layers. Commonly detected moulds in silage are *Penicillium roqueforti* and *P. paneum*, *Monascus ruber*, *A. fumigatus*, *Byssoschlamys nivea*, *Mucoraceae* (in particular *Rhizopus nigricans*) and *Chrysosporium sitophila* (8, 9). No mycotoxins from *Mucoraceae* and *C. sitophila* are documented. The predominant mould species in silages is *P. roqueforti*, which is tolerant to acidic conditions and able to grow at oxygen levels as low as 0.1% (v/v). At silage surfaces it usually forms white to grey coloured spots or layers. Occasionally, particularly in maize silage, the species forms typical green to blue coloured balls or lumps of mouldy silage approximately 50 to 100 cm below the top surface. *P. paneum* is closely related to *P. roqueforti* and these species and cannot be differentiated visually on silage. *P. roqueforti* and *P. paneum* isolates from silage are capable of producing a wide range of mycotoxins *in vitro* under laboratory conditions, including for instance different roquefortins, mycophenolic acid, PR-toxin, festuclavine and agroclavine (10, 11). *P. paneum* additionally produces patulin. However, a number of these mycotoxins are probably not formed in silage or may not be stable under conditions prevailing in silage (as discussed later in this paper). *A. fumigatus* is a heat-tolerant mould species that is particularly detected in heavily moulded parts of silage and is capable of producing a large number of different toxic metabolites, including the potent toxin gliotoxin, verruculogen, fumitremorgens, fumigaclavines and trypacidin (12, 13). Apart from production of mycotoxins, the occurrence of *A. fumigatus* in silage is considered a health risk because inhalation of spores of this mould can cause lung disease (aspergillosis) in animals and man. An increasing amount of research in the area of

mycotoxins is dealing with *A. fumigatus* mycotoxins. *M. ruber* forms red-purple spots on silage surfaces. Mycotoxins produced by this species are monacolin K and citrinin. *B. nivea* is a producer of patulin.

STABILITY OF MYCOTOXINS IN SILAGE

Information about the stability of mycotoxins in silage is not fully conclusive. There are data indicating that certain field-derived and ensilage-derived mycotoxins are degraded in silage. However, reports in the literature about this subject are contradictory. This possibly relates to the fact that the conditions in silage are not homogeneous and not constant. In particular exposure of silage to oxygen leads to conditions that are continuously changing (for instance pH, temperature and chemical composition). As a result the composition of the microflora changes accordingly and a succession of different microbial groups occurs. In advanced stages of aerobic deterioration of silage high concentrations moulds and bacilli are present. To some extent this situation has similarity with composting processes. Under such conditions, probably most mycotoxins will be degraded.

Zearalenone is generally regarded as being stable in silage. No effect of ensiling on the zearalenone concentration was detected in studies in which the level of zearalenone was monitored during up to nine months of ensilage (14, 15). This finding is consistent with data showing that the average and range of zearalenone concentrations in maize silage and unfermented maize products used as feed ingredient are similar (16). With respect to the stability of DON in silage there is contradictory information. In a study investigating DON stability in wheat and maize silage it was concluded that ensiling induced a strong reduction of DON (17). However, in other studies no effect of ensiling on DON concentration was detected (15, 18). Furthermore, the average and range of DON concentrations in maize silage and unfermented maize products used as feed ingredient are similar (16). The conclusion is that under most conditions DON is stable in silage or may be degraded to a limited extent. Aflatoxin B₁ produced in maize in the field has been found to be degraded slowly in maize silage (19). This observation was confirmed in a recent French study, in which a 3-fold decline of aflatoxin B₁ was detected during nine months storage of maize silage (15). Likewise, partial degradation of

ochratoxin A, a mycotoxin that is associated with small grain cereals, has been observed in ensiled barley (20). No information is available about the fate of fumonisins in silage, but probably these mycotoxins are stable. Contradictory information is available about the fate of ergot alkaloids produced by *Claviceps* and *Neotyphodium* species in silage. Health problems of cattle have been associated with high concentrations of ergovaline in silage from endophyte infected perennial ryegrass (21) and with high concentrations of ergocryptine in silage from maize that was contaminated with a weed containing *Claviceps* ergots in the field (22), indicating that these substances were stable (at least partially) in silage. On the other hand, the concentration of *Claviceps purpurea* ergot alkaloids (ergometrine, ergotamine and ergocryptine) in extensively managed grasslands strongly reduced when the grass was ensiled (23).

The *Penicillium* mycotoxins roquefortin C and mycophenolic acid are stable in silage, whereas PR-toxin and patulin are presumably unstable. In contrast to roquefortin C and mycophenolic acid, PR-toxin and patulin are seldom detected in silage. Experiments with blue-veined cheeses manufactured with *P. roqueforti* strains showed that PR-toxin was degraded and detoxified as a result of a chemical reaction with ammonia and free amino acids (24). Many silage types contains relatively high concentrations of ammonia and free amino acids, so reaction with these compounds may be the reason that PR-toxin is often undetectable. For patulin a similar mechanism may apply. Patulin is known to react with SH-groups of cysteine and other sulphur containing amino acids in protein rich environments and to be inactivated in fermented foods, such as wine, beer and cheese (25, 26).

OCCURRENCE OF MYCOTOXINS IN SILAGE

There is considerable information about the occurrence and concentrations of mycotoxins in commodities and feed ingredients (1, 27, 28). But, despite the fact that silage usually constitutes a major fraction (often more than 50%) of the diet of dairy cattle in many parts of the world, information on the incidence and concentrations of mycotoxins in silages is relatively scarce, in particular for silages other than maize silage. DON, zearalenone, roquefortin C and mycophenolic acid appear to be the most frequently occurring mycotoxins in silage.

Table 2 gives an overview of results from surveys in Europe and the USA for DON and zearalenone in silage, conducted between 1989 and 2007. The data show that the incidence of DON in maize silage was high: in six out of seven surveys the incidence was 72 to 100%. In one survey the incidence was 42%, but the quantification limit of the detection method was relatively high in this study (0.5 mg/kg). The average DON concentration of positive samples in these surveys varied between 0.60 and 1.85 mg/kg. The average of the seven surveys for DON in maize silage is 1.1 mg/kg. The incidence of zearalenone in maize silage was high too, but generally lower than that of DON: in five out of six surveys the incidence was 32 to 59%, in one it was 96%. The average zearalenone concentration of positive samples varied between 0.05 and 0.45 mg/kg, and the overall average of the surveys was 0.17 mg/kg. Worth mentioning is that the average DON and zearalenone concentrations detected in samples of maize collected in a worldwide survey of commodities and feed ingredients between 2003 and 2005 (28) were similar to averages of the maize silage surveys. This is consistent with the view that DON and zearalenone are stable in silage. Information about the occurrence of DON and zearalenone in other silages than maize silage are scarce. In a Dutch survey between 2002 and 2004, DON was not detected in 120 grass silage samples and in 3 of 30 (10%) wheat silages, whereas zearalenone was detected in 7 of the grass silages (6%) and none of the wheat silages (32).

Fumonisin contamination of maize is widespread, as indicated by the high incidence of fumonisins in maize and maize by-products intended for use in animal feed (27, 28). Incidence of fumonisins in maize silage is likely to be high too, since evidence indicating degradation of fumonisins in silage is lacking. This is confirmed by the results of a survey in Midwestern USA in 2001 and 2002, in which fumonisin B₁, fumonisin B₂, and fumonisin B₃ were detected in, respectively, 97%, 72% and 57% of maize silages and average concentrations in positive silages were, respectively, 0.615, 0.093, and 0.051 mg/kg (35). In contrast, in the Dutch survey described earlier fumonisin B₁ and B₂ were detected only in 1.4% of the maize silages (32). This low incidence probably reflects that the environmental conditions of forage maize growth in the Netherlands are not favourable for infection by fumonisin producing moulds (*F. verticillioides*).

The occurrence of ensilage-derived mycotoxins produced by *P. roqueforti* and *P. paneum*, *A. fumigatus* and *Monascus ruber* usually relates to the preservation quality of silage. Growth of these moulds in silage is dependent on infiltration of oxygen during storage or during feeding-out. The predominance of *P. roqueforti* in silage can be explained by its tolerance to high concentrations of carbon dioxide and acetic acid and its ability to grow at low oxygen levels (9). Growth of moulds is usually highest in surface layers, essentially because these layers have the highest exposure to oxygen. Consequently, the distribution of ensilage-derived mycotoxins in silage is highly heterogeneous. The highest levels are occurring in visibly moulded areas and in the typical green-blue lumps of mouldy silage occasionally occurring in maize silages just below the top surface.

The heterogeneity of roquefortin C and mycophenolic acid in maize silage is depicted in Figure 1, which shows the incidence and average concentration of roquefortin C and mycophenolic acid in samples taken from the centre, surface layer and surface areas with visible moulds of maize silage at 16 Dutch dairy farms (33). Incidence and average concentration of both mycotoxins were highest in visibly moulded silage. For example, the average roquefortin C concentration in samples of visibly moulded silage was 16 times higher than that in silage surface samples and 270-fold higher than that in silage centre samples. Similar observations were made in a recent German study, not only with respect to the occurrence of roquefortin C and mycophenolic acid but also with respect to the *M. ruber* mycotoxin monacolin K and a number of *A. fumigatus* mycotoxins, including gliotoxin, verruculogen and fumigaclavin C (13). A selection of the results of this study is shown in Table 3.

Very low incidences of roquefortin C and mycophenolic acid in maize and grass silages were found in a Dutch survey, in which samples were analyzed that were taken relatively shortly after ensiling (3 to 6 weeks) from completely sealed silages that were not yet in use for feeding purposes. Roquefortin C was detected in none of 140 maize silages and in one of 120 grass silages at a low concentration (0.08 mg/kg). Mycophenolic acid was detected neither in maize nor in grass silages (32). These observations emphasize the effect of storage conditions and storage time on the occurrence of this class of mycotoxins in silage. Remarkably, low levels of roquefortin C,

mycophenolic acid and patulin were detected in freshly harvested maize prior to ensiling in a recent study conducted in Pennsylvania, USA (36). This finding indicates that *Penicillium* mycotoxins may also be formed in maize in the field, at least under the environmental conditions of maize growth in Pennsylvania, USA.

In a survey of mycotoxins occurring in the total diet of high-yielding dairy cows conducted in 2005 in the Netherlands DON, zearalenone, roquefortin C and mycophenolic acid were identified as the mycotoxins with the highest incidence (33). As expected, roquefortin C and mycophenolic acid were detected in ensiled feeds only. DON and zearalenone were detected in compound feed, feed commodities and ensiled feeds. Maize silage was found to be the most important source of all of these four mycotoxins in the diet. Maize silage represented on average 30% of the total daily feed intake of the animals, but contributed about 80% of the total dietary intake of DON and zearalenone and more than 95% of that of roquefortin C and mycophenolic acid. The greater contribution of maize silage to the intake of ensilage-derived mycotoxins in comparison with grass silage (which represented about 45% of the daily feed intake) is an indication that problems with aerobic deterioration were more serious in maize silage than in grass silage.

PREVENTION

Regarding prevention strategies, a distinction is made between field-derived and ensilage-derived mycotoxins. Prevention of field-derived mycotoxins focuses on two areas: reduction of the infection pressure of moulds and reduction of the susceptibility of the plant to fungal infections (1). Codex Alimentarius issued codes of practice for the reduction of mycotoxins in cereal crops (37). Recommendations in these codes of practice are summarized in Table 4.

The most important factor prevention strategy for ensilage-derived mycotoxins is to prevent exposure of silage to oxygen. Oxygen has a detrimental effect on silage quality because it enables growth of different groups of acid-tolerant aerobic microorganisms, which eventually is leading to complete deterioration of the silage. This process is usually initiated by yeasts, which oxidize the preservative acids present in silage. As the process

proceeds, temperature and pH rise and bacilli, moulds and other aerobic micro-organisms start to proliferate (9).

At ensiling, air is trapped in the ensiled mass. This oxygen is rapidly consumed (within hours) by respiratory activity of plant material and (facultative) aerobic microorganisms. Once filled, the silo should be sealed as quickly as possible, for instance with sheets of plastic or foil. Where appropriate, additional measures should be taken to prevent damages of the seal by, for instance, birds or rodents. However, since in practice the seal of a silo is never completely airtight, it is inevitable that surface layers will be exposed to air and some air will infiltrate the silage during the storage period. A high packing density of the silage is important because it restricts air infiltration into the silage during storage and after opening of the silo for feeding, when exposure to air becomes inevitable at the silage face. Another factor of importance is the silage removal rate during feeding. Maintaining a high silage removal rate minimizes infiltration of air into the silage behind the face. Finally, when preventive measures have not been successful, visibly moulded silage should be discarded before feeding, since these areas are hot-spots of ensilage-derived mycotoxins, as discussed previously.

METABOLISM OF MYCOTOXINS IN RUMINANTS AND IMPACT ON FOOD SAFETY

The significance of a mycotoxin occurring in feed with respect to animal health and the safety of animal food products for consumers is dependent on its metabolism in the animal, its toxicological effects in man and animals, and its carry-over from feed into milk, meat or organs. After intake via silage or another feed, mycotoxins, like other xenobiotics, follow the typical pharmacokinetic cascade of uptake from the gastrointestinal tract to the blood, internal distribution, metabolism, storage/remobilization and excretion. The rumen has an important function in the metabolism of mycotoxins in ruminants. It contains a complex and dense microflora with a high biodegradative power. Some mycotoxins are rapidly metabolized in the rumen into less toxic metabolites, some are transformed into equally toxic or more toxic metabolites and some are not transformed at all (5, 38). DON and ochratoxin A are examples of mycotoxins that are transformed into less toxic metabolites in the rumen. For that reason cattle are less

sensitive to these mycotoxins than non-ruminant animals such as pigs. Zearalenone is transformed in the rumen into different metabolites, with varying toxic activities. Fumonisin and aflatoxin B₁ are not metabolized in the rumen. Aflatoxin B₁ is transformed into aflatoxin M₁ in the liver of ruminants. Aflatoxin M₁ is less mutagenic and genotoxic than aflatoxin B₁, but the cytotoxicity of aflatoxin M₁ and B₁ is similar. Information concerning the metabolism of *Claviceps* and *Neotyphodium* alkaloid mycotoxins and *A. fumigatus* mycotoxins is lacking. Research on the metabolism of roquefortin C and mycophenolic acid in cattle is currently in progress.

Aflatoxin B₁ is the only mycotoxin with significant carry-over into milk: between 1 and 6 percent is excreted in milk (as aflatoxin M₁). Carry-over rates of DON, zearalenone, fumonisin B₁, ochratoxin A and the alkaloid ergovaline appear to be at least about 100-fold lower (5, 38). Carry-over rates of other mycotoxins frequently occurring in silage are not experimentally assessed. However, there are no indications that significant transfer of these mycotoxins to milk occurs.

CONCLUSIONS

Silage can be contaminated with a wide range of mycotoxins, originating from infection of the crop in the field or from growth of moulds in silage during storage or feeding-out. Prevention of contamination of silage by mycotoxins requires different strategies. Field-derived mycotoxins can be reduced by application of proper agricultural practices in crop production. Ensilage-derived mycotoxins can be reduced by application of adequate silage management, with emphasis on prevention of aerobic spoilage. Relatively little information is available about the effects that the ensilage-derived mycotoxins produced by *Penicillium* species and *A. fumigatus* can have on animal health and productivity. This topic should receive priority in future investigations.

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Table 1. Major mycotoxigenic moulds and mycotoxins in silage crops and silages.

Mycotoxin group	Major toxin(s)	Mould species	Crop(s)	Field- or ensilage-derived
Aflatoxins	Aflatoxin B ₁ (M ₁), B ₂ , G ₁ , G ₂	<i>Aspergillus flavus</i> , <i>A. parasiticus</i>	Maize	Field
Trichothecenes	Type A: T ₂ , diacetoxyscirpenol	<i>Fusarium langsethiae</i> , <i>F. poae</i> , <i>F. sporotrichioides</i>	Maize, Sg cereals ¹	Field
	Type B: DON, nivalenol	<i>F. graminearum</i> , <i>F. culmorum</i>	Maize, Sg cereals, grass	Field
Fumonisin	Fumonisin B ₁ , B ₂	<i>F. verticillioides</i> , <i>F. proliferatum</i>	Maize	Field
Resorcylic acid lactones	Zearalenone	<i>F. graminearum</i> , <i>F. culmorum</i>	Maize, Sg cereals, grass	Field
Ochratoxins	Ochratoxin A	<i>A. ochraceus</i> , <i>Penicillium verrucosum</i>	Sg cereals	Field
Alkaloids	Clavines, lysergic acid amide, ergotamine	<i>Claviceps purpurea</i>	Sg cereals	Field
	Lolitre B, ergovaline	<i>Neotyphodium lolii</i> , <i>N. coenophialum</i>	Grass	Field
<i>P. roqueforti</i> toxins	Roquefortin C, mycophenolic acid	<i>P. roqueforti</i> , <i>P. paneum</i>	All types of silages	Ensilage
<i>A. fumigatus</i> toxins	Gliotoxin, fumigaclavines	<i>A. fumigatus</i>	All types of silages	Ensilage
<i>M. ruber</i> toxins	Monacolin K, citrinin	<i>Monascus ruber</i>	All types of silages	Ensilage

¹ Sg cereals: Small grain cereals (wheat, triticale, rye, barley).

Table 2. Incidence and average and maximum concentrations of the *Fusarium* mycotoxins DON and zearalenone (ZEA) in silage in different surveys.

Mycotoxin	Silage crop	Location	Year(s)	Percentage positive (total number) ¹	Concentration (mg/kg) ²		Reference
					Average (of positive samples)	Maximum	
DON	Maize	North Carolina, USA	1989-1993	76% (106)	1.85	-	29
DON	Maize	Austria	1995-1999	91% (418)	0.75	2.8	30
DON	Maize	Germany	1998	79% (24)	1.61	9.86	16
DON	Maize	Pennsylvania, USA	2001-2002	42% (62)	0.6	3.7	31
DON	Maize	Netherlands	2002-2004	72% (140)	0.85	3.14	32
DON	Maize	Netherlands	2005	100% (16)	0.93	2.39	33
DON	Maize	Denmark	2007	100% (20)	1.06	5.09	34
DON	Wheat	Netherlands	2002-2004	10% (30)	0.62	1.17	32
ZEA	Maize	North Carolina, USA	1989-1993	32% (93)	0.45	-	29
ZEA	Maize	Germany	1993-1995	38% (44)	0.05	0.17	16
ZEA	Maize	Austria	1995-1999	59% (149)	0.07	0.6	30
ZEA	Maize	Germany	1998	96% (24)	0.13	1.07	16
ZEA	Maize	Netherlands	2002-2004	49% (140)	0.17	0.94	32
ZEA	Maize	Netherlands	2005	50% (16)	0.15	0.48	33
ZEA	Grass	Netherlands	2002-2004	6% (120)	0.09	0.31	32
ZEA	Grass	Netherlands	2005	13% (16)	0.13	0.21	33

¹ The percentage of positive samples and total number of samples analysed.

² Concentration in dry matter.

Table 3. Incidence and average and maximum concentrations of the *A. fumigatus* mycotoxins gliotoxin, verruculogen and fumigaclavin C and the *M. ruber* mycotoxin monacolin K in maize silage samples of different quality (normal versus visibly moulded), reported by Richter *et al.* (13).

Mycotoxin	Silage quality	Percentage positive ¹	Concentration (mg/kg)	
			Average (of positive samples)	Maximum
Gliotoxin	Normal	2.5%	0.01	0.01
Gliotoxin	Moulded	13.9%	0.09	0.51
Verruculogen	Normal	5.9%	0.01	0.02
Verruculogen	Moulded	13.9	0.05	0.25
Fumigaclavin C	Normal	3.4%	0.03	0.04
Fumigaclavin C	Moulded	46.3%	0.92	18.8
Monacolin K	Normal	6.8%	0.13	0.45
Monacolin K	Moulded	14.8%	7.32	54.6

¹ The percentage of positive samples; 118 samples of normal quality and 108 visibly moulded samples were analysed.

Table 4. Recommended agricultural practices for the prevention of development of field-derived mycotoxins (37).

Apply crop rotation, to reduce infection pressure

Remove crop residues from field, for instance by deep ploughing, to reduce infection pressure

Use seed varieties developed for resistance to fungal infections

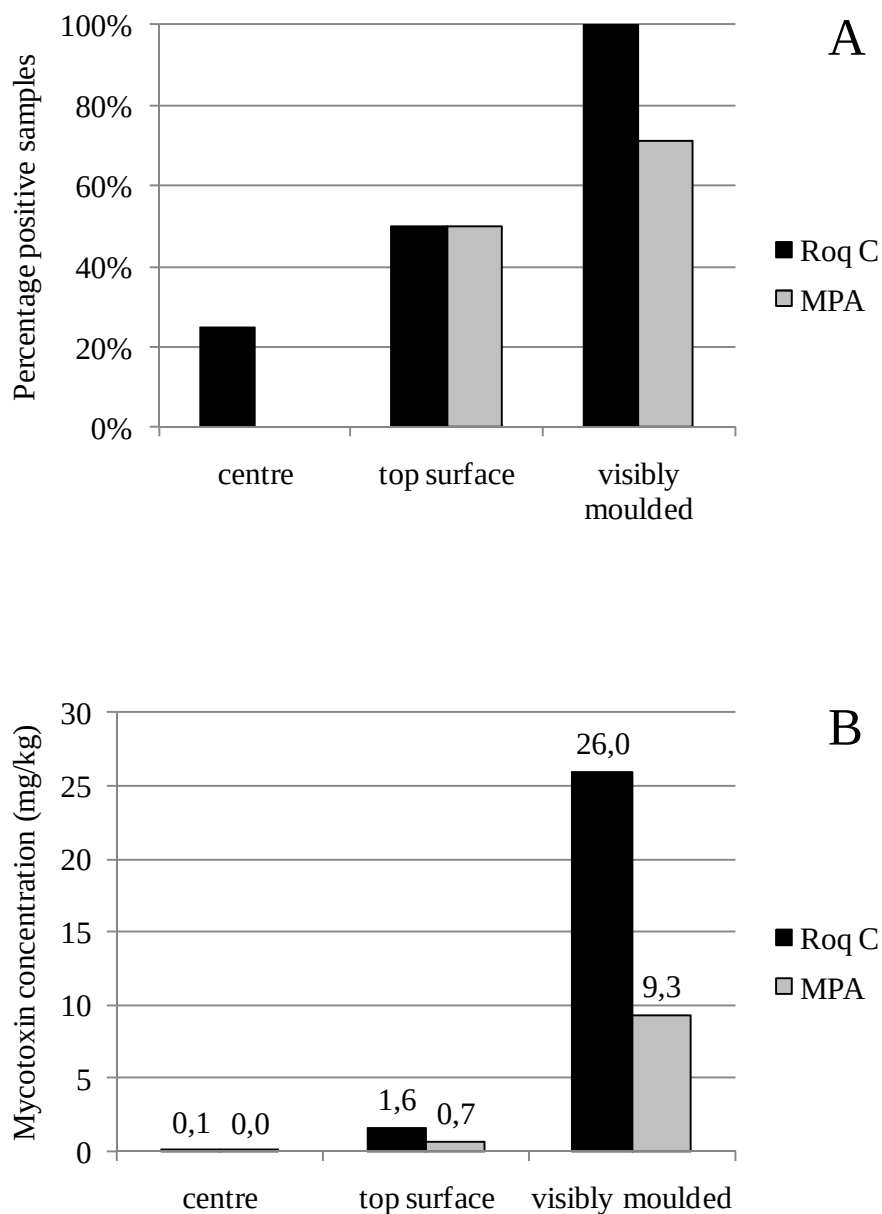
Apply fertilization in conformity to crop demand, to avoid plant stress

Apply good agronomic practices (irrigation, weed control, plant spacing) and avoid plant stress from high temperatures and drought

Apply proper phytosanitary measures on seeds and crops, to avoid insect damage and fungal infections

Minimize mechanical damage, to avoid plant stress and fungal infections

Figure 1. The incidence (graph A) and average concentrations (graph B) of roquefortin C (Roq C) and mycophenolic acid (MPA) in samples taken from the centre, top surface layer and visibly moulded areas of 16 maize silages at dairy farms, reported by Driehuis *et al.* (33).



MYCOTOXINS IN SILAGE

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Introduction

Mycotoxins are secondary metabolites secreted by molds mostly belonging to the *Aspergillus*, *Penicillium* and *Fusarium* genera (Yiannikouris and Jouany, 2002). The ubiquitous nature of mycotoxins and the severity of their effects on human health make them a major food safety concern. The Food and Agriculture Organization (FAO) estimates that 25% of all crops are contaminated with mycotoxins (CAST, 1989). Direct costs of disposal of condemned food and feed ingredients and indirect costs of regulatory enforcement and quality control measures caused by fungal toxin contamination in the US were estimated at approximately \$1.4 billion (CAST, 2003). The social importance of mycotoxins are evident from the 25,000 to 166,000 new causes of liver cancer annually caused by aflatoxins (Liu and Wu, 2010).

Mycotoxins also negatively affect domestic animals causing suppression of the immune system, imbalance of hormonal function, and reduction of nutrient utilization, which result in decreased performance and eventually death (Whitlow and Hagler, 2005). For the farm system, the costs associated with feeding animals contaminated diets occur in the form of poor animal performance and milk disposal due to high concentration of toxins (Applebaum et al., 1982; Masoero et al., 2007; Kutz et al., 2009). Milk with aflatoxin concentrations above 0.05 µg/kg are considered illegal to be marketed in USA, Brazil, Argentina, and countries belonging to the southern common market while on Europe the action level is 0.05 µg/kg (FDA, 2000; ANVISA, 2002; EFSA, 2004a).

Silage making is based on the principle of forage conservation under anaerobic conditions. Epiphytic or inoculated lactic acid bacteria naturally ferment carbohydrates in the forage and produce acids, which reduce the pH and consequently reduce the growth of undesirable microorganisms (Garon et al., 2006). Anaerobiosis and low pH inhibit the growth of most of fungi, thus ensiling is an effective strategy to prevent the growth of and mycotoxin production from many field and storage fungi. However, the conditions that prevent the growth of the majority of fungi in silage also provide a more conducive environment for the growth of acid and low oxygen-tolerant species, such as *Penicillium roqueforti*, *Aspergillus*

fumigatus, *Byssoschlamys nivea*, and various *Fusarium* species (Pahlow et al., 2003). Gonzales-Pereyra et al. (2008) also noted that inadequate management of silage can impair fermentation, promote aerobic conditions within the silage mass, and favor the growth of fungi that are normally less tolerant of acidic or anaerobic conditions such as *Aspergillus flavus*. Furthermore, once silos are opened, the ensuing aerobic conditions often allow the growth of spoilage-causing yeasts, which metabolize lactate to CO₂ and thereby increase the silage pH. This elevated pH predisposes toxigenic fungi to grow actively, particularly in poorly managed silages during the feed-out phase.

Mycotoxin contamination is a ubiquitous problem due to the high adaptive capacity of toxigenic fungi, which allows them to proliferate in all stages of feed production such as on the field and at harvest, storage, and feed-out. In spite of their adaptive capacity, various techniques can be used to prevent or at least minimize contamination of feeds with mycotoxins and effective detoxification methods are also available. This review intends to briefly discuss some of the mycotoxins frequently found in silages and to discuss strategies for preventing mycotoxin contamination or decontaminating mycotoxin-infested feed ingredients.

Mycotoxins

Mycotoxins cause several undesirable effects in humans and animals. More than 400 mycotoxins are known to occur naturally, however, only a few of them have been extensively studied (Whitlow and Hagler, 2005). Mycotoxins that are frequently present in silages include deoxynivalenol (DON), zearalenone (ZEA), fumonisin, roquefortine C. Aflatoxin can also be present in silages made in hot and humid environments.

Deoxynivalenol

Deoxynivalenol (DON) is a toxin produced by *Fusarium* species (*F. graminearum*, *F. sporotrichioides*, *F. culmorum*, *F. poae*, *F. roseum*, *F. tricinctum*) it is also known as vomitoxin because it tends to cause emesis in swine. Other than vomiting, DON causes feed refusal, diarrhea, reproductive problems, and eventually death in non-ruminant animals. The effect of DON in dairy cattle is not well established but decreases in animal performance in dairy herds have been associated with the toxin (Whitlow et al., 1994). Deoxynivalenol has also been related to altered rumen fermentation (Seeling et al., 2006) and reduced flow of protein to the duodenum (Danicke et al., 2005). In a survey on the presence of mycotoxins in

feed ingredients, Driehuis et al. (2008) observed that corn silage was the main source of DON and zearalenone (ZEA) in diets of dairy cattle. These authors reported an average concentration of DON in corn and grass silage samples of 550 µg/kg and a maximum concentration of 1,250 µg/kg. They estimated that these high DON concentrations could result in intakes of 8.4 µg of DON /kg of BW or 5 mg per cow/day. After reviewing several studies, DiCostanzo et al., (1995) concluded that beef cattle are able to tolerate up to 21 mg/kg of DON. Charmley et al. (1993) demonstrated that a contamination level of 6 mg of DON /kg of diet DM did adversely affect on milk yield or cause carry-over of the toxin into milk. Data available on adverse effects of DON on dairy cattle is limited, and insufficient to allow the establishment of a maximum tolerance level, however the guidance value is 5mg/kg (European commission, 2006, Driehuis et al., 2008). The Food and Drug Administration (FDA) advisory guideline for DON are 10 and 5 mg/kg DM of diet for beef and dairy cattle respectively (FDA, 2010). The concern about the intake of DON by dairy cattle is related to its' potential negative effect on animal health and production, however due to the low transfer of this toxin to milk, DON is not considered a contaminant that reduces the safety of dairy products (EFSA, 2004b).

Fumonisin

Fumonisin are mainly produced by two species of *Fusarium*, *F. verticilloides* (formely *F. moniliforme*) and *F. proliferatum* (Whitlow and Hagler, 2005). The structural formula of fumonisin is similar to sphingosine, which is a component of sphingolipids that abound in nerve tissue. The toxicity of this mycotoxin results from interruption of sphingolipid biosynthesis, therefore it causes paralysis, nervousness, and ataxia in horses, and pulmonary edema in swine (Marasas et al., 1988; Ross et al., 1990; Diaz and Borermaans, 1994). The toxin tends to be less aggressive in ruminants than monogastrics, however fumonisin has been shown to be hepatotoxic and nephrotoxic to calves receiving 1 mg/kg body weight of the toxin (Mathur et al., 2001). The latter authors reported hepatocellular apoptosis and renal tubular necrosis within 7 days of dosing the toxin. Similar results were found in a study with beef calves supplemented with 148 mg/kg of total fumonisin in the diet for 31 days (Osweiler et al., 1993). Diaz et al. (2000) demonstrated that dosing of 100 mg/kg of fumosin reduced feed intake and milk yield in dairy cattle. Nevertheless, carry-over of the toxin into milk is minimal (Scott et al., 1994).

Gonzalez-Pereyra et al. (2008) reported that fumonisin levels in corn silage varied from 340 to 2490 µg/kg DM. These authors reported that samples from different locations in the silo had different concentrations of the toxin and those from the top layer and side walls had high pH values, which could favor development of the toxin.

Fusarium species tolerate low winter temperatures and colonize crop residues such as maize stalks and rice and wheat stubble, and these become major sources of inocula as temperatures increase in early spring (Binder, 2010). Fungal spores also become airborne during rainy season and can travel long distances causing mycotoxin contamination epidemics (Binder, 2010; Lanier et al., 2010). The Food and Drug Administration (FDA) advisory guideline for fumonisin is 15 mg/kg DM of diet for lactating dairy cows (FDA, 2001).

Zearalenone

Zearalenone is an estrogenic metabolite produced by several species of *Fusarium* such as *F. graminearum*, *F. culmorum*, *F. crookwellense* (Saeger et al., 2003). Despite of the structural dissimilarity between zearalenone and steroidal estrogens, ZEA can cause numerous reproductive problems including, hyperestrogenism, mammary gland enlargement, and vaginitis (Diekman and Green, 1992). Ruminants are less susceptible than pigs or chickens due to conversion of ZEA to its' hydroxyl-metabolites, α alpha and β zearalenol, by ruminal flora (Kiessling et al., 1984; Kennedy et al., 1998). Although α -zearalenol is three to four times more estrogenic than ZEA, its lower rate of absorption and interconversion to β zearalenol in the liver helps to decrease negative effects of ZEA (Fink-Gremmels, 2008). Despite their lower susceptibility to this toxin, studies show that high levels of the toxin may negatively affect dairy cattle. For instance, conception rates were decreased by 25% in dairy heifers receiving 250 mg of ZEA (Weaver et al., 1986).

Whitlow and Hagler (2005) reported a 30% incidence of ZEA contamination in 461 corn silage samples and the average contamination level was 525 µg/kg DM. Driehuis et al. (2008) reported ZEA contamination in 13 and 50% of grass and corn silage samples with average concentrations of 180 and 146 µg/kg respectively. Reed and Moore (2009) reported that sorghum silage contained 660 µg/kg of ZEA and concentration up to 79.80 mg/kg in *Medicago sativa*. The main concerns with this toxin focus on its' negative effect on animal health and reproduction because like fumonisin the level of transfer into milk is considered negligible (Seeling et al., 2005). There are no zearalenone action limits, guidance, or advisory

levels established by FDA at this time. The guidance value for zearalenone in Europe is 500 µg/kg (European commission, 2006).

Aflatoxin

Aflatoxin is a mutagenic, carcinogenic, and toxic secondary metabolite produced *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (Creppy, 2002). Due to the ability of *Aspergillus* to grow over a broad range of temperatures and humidities, aflatoxin is a ubiquitous contaminant of food and feed ingredients worldwide (Phillips, 1999). Feeding aflatoxin -contaminated diets to lactating cows reduces their health and performance and also causes transfer of the toxin to milk and dairy products (Diaz et al., 2004).

The symptoms associated with aflatoxin ingestion or inhalation include: inappetence, lethargy, ataxia, enlargement of the liver, decreased rumen motility, and reduced feed efficiency and milk production (Whitlow and Hagler, 2005; Guthrie and Bedell, 1979; Mathur et al., 1975). Aflatoxin also reduces the immune response to opportunistic diseases and interferes with the efficiency of vaccines (Diekman and Green, 1992; Marin et al., 2002). However, these symptoms are not specific to aflatoxicosis, which makes precise diagnosis of the condition difficult (Columbre, 1993). Garret et al. (1968) demonstrated increased liver weight in beef cattle fed a diet containing 100 µg/kg of aflatoxin. Similar concentrations also cause decrease in animal production and compromise animal health (Patterson and Anderson, 1982). Applebaum et al. (1982) showed that high-producing animals are more sensitive to impure aflatoxin contaminated diet than diets contaminated with pure aflatoxin B₁. Queiroz et al. (2010) observed increase concentrations of plasma adhesion molecules and haptoglobin, an acute phase protein when dairy cattle were fed an aflatoxin-contaminated diet.

The incidence of aflatoxin is relatively low in silages compared to other mycotoxins, probably due to low tolerance of *Aspergillus flavus* and *A. parasiticus* to the acidic and anaerobic silage environment. However, data in literature report high levels of aflatoxin in poorly made or managed silages or in silages made with diseased corn plants (Queiroz et al., 2009). Gonzalez-Pereyra et al. (2008) reported an aflatoxin B₁ (AFB₁) concentration of 156 µg/kg DM in corn silage stored in a trench type silo without proper sealing. Richard et al. (2009) observed aflatoxin concentrations of up to 60 µg/kg DM in corn silage.

The rate of aflatoxin transfer to the milk varies from 1 to 6% (EFSAa, 2004). Due to the severity of aflatoxin effects on human health, it is the only one of the 400 known mycotoxins that has an action level regulated by governmental agencies. The aflatoxin Action

Level established by the FDA for fluid milk is 0.5 µg/kg and it is 20 µg/kg for feed ingredients offered to dairy cattle. Therefore, their high aflatoxin concentrations would render the corn silages mentioned above unsafe for feeding to dairy cattle.

Factors Affecting the Extent of Mycotoxin Contamination

Mycotoxins are produced through different pathways involved in secondary metabolism by fungi (Steyn, 1998). Pathways involved in secondary metabolism are not essential for cell growth in microorganisms and are not the main synthetic or catabolic pathways. Rather, secondary metabolism processes that result in mycotoxin contamination are caused by interaction and response of fungi to environmental conditions (Yiannikouris and Jouany, 2002). Thus, the extent of feed contamination by mycotoxins is related to environmental stresses and physicochemical parameters such as availability of free-water, prevailing temperature, oxygen, and pH and availability of substrate (Nelson, 1993). Common environmental problems associated with mycotoxin contamination of feed ingredients are: insect or animal damage, plant stress caused by extreme weather conditions such as hail and the attendant lodging, or by contamination with opportunistic fungal diseases (Queiroz et al., 2010). Mycotoxin contamination of silage is frequently associated with inadequate silage management practices.

Preventing Mycotoxin Contamination of Feeds

Preventing mycotoxin contamination is a complex task because many interplaying factors contribute to establishing and predisposing fungi to produce mycotoxins. Risk-management concepts can help to identify control points within the feed production chain that can be monitored to minimize mycotoxin contamination in feed ingredients. As mentioned earlier, infestation by toxigenic fungi can occur in the field, during storage of crop products or when feed is offered to animals. The following section outlines the different phases of feed production and corresponding strategies to prevent mycotoxin contamination.

Field or Pre-harvest stage

Prevention of mycotoxin contamination on the field is desirable, but not always achievable. Agronomic practices to minimize environmental stress and maximize plant performance can significantly decrease mycotoxin contamination (Binder, 2010). Some of

such practices include the use of varieties or hybrids that are adapted to the growing area and resistant to fungal diseases, control of weeds, application of fungicides and pesticides, use of irrigation, timely crop rotation, proper fertilization (Edwards, 2004; Whitlow and Hagler, 2005). Despite their theoretical potential, Duncan et al. (1994) noted that fungicides have shown little efficacy at preventing the growth of *A. flavus* and thereby preventing aflatoxin contamination. It is important to note that no matter how good the management at the field is, no technique completely eliminates the possibility of mycotoxin contamination of crops on the field.

Harvest phase

Crop and forage harvests should be carefully timed to coincide with the recommended maturity for optimizing yield, DM concentration, and nutritive value for each crop. Excess moisture in harvested crops and forages can predispose to spoilage of grains and prevent efficient silage fermentation.

Harvesting equipment should be properly maintained and calibrated to avoid lodging of or physical damage to crops at harvest because contact with soil and kernel damage can predispose to fungal growth and mycotoxin production (Whitlow and Hagler, 2005). Immediate storage of harvested feeds in appropriate storage facilities is also necessary to minimize the risk of mycotoxin contamination.

Post-harvest stage

Proper storage of feeds is critical to prevent the development of mycotoxins. Special attention should be paid to the moisture concentration of stored feeds. Moisture levels should be kept under 15% for dried feeds and artificial or natural drying should be employed where necessary. Drying feeds to moisture contents below 13% compensates for uneven moisture concentrations throughout the feed or forage mass (Whitlow and Hagler, 2005).

When poor storage conditions allow fungal growth, it is not uncommon to detect infestation with more than one species because during storage, grains are colonized by a succession of fungi, which result in contamination with different toxins (Binder, 2010). Other factors that should be considered to prevent mycotoxin contamination during storage are appropriate temperature and adequate aeration (Shapira and Paster, 2004). High temperatures increase the water activity leading to mold growth, while inadequate aeration of grains allows moisture accumulation, which increases the chances for spreading of fungi. Special attentions

should be paid to the physical structure of silo to avoid rehydration of the grains by rain or water sources and to prevent damage caused by insects or rodents. As mentioned above, damaged grains favor mycotoxin development thus mechanical separation of broken kernels is recommended.

Strict adherence to excellent silage management principles and practices at all stages of silage making and feeding are essential to prevent mycotoxin contamination of silages. Such principles and practices include the following: Choosing disease resistant hybrids, ensuring that appropriate agronomic practices are employed to minimize lodging, disease infestation or stressing of crops, harvesting at the appropriate maturity stage that optimizes nutritive value, yield and DM concentrations, chopping forages to lengths that facilitate compaction without reducing the effective fiber concentration, filling silos rapidly, packing or consolidating silage to achieve a density of $\geq 200 \text{ kg/m}^3$ to reduce porosity and prevent air pockets in the silage, proper management of the silo face and feeding the silage at a rate that minimizes spoilage during feed-out.

The low pH and anaerobic conditions in silage inhibit the growth of most spoilage and toxigenic fungi (Pahlow et al., 2003). Bacterial inoculants and chemical additives have been successfully used to enhance the acidity by promoting a rapid drop in pH at ensiling. Inoculants and additives are also used to increase aerobic stability, and reduce the spoilage fungal population in silages (Huisden et al., 2009; Mari et al., 2009; Pedroso et al., 2010). However, few trials have evaluated if such additives and inoculants can prevent or reduce mycotoxin contamination of silage. Queiroz et al. (2009) reported when corn plants infested with Southern rust disease were ensiled, that aflatoxin concentration was 200 times above the FDA Action Level. However, inoculation of the diseased plants with an inoculant containing *L. buchneri* and *P. Pediococcus* resulted on silages with no aflatoxin. *Lactobacillus buchneri* is a heterofermentative bacteria, which can convert lactic acid to acetic acid and 1,2-propanediol (Oude Elferink et al., 2001). Acids such as acetate and propionic acid have proven antifungal effects (Moon, 1983) and as such, they are added to silage directly or indirectly through the use of bacterial inoculants to inhibit the growth of yeasts and molds (Kung et al., 2003). Numerous studies demonstrating the antifungal effects of acids, mold inhibitors and inoculants can be found on the literature, however mold inhibitors and inoculants often have no effect on mycotoxins that have been already synthesized (Binder, 2010).

Another strategy to reduce the growth of toxigenic fungi in silage involves limiting oxygen ingress into silos. Amaral et al. (2010) evaluated the efficacy of using traditional

plastic or a film that limits oxygen infiltration for covering silos. They reported that unlike traditional plastic, the new oxygen barrier film inhibited synthesis of aflatoxin in corn silage in Brazil. This experiment emphasizes the importance of maintaining anaerobic conditions during and after ensiling to avoid mycotoxin contamination of silage.

Mycotoxin Detoxification Methods:

Unquestionably, the feed industry and feed producers should target prevention of mold growth to prevent mycotoxin contamination of feeds. Despite the existence of several notable advances in pre and post-harvest fungal inhibition technologies, feed contamination with mycotoxins is still very common.

Silage traditionally represents a significant proportion of the diet of dairy cows and its' year-round, widespread use in some countries and seasonal use at times of low pasture availability in others, emphasize the importance of ensuring that mycotoxin contamination of silage is minimized. Yet no method effectively or completely detoxifies silages contaminated with mycotoxins. However, numerous products that improve aerobic stability via their antimycotic action may indirectly decrease the risk or level of mycotoxin contamination of silage.

Dilution of contaminated ingredients is an effective decontamination strategy for contaminated grains. However, it is less appropriate for contaminated silage because it would probably reduce the feedout rate and may necessitate partial utilization of the silage on the silo face. These factors would lead to mold growth and intensification of the contamination problem (Whitlow and Hagler, 2005). The other post-harvest methods for detoxifying contaminated feed ingredients can be classified into the following groups:

Biological methods

Microorganisms can reduce mycotoxin production by competition for nutrients and space with toxigenic molds (Bhatnagar et al., 1994). Nontoxigenic strains of *A. flavus* and *A. parasiticus* have been successfully used to compete with wild toxigenic strains and decrease aflatoxin contamination in cottonseed (Cole and Cotty, 1990). Microorganisms also can be used to metabolize and reduce mycotoxins within the environment. Kiessling et al. (1984) reported that ruminal microbes can metabolize ochratoxin A, zearalenone, T-2 toxin, and diacetoxyscirpenol but had no effect on aflatoxin and deoxynivalenol. Binder et al., 2000)

were the first to isolate a pure strain of Eubacterium, referred as BBSH 797 that is able to transform the epoxy group of DON.

Physical Methods

Manual removal of contaminated grains is the simplest method of physical removal of mycotoxins, but it is extremely time consuming and in many cases, impractical. Automated physical removal of moldy, damaged, or inadequately developed kernels and grains is one of the widely used decontamination techniques. Partial removal of mycotoxins is also achieved by cleaning the grain or milling, however this can lead to increased toxin levels in the bran though levels in the flour are decreased (Bata and Lastity, 1999). During milling or dehulling, the external layers of grains, which are more susceptible to fungal action are removed (Siwela et al., 2005). This process results in a toxin concentrated dust by-product (Sorenson et al., 1981), and requires a significant input of energy, which in turn, increases costs and makes the method less suited for on-farm use. Also, because mycotoxins diffuse away from the mycelia region, physical segregation of moldy material does not guarantee efficient removal of aflatoxin (Park et al., 1981).

Mycotoxins can be relatively heat stable; therefore thermal detoxification produces variable results. Rater and Matissek (2008) noted that aflatoxin B₁ inactivation by thermal treatment was affected by the type of contaminated matrix, time of heat exposure, and heat intensity. The authors reported that after 30 min under 150 °C, 100% of pure AFB₁ had been degraded, however, when the toxin was mixed with carbohydrates, the inactivation efficacy was only 50%. The same authors reported that increasing the temperature to 180 °C caused a 70% reduction in AFB₁ concentration when the toxin was mixed with carbohydrates during the same time of exposure.

Irradiation is a physical method that reduces infestation by undesirable *Aspergillus* species and decreases contamination with AFB₁. Irradiation of contaminated feed with ultraviolet light, microwaves, or gamma rays have decreased aflatoxin contamination (Yousef, 1986). Corn grain samples contaminated with AFB₁ and AFB₂ were totally decontaminated after exposure to 10 kGV of gamma radiation (Aquino et al., 2005). Ultraviolet light is unquestionably the cheapest method of irradiation. Reduction of AFB₁ and total aflatoxin varied from 60.5 to 75% when contaminated feed samples were subjected to an extensive period (30 h) of sunlight exposure (Herzallah et al., 2008). However most irradiation methods are unaffordable to producers and some are inaccessible. The sunlight

treatment is cheap but only effective to treat grains on the surface of a batch. Therefore only a small amount of feed can be treated at a time. This and the long exposure period make this method impracticable for large quantities of feed. In fact, none of these physical methods is practicable or effective for decontaminating silage on dairy farms due to the large quantities involved.

Chemical methods

About 100 compounds are known to inhibit to some extent aflatoxin production (Zaika and Buchanan, 1987). Most of them work by inhibiting the growth of fungi, inhibiting aflatoxin synthesis, or both. Dichlorvos is an organophosphate insecticide which inhibits aflatoxin synthesis but not fungal growth (Buchanan et al., 1983). Caffeine is also capable of decreasing aflatoxin biosynthesis and it negatively affects mold growth (Buchanan et al., 1983). Rodriguez and Mahoney (1994) reported that non ionic surfactants such as polyoxyethylene 10 lauryl ether reduced the synthesis of aflatoxin from *A. flavus*. Practical use of these chemicals on farms may be restricted due to their costs and harmfulness to human health.

Ammoniation is one of the most widely used methods of decontamination of toxins in commodities and it is accepted as a decontamination practice in many countries including Brazil (CAST, 2003). Ammonia can modify the molecular structure of aflatoxin and decrease aflatoxin concentration in feed ingredients by about 99% (Hoogenboom et al. 2001). The adoption of this technique on farms is highly dependent on training and investment due to the risks associated with this procedure. Ammoniation of forages, though effective and feasible for forage conservation, is not as effective at decontaminating mycotoxins as ammoniation of grains under high pressure and temperature. Also, under high temperatures, ammonia reacts with sugars in forages to form 4-methylimidazole, which causes hyperexcitability in cattle (Kerr et al., 1987).

Ozonation is a very effective method to detoxify contaminated feed. Reduction of 92% of corn contamination with aflatoxin was reported when samples were treated with ozone gas (12% of total weight) for 96 h (Prudente and King, 2002). Total degradation of aflatoxin in corn samples was reported when 20% of ozone was applied (Mckenzie, 1997). The same authors noted that AFB₂ and AFG₂ were more resistant to degradation than the AFB₁ and AFG₁ forms, which are rapidly degradable when ozone is applied at 2% of the sample weight. Ozonation destroys the structure of AFB₁ by interacting with a double bond of the

furane ring in the toxin molecule (Puzyr et al., 2010). This procedure has also been used to destroy DON, moniliformin, cyclopiazonic acid, ochratoxin A, patulin, secalonin acid D, and zearalenone (Young et al. 1986; Zhang and Li, 1994; McKenzie et al. 1997). Despite the advantages of ozonation over most other chemical treatments, drawbacks include loss of nutritive compounds and potential formation of fat-soluble reaction products with some mutagenic potential (Prudent and King, 2002).

Most of the decontamination strategies described above are mostly unsuitable for silages and other feeds on dairies due to the large quantities that would be treated.

Enterosorbents

Enterosorbents are substances that can bind to toxins in the gastrointestinal tract of animals, reducing their bioavailability and associated toxicities (Philips, 1999). They represent an alternative method to avoid the inaccessibility or high cost of physical methods and the hazards of chemical methods. Hydrated sodium calcium aluminosilicates (HSCAS) are clay-based products that form a stable complex with AFB₁, which cannot cross the luminal membrane in the gastrointestinal tract (Spotti et al., 2005). In vitro evaluation of the binding capacity of HSCAS demonstrated that 80 to 99 % of aflatoxin present in various solutions (buffer, water, or rumen fluid) was bound to the clay (Moschini et al., 2008). In vivo trials with HSCAS have shown less complete, but nevertheless useful levels of detoxification. Diaz et al. (2004) evaluated different sequestering agents and reported a wide range of efficacy at reducing AFB₁ in milk (31 to 65%) when a diet containing 100 µg/kg of AFB₁ was fed to lactating dairy cows. Kutz et al. (2009) reported 44 to 48 % of toxin reduction in milk when mycotoxin binders were used in the diet. These results emphasize the importance of carefully selecting and screening potential sequestering agents before evaluating them in animals under practical farming conditions. Satisfactory decontamination has been achieved with the use of inorganic sequestering agents such as HSCAS but these have discriminating affinity to aflatoxin, having no effect on other toxins. For instance, the inclusion of HSCAS in diets has not changed the estrogenic effect of zearalenone (Bursian et al., 1992). When used at 0.5 or 1% of the diet, HSCAS did not mitigate the negative effects of DON on the daily gain of nonruminant animals.

Other types of enterosorbents such as activated carbon, glucomannan, and peptidoglycans have also been evaluated. Diaz et al. (2004) studied the effect of bentonites, esterified glucomannan (yeast cell wall), and activated carbon as sequestering agents to

reduce AFB₁ absorption and transfer to milk. The authors reported that esterified glucomannan fed at 0.05% of diet DM was similarly effective as sodium bentonites fed at 1.2%. Both products decreased milk aflatoxin concentration by 58.5% and 64.6%, respectively. In the same experiment activated carbon showed no significant reduction in aflatoxin M₁. The results of using these binders are fairly dependent on the extent of aflatoxin contamination in the diet. For instance, the same esterified glucomannan product that reduced milk AFM₁ concentration when dietary AFB₁ concentration was 55 µg/kg of diet DM (Diaz et al., 2004) had no effect on milk aflatoxin when dietary concentration of AFB₁ was 100 µg/kg of diet (Kutz et al., 2009). Cholestyramine, an insoluble quaternary ammonium anion exchange resin was used (2.5g/kg) to decrease the toxic effect of 6 mg/kg of zearalenone in mice (Underhill et al. 1995). Avantaggiato et al. (2005) observed that cholestyramine effectively binds to zearalenone and fumonisins. The authors also reported that activated carbon was the only absorbent, out of 21 products, capable of binding deoxynivalenol and nivalenol.

Conclusions

Mycotoxins are a major food safety concern due to their effects on animal and human health and their ubiquitous presence in food and feed ingredients. Despite of all the scientific efforts towards development of techniques to prevent mycotoxin contamination, none of the existing techniques is foolproof. Most of the detoxification techniques focus on elimination of mycotoxins present in grains. Special attention needs to be taken when considering mycotoxin contamination in silages because there are no effective detoxification methods for silage. Fortunately, promising contamination prevention strategies have been obtained with fermentation aids, including acids and inoculants, better silo covering films, and the use of enterosorbents. These techniques do not eliminate the problem, but can ameliorate it by inhibiting the growth of toxigenic fungi or by reducing absorption of the toxin by the animal.

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Aerobic stability and instability of silages caused by bacteria

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Introduction

The ensiling process is often divided into 3 phases: aerobic, fermentation, and aerobic deterioration phases. The fermentation phase is sometimes divided into 2 phases: fermentation and storage. Each phase has a considerable impact on the quality of silage fed to animals, and diverse microorganisms can be successively activated and deactivated. Occurrence of and changes in the microbial population depend mainly on crop composition and silo management, i.e., crop species, dry matter (DM) content, water-soluble carbohydrate (WSC) content, buffering capacity, degree of anaerobiosis, and contamination of soil and manure. The microorganisms identified predominantly in silages are facultative anaerobes; hence, many bacteria and yeasts have the potential to participate in all phases of the ensiling process.

If good anaerobiosis is achieved by prompt sealing, air trapped in the forage is rapidly consumed by plant respiration and aerobic microorganisms. This may terminate the aerobic phase within a few hours and initiate the fermentation phase, during which facultative and obligate anaerobes compete for substrate (Driehuis and Oude Elferink, 2000). The fermentation phase may continue for several weeks to a year. Lactic acid bacteria (LAB) are the most important microorganisms in this phase, because if they can overwhelm other bacteria, acidification would rapidly and loss of nutrients would be minimized. In the initial stage of the fermentation phase, however, large numbers of enterobacteria occasionally proliferate and produce large amounts of ethanol and 2,3-butanediol (McDonald et al., 1991). Anaerobic yeast growth could also occur vigorously in some cases, resulting in fermentation of sugars to ethanol and CO₂. These alcohols do not contribute to acidification and preservation, and thus indirectly impair silage stability during the

subsequent aerobic deterioration phase. Growth of *Clostridium* spp. during the late stage of fermentation is a concern, but the growth will not be intensified if acidification is sufficient and anaerobiosis is secured. With respect to aerobic stability, silages with high butyric acid content resist deterioration.

In the aerobic deterioration phase, air penetrates into a hitherto anaerobic environment; aerobic and facultative anaerobic microorganisms gain another opportunity to proliferate. Yeasts, molds, and acetic acid bacteria are mainly associated with aerobic deterioration, whereas *Bacillus* spp., LAB, and *Enterobacteria* may also abound in the aerobic deterioration phase (Merry and Davies, 1999). Several studies have shown that *Clostridium* spp. (obligate anaerobes) grow in air-exposed silages (Jonsson, 1991; Vissers et al., 2007).

Under aerobic conditions, yeasts can oxidize lactic acid and raise silage pH, which in turn facilitates the growth of other microorganisms. Therefore, yeasts are considered to be primarily responsible for the initiation of aerobic spoilage, and a population of 10^5 cfu/g at silo opening is regarded as a critical level, above which silages may deteriorate in the presence of air (McDonald et al., 1991). The roles of bacteria in initiating aerobic deterioration would not be greater than the roles of yeasts. Among acetic acid bacteria, *Bacillus* spp., *Enterobacteria*, and *Clostridium* spp., only acetic acid bacteria have been shown to initiate spoilage in the absence of simultaneous or previous yeast growth (Woolford and Wilkie, 1984). Therefore, acetic acid bacteria have been considered the most important targets for spoilage control, with the others being follow-up or opportunistic bacteria that may grow after increase in pH and acid oxidation. However, acetic acid bacteria are detectable almost exclusively in corn silages (Oude Elferink et al., 2001). This is in contrast to the fact that production of ethanol, the major substrate for acetic acid bacteria, is often minimal in corn silages compared to low-moisture grass silages. Furthermore, Kan and Nishino (2011) have shown that inoculation of *Acetobacter pasteurianus*, isolated from bunker-made untreated corn silage, does not enhance aerobic spoilage of corn silage, irrespective of the time of inoculation, i.e., either before ensiling or after silo opening. It is reasonable to state that obligate aerobic bacteria may initiate

aerobic spoilage by metabolizing fermentation products. However, the role of acetic acid bacteria is not conclusive and awaits further investigation.

For the suppression of microorganisms, undissociated acid is of primary importance, and dissociated molecules have minor effects (Courtin and Spoelstra, 1990; Muck and Pitt, 1991). Undissociated acid can passively diffuse into microbial cells through the cell membrane and split into anions and protons inside the cell, depending on the internal pH, thereby acidifying the cytoplasm and disrupting proton-motive force, as well as inhibiting substrate transport, energy-yielding processes, and macromolecular synthesis (Ostling and Lindgren, 1993). The pH has a marked influence on the proportion of acids present in undissociated or dissociated forms (Table 1); hence, the inhibitory activities of lactic, acetic, and propionic acids largely depend on pH (Matsuda et al., 1994). Lactic acid exhibits a strong antibacterial activity under acidic conditions, such as the antibacterial activities in well-preserved silages (pH 4.0); under insufficiently acidic conditions (pH 5.0 and 6.0), the activity of lactic acid against non-LAB is lower than the activity of acetic and propionic acids. Inhibition of yeasts and molds by lactic acid, however, hardly occurs at any pH; concentration of lactic acid at 50 g/L is equivalent to 115 g/kg DM in silages with a DM content of 300 g/kg. The minimum inhibitory concentrations of acetic acid and propionic acid against yeasts and molds at pH 4.0 are 5 g/L and 2.5 g/L, respectively, indicating that the antifungal activity of acetic and propionic acids is more than 10 and 20 times greater, respectively, than that of lactic acid. The antifungal activity is markedly reduced if the surrounding pH increases to 5.0 and 6.0, but acetic and propionic acids are yet better for fungal suppression than lactic acid is. During the actual ensiling process, lactic, acetic, and propionic acids exhibit synergistic action against yeasts (Moon et al., 1983). However, lactic acid may contribute minimally to the antifungal effects of fermentation products. Many yeast species such as *Pichia* and *Candida* spp. can assimilate lactic acid under aerobic conditions. This is yet another reason that yeasts are primarily responsible for the initiation of aerobic spoilage.

Silages considerably contribute to ruminant feeding in temperate and cold regions

(Wilkinson, 1999). In Japan, silages account for more than 70% of conserved forages (Ohmomo et al., 2002). Owing to the expansion of big bale machinery, preparation of low-moisture silages with long cut lengths, restricted fermentation, and insufficient acidity (pH >5.0) has increased. This may have raised the risk of contamination with undesirable *Enterobacteria*, *Bacillus* spp., yeasts, and molds, leading to lowered stability in both the fermentation and aerobic deterioration phases. In large-scale farming, by contrast, silage is more often made in bunker silos, in which a large area of the silo face is inevitably exposed to air. Management of aerobic deterioration is now considered critical and must be researched further.

Prediction of the onset of silage deterioration and the types of microorganisms that may initiate and carry out the spoilage on the basis of silo opening analysis is yet impossible. In this regard, a high concentration of acetic acid is useful against bacteria and fungi, and *Lactobacillus buchneri* has been considered a promising agent. However, alternatives to the heterofermentative LAB are necessary, because excessive acetic acid production remains a concern. Improvements, especially in the area of novel bacterial inoculant preparation, are necessary. A goal is to discover homofermentative or facultatively heterofermentative LAB with antifungal activity, and sources from which this target can be achieved are available.

Table 1. Minimum inhibitory concentrations (g/L) at different pH of lactic, acetic, and propionic acids against bacteria and fungi (Matsuda et al., 1994)

Microorganisms	Lactic acid			Acetic acid			Propionic acid		
	pH	pH	pH	pH	pH	pH	pH	pH	pH
	4.0	5.0	6.0	4.0	5.0	6.0	4.0	5.0	6.0
Bacteria									
<i>Escherichia coli</i>	1.25	15	25	5	15	35	<0.5	0.5	1.25
<i>Bacillus cereus</i>	5	25	40	nd	2.5	15	nd	0.5	0.5
<i>Bacillus licheniformis</i>	2.5	25	40	nd	0.5	5	nd	2.5	5
<i>Staphylococcus aureus</i>	nd	20	35	nd	5	30	nd	0.5	1.25
<i>Lactobacillus casei</i>	2.5	15	30	5	20	>50	5	15	>50

<i>Lactobacillus brevis</i>	2.5	15	40	5	25	>50	5	15	>50
<i>Lactobacillus helveticus</i>	nd	15	20	1.25	10	30	2.5	10	45
<i>Pediococcus pentosaceus</i>	5	15	25	2.5	15	40	<0.5	10	45
Yeasts and molds									
<i>Saccharomyces cerevisiae</i>	>50	>50	>50	5	15	>50	2.5	5	25
<i>Pichia anomala</i>	>50	>50	>50	2.5	10	35	1.25	5	25
<i>Candida krusei</i>	>50	>50	>50	5	20	30	2.5	10	>50
<i>Aspergillus oryzae</i>	>50	>50	>50	0.5	1.25	35	0.5	1.25	2.5

Medium composition: glucose 10 g/L, peptone 5.0 g/L, yeast extract 3.0 g/L, KH₂PO₄ 0.3 g/L, K₂HPO₄ 0.3 g/L, and MgSO₄ 0.1 g/L.

Bacteria detectable in deteriorated silage

Enterobacteria

Enterobacteria are facultative anaerobes and can survive in the silage environment if acidification is insufficient. They can also be detected in deteriorated silage after increase in pH, which is a result of lactic and acetic acid oxidation. Lindgren et al. (1985) counted a high number of enterobacteria ($>10^8$ cfu/g) in severely deteriorated corn silage, even though the pH was low and lactic acid concentration was high at silo opening. Because most enterobacteria do not proliferate at pH lower than 4.5, they are regarded as follow-up microflora; however, spoilage-associated species have not been well studied. Li and Nishino (2011a, b) identified, by culture-independent community analysis, *Erwinia*, *Rahnella*, *Pantoea*, *Morganella*, and *Klebsiella* spp. in deteriorated grass and corn silage. *Erwinia* and *Rahnella* spp. were also found as the dominant enterobacteria in fresh grass (Heron et al., 1993), but their presence in the aerobic deterioration phase was not examined.

The number of enterobacteria in standing crops sometimes exceeds that of LAB, and an increase in the numbers of both enterobacteria and LAB has been observed during wilting. Manure application may increase the number of enterobacteria in standing crops; however, the number will decrease with time (Ostling and Lindgren, 1991). Manure application, therefore, does not affect the

proportion of enterobacteria in silage crop, but slow acidification may occur if the growth of enterobacteria is vigorous during initial stages of the fermentation phase. Contamination with *Escherichia coli* is an important risk factor in terms of animal health and food hygiene (Wilkinson, 1999), but *E. coli* constitutes a small proportion (approximately 10^{-3} times) of the total number of enterobacteria during ensiling (Ostling and Lindgren, 1991).

The minimum inhibitory concentration of undissociated lactic acid against enterobacteria is 0.5–0.8 g/L, which is much lower than the normal content (approximately 10 g/L) observed in silages with desirable lactic acid fermentation (Wilkinson, 1999). Therefore, even when large numbers of enterobacteria are present in silage crops, they may not be prevalent, and spoilage will be initiated by microorganisms other than enterobacteria. However, if the growth of LAB is retarded by extensive wilting or delayed sealing, enterobacteria may predominate and produce ethanol and 2,3-butanediol at levels higher than the levels of lactic and acetic acids. This alcoholic fermentation may retain the silage pH at above 5.0, and low concentrations of undissociated acids may facilitate the growth of aerobic yeasts and molds. Proliferation of enterobacteria during the initial stages of ensiling could therefore be regarded as a causal factor of aerobic spoilage.

Bacillus

Bacillus spp. are spore-forming obligate or facultative anaerobes. Their density varies (10^1 – 10^5 cfu/g) during silo opening, with larger populations observed in grass silage than in corn silage (Te Giffel et al., 2002). Soil is the main habitat of *Bacillus* spp.; therefore, their numbers can increase if the silage is contaminated with soil or manure at harvest. Silage in the surface layer of a bunker silo may also have a high number of *Bacillus* spp., because air would infiltrate more into the silage mass located close to the surface compared with silage mass in deeper positions (Lindgren et al., 1985).

Inoculation of *Bacillus* spp. during silo opening did not enhance aerobic deterioration of γ -irradiated corn silage (Woolford and Wilkie, 1984). Thus, although a marked increase in the

number of *Bacillus* spp. (Lindgren et al., 1985) and change in bacterial composition from LAB to *Bacillus* spp. (Inglis et al., 1999) were observed after aerobic exposure, *Bacillus* spp. are not regarded as initiators of aerobic deterioration. Many *Bacillus* species, including *B. cereus*, *B. coagulans*, *B. circulans*, *B. pumilus*, and *B. licheniformis*, are found in deteriorated silage (Woolford, 1990; Inglis et al., 1999; Li and Nishino, 2011a, b); however, whether the various species prefer specific crops and impair aerobic stability remains unknown.

Silage is identified as the main source of aerobic and anaerobic spores in milk (Te Giffel et al., 2002). *Bacillus* spp. are ubiquitous in nature; therefore, silage and milk will inevitably be contaminated by a few *Bacillus* spores. The species detected in silage have been shown to cause food spoilage; hence, *Bacillus* growth and spore formation must be discouraged both during ensiling and after exposure to air.

Clostridium

Clostridium spp. are also spore formers but are unable to grow under aerobic conditions. The number of proteolytic and lactic acid-assimilating species may increase during the later stage of fermentation phase and produce ammonia and butyric acid, thereby lowering the nutritive value of silages (McDonald et al., 1991). Among fermentation products, butyric acid is a strong inhibitor of fungal growth; therefore, from an aerobic spoilage inhibition perspective, silages with high butyric acid content are stable.

Because *Clostridium* spp. are obligate anaerobes, their presence in silages has been related to anaerobic instability in the late fermentation phase and not to aerobic instability in the aerobic deterioration phase (Driehuis and Oude Elferink, 2000). However, Jonsson (1991) observed an increase in *Clostridium* spores from 10^5 cfu/g at silo opening to 10^6 or 10^7 cfu/g after aerobic spoilage in *C. tyrobutyricum*-inoculated grass silage. Furthermore, Vissers et al. (2007) reported that *Clostridium* spores at density higher than 10^5 cfu/g were observed in grass and corn silages, especially in the surface layers and in the molded spots of bunker and clamp silos. Growth of strictly

anaerobic *Clostridium* spp. in air-exposed silages is difficult to envision but may be explained as follows: Oxygen penetrating the silage mass might have initiated the growth of aerobic and acid-tolerant microorganisms (both bacteria and yeasts), which might have oxidized residual sugars and fermentation acids and increased the silage pH. When oxygen was completely consumed, anaerobic niches with an elevated pH might have developed, enabling the multiplication of *Clostridium* spp. in the deteriorated silage. While this hypothesis needs to be validated by further experiments, survival of *Clostridium* spp. in the fermentation and aerobic deterioration phases should be minimized.

Acetic acid bacteria

Besides yeasts, acetic acid bacteria, which are Gram-negative and catalase-positive rods, are known to play a role in initiating the aerobic deterioration of corn silages. The species isolated from silages belong to the *Acetobacter* genus, although 10 genera are presently recognized as members of the acetic acid bacteria family (Yamada and Yukphan, 2008). Oxygen is considered necessary for the growth of acetic acid bacteria; however, a strain capable of surviving under anaerobic conditions has been identified (Du Toit et al., 2005). Oxidation of ethanol to acetic acid is the best-known characteristic of acetic acid bacteria; however, oxidation of lactic and acetic acids to CO₂ and H₂O is also an important function of these bacteria.

Inoculating corn silage with acetic acid bacteria accelerates the onset of heating in the presence of air (Spoelstra et al., 1988). In this study, ethanol was first oxidized to acetic acid without an increase in the silage pH. Thereafter, when ethanol was depleted, lactic and acetic acids were oxidized to set off distinctive spoilage. These reactions were observed with or without simultaneous proliferation of yeasts, indicating that acetic acid bacteria can be solely responsible for initiating aerobic deterioration. An interesting property of acetic acid bacteria in ensiling is crop difference; acetic acid bacteria are found exclusively in corn and other cereal silages (Oude Elferink et al., 2001). Although extensive ethanol fermentation may occur at a higher rate in low-moisture grass silages

than in corn silages, acetic acid bacteria are seldom detected in aerobically stable or unstable grass silages. The reason for the absence of acetic acid bacteria in grass silages is unknown and needs to be examined.

The role of acetic acid bacteria in ensiling and aerobic spoilage is still inconclusive. Kan and Nishino (2011) examined the effects of *A. pasteurianus* inoculant, which was isolated from bunker-made corn silage. Their study revealed that addition of *A. pasteurianus* (10^5 cfu/g) during ensiling elicited a slight improvement in aerobic stability of corn silage but not grass silage. Spoilage was not accelerated in either corn or grass silage when *A. pasteurianus* (10^5 cfu/g) was added after silo opening, although survival of *A. pasteurianus* during the spoilage process was confirmed by the detection of 16S rRNA and PQQ-dependent alcohol dehydrogenase genes. Acetic acid bacteria are acid tolerant, aerobic, and capable of oxidizing ethanol, lactic acid, and acetic acid; hence, the hypothesis that acetic acid bacteria and yeasts initiate aerobic deterioration is undoubtedly reasonable. However, unaccountable differences exist between grass and cereal silages, and acetic acid bacteria are not usually counted despite the wide recognition of their importance in aerobic deterioration. Microbial ecology of yeasts and other bacteria is well understood, but there is room for improvement in our understanding of acetic acid bacteria in forage conservation.

Lactic acid bacteria

Lactic acid bacteria are facultative anaerobes, and a large population is occasionally detected in aerobically deteriorated silages. The species identified are mainly *Lactobacilli*, i.e., *L. plantarum*, *L. brevis*, *L. buchneri*, and *L. bulgaricus*, but *Enterococcus* and *Pediococcus* spp. have also been detected (Woolford, 1990). Lactic acid bacteria are acid tolerant and well adapted to silage environment, and thus, major populations found at silo opening can be LAB with desirable lactic acid fermentation. These surviving LAB may have a greater opportunity to prevail over the other bacteria after silo opening. Furthermore, some LAB have been shown to metabolize lactic acid into acetic acid and CO₂ under aerobic conditions in the absence of sugars (Quatravaux et al., 2006).

Inoculation of LAB, however, did not enhance aerobic deterioration of γ -irradiated corn silage (Woolford and Wilkie, 1984). Further, changes in silage pH may be minimal even if lactic acid is oxidized to acetic acid and CO₂. Because LAB have limited proteolytic and deaminating activity, the growth of LAB during the aerobic deterioration phase may not increase the concentrations of amines and ammonia. Therefore, although some LAB species exhibit distinctive persistence during the spoilage process (Li and Nishino, 2011b), LAB are considered to have little or marginal impact on the aerobic instability of silages.

Bacteria capable of spoilage inhibition

Propionibacteria

Because the antifungal activity of propionic acid is greater than that of lactic and acetic acids, propionibacteria became the popular choice for inhibiting aerobic deterioration in the 1980–1990s. Propionibacteria can ferment glucose or lactic acid to produce acetic and propionic acids, with the latter being produced at higher levels than the former (Merry and Davies, 1999). Thus, if the bacteria were successfully grown in rapidly fermenting silages where lactic acid bacteria play a major role, the concentration of antifungal propionic acid could increase substantially. However, silages may not be suitable for the growth or survival of propionibacteria, because only a few groups have successfully isolated propionibacteria from silages. Published results indicate inconsistent or unreliable effects of propionibacteria on propionic acid production and stability improvement (Merry and Davies, 1999). Therefore, after the appearance of *L. buchneri* as an alternative inoculant, attention shifted to the heterofermentative LAB species.

Lactic acid bacteria

The majority of aerobic spoilage is initiated by yeasts, but attention should always be paid to suppressing the growth of bacteria. In this regard, synergistic action of antibacterial lactic acid and antifungal acetic acid is better than the singular action of propionic acid-producing bacteria. Because

homofermentative LAB exclusively produce lactic acid, inoculation of LAB species often fails to improve or sometimes worsens aerobic stability. Inoculation of heterofermentative LAB can increase antifungal acetic acid content, but typical species such as *L. brevis* and *L. fermentum* may not yield sufficient levels (>50 g/kg DM) of acetic acid to prevent spoilage (Danner et al., 2003; Wang and Nishino, 2009). Inoculation of *L. buchneri*, in contrast, can yield sufficient acetic acid levels if added at 10^{5-6} cfu/g during ensiling, because *L. buchneri* can metabolize lactic acid to acetic acid and 1,2-propanediol under anaerobic conditions (Oude Elferink et al., 2002). This metabolism reduces the level of lactic acid during fermentation; hence, oxidation of lactic acid by yeasts can also be reduced after silo opening. Anaerobic degradation of lactic acid into acetic acid is also observed with *L. plantarum* (Lindgren et al., 1990); however, the activity is limited to conditions in which sugars are unavailable, and thus, manipulation of acetic acid production using this metabolism is rather insecure and unreliable. In silages inoculated with *L. buchneri*, 1,2-propanediol can be degraded anaerobically by *L. diolivorans* into propionic acid and 1-propanol (Krooneman et al., 2002), both of which can further promote stability after exposure to air (Danner et al., 2003). Because excessive acetic acid production may suppress silage intake, commercial preparations are often mixed with homofermentative LAB species to attenuate the activity of *L. buchneri*.

Increasing attention has been directed toward the use of antifungal substances produced by LAB. Although most studies on antimicrobial activity have focused on antibacterial effects, a few studies have described inhibitory effects of LAB on spoilage yeasts and molds. If homofermentative or facultatively heterofermentative LAB with strong antifungal activity are identified, concerns about DM recovery and feed intake can be minimized. A leading strain for this concept is *L. plantarum* MiLAB 393, which was isolated from grass silage (Strom et al., 2002). This strain produces 3 antifungal substances, i.e., phenyllactic acid, cyclo(L-Phe-L-Phe), and cyclo(L-Phe-*trans*-4-OH-L-Phe). The same research group that isolated these antifungal substances screened more than 1,200 LAB isolates by using *Aspergillus fumigatus* as an indicator and found strong inhibitory activity in 42 isolates (Magnusson et al., 2003). *Lactobacillus coryniformis*, *L.*

plantarum, and *Pediococcus pentosaceus*, which were frequently identified, produced unknown antifungal substances other than phenyllactic acid, cyclo(L-Phe-L-Phe), and cyclo(L-Phe-*trans*-4-OH-L-Phe). None of the antifungal isolates could inhibit the growth of *Pichia anomala* and *Penicillium roqueforti*, both of which are important spoilage fungi in silages; however, these works should be expanded to develop sound LAB inoculants besides *L. buchneri*.

Silage and fermented foods of long shelf life may be a good source of novel LAB inoculants. High survival and well adaptation to the fermentation process are key factors, and propionibacteria have failed to fulfill these criteria. In this regard, total mixed ration (TMR) silage is an interesting material. In Japan, some companies and contractors manufacture commercial TMR silages by mixing wet byproducts with dry feed. Wet brewers grains and soybean curd residue are often used as the main ingredients, and feedstuffs such as grass hay, legume hay, cracked corn grain, soybean meal, and wheat bran are added in lower proportions. A beneficial property of TMR silages is their high stability in the presence of air; no heating would occur even in summer, although non-ensiled TMR mixtures will easily deteriorate within a day or 2. Furthermore, the TMR silage was stable even with large yeast populations (10^6 cfu/g) at silo opening, despite the fact that silage with $>10^5$ cfu/g of yeasts is prone to deterioration in the presence of air (Nishino et al., 2004; Wang and Nishino, 2008). We isolated *L. buchneri* as the predominant LAB from a TMR silage containing wet brewers grains (Wang and Nishino, 2008), and detected sourdough LAB, i.e., *L. panis*, *L. hammesii*, *L. mindensis*, *L. frumenti*, *L. farciminis*, and *L. pontis* (Meroth et al., 2003; De Vuyst et al., 2009), in commercial TMR silages collected from several regions and manufacturers (Wang and Nishino, 2010). Both TMR silage and sourdough have a long shelf life; hence, the ability of sourdough LAB to inhibit aerobic spoilage is worth investigating.

Conclusions

Because yeasts are well known to play a major role in initiating spoilage, less attention has been paid lately to the involvement of bacteria in the aerobic deterioration process. The role of

bacteria had been investigated intensively in the 1970–1980s, and those studies have greatly contributed to our present understanding. However, there is room for further improvement with respect to how and when *Acetobacter* spp. and *Clostridium* spp. can be activated, and whether previous culture-based knowledge can be modified by incorporating culture-independent analysis. The search for novel homofermentative LAB inoculants capable of producing antifungal substances seems attractive and challenging. To achieve these goals, research is indeed worth continuing.

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Control of Losses during the Haymaking Process

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Abstract

Haymaking is the process of converting high-moisture fresh forage into a low-moisture stable product. A large quantity of water must be evaporated, usually by natural wilting under the sun and wind. Hay is produced after several mechanical operations which include mowing, conditioning, windrow handling, and packaging into a bale or another convenient form. Hay is then placed into storage where it remains stable if the moisture content is relatively low. Mechanical losses in the field can vary from 6 to 30%. Hay storage losses are typically another 5 to 10% but can be greater if moisture is too high or forage is exposed to rain. Good management is essential to minimize field and storage losses, and preserve a high nutritive value of stored hay.

Introduction

Forage conservation systems are often defined with respect to the moisture content at harvest and storage. Direct-cut silage contains typically 70 to 80% moisture; wilted silage, 55 to 70% moisture; haylage, 45 to 55% moisture; and baleage, 25 to 55% moisture. All these systems must be sealed to ensure anaerobic conservation whereas hay storage systems are open to air movement. For this reason, hay conservation requires very low moisture content, usually below 15 to 20%, to minimize oxidative reactions and microbiological deterioration.

In several parts of the world today, silage systems are widely and successfully practiced. An important advantage of silage is the short wilting time required in the field: just a few hours when forage is stored at 70% moisture, a full day under non-rainy conditions to reach 60% moisture, a second day under sunny or windy conditions to reach about 40% moisture content. Meanwhile, hay making can require as many as four days of field drying without rain to reach the target moisture content below 20%. For this reason, hay systems have a greater risk of rain-induced and respiration losses due to prolonged wilting than silage systems.

Despite challenges with field operations, hay remains an important feed for livestock because of its recognized nutritive value. Fifty years ago, Gordon et al. (1961) observed that feed acceptance, milk production and weight gain by dairy cows were greater for alfalfa hay than silage. More recently, Petit and Tremblay (1992) noted that hay contained more undegraded protein leaving the rumen than silages; this “by-pass” protein is beneficial for lower tract digestion. Verbič et al. (1999) also estimated that hay had more metabolizable protein than direct-cut silage or wilted silage. In addition, they observed that voluntary intake by sheep was greater for hay than for silage.

Losses occur at different stages during the forage conservation. In the case of silage systems, field losses tend to be small because wilting time is short (Buckmaster et al., 1990; Savoie and Jofriet, 2003). In the case of haymaking systems, field losses tend to be high because of extended wilting, more plant cell oxidation and more mechanical treatments to accelerate drying. Overall dry matter (DM) losses during haymaking have been estimated to range between 18 and 30% (Rees, 1982; Rotz and Muck, 1994).

The present paper describes the main sources of loss during hay making. It proposes various options of field and storage management to reduce losses and conserve high quality hay.

Main sources of loss in the field

Two types of losses occur in the field: mechanical and non-mechanical (due to oxidation and leaching). Mechanical losses are usually related to one of four harvest steps: mowing, conditioning, windrow handling, and baling (McGechan, 1989). Plant

species, stage of development at mowing, and timing of operations influence the amount of mechanical losses (Koegel et al., 1985a). Legumes require gentler operations than grass to minimize leaf loss (Dernedde and Honig, 1979 cited by Rees, 1982). As plants get older, they become drier and more brittle, which can contribute to increased mechanical losses (McGechan, 1989; Shinnars et al., 1991).

Non-mechanical losses, sometimes called invisible losses, are due mainly to oxidation and leaching. They reduce the nutritional value of forage because the more digestible components are the first to oxidize and be leached (Rotz, 1995). They usually represent 5 to 10% of DM (Rotz and Abrams, 1988). The following sub-sections review the mechanical and non-mechanical losses in the field.

Mowing height and windrow width

Mowing height can influence harvested yield and nutritive value of forages. For example, alfalfa mowed at 10 cm above the ground over a four-year period resulted in up to 38% less yield than alfalfa mowed at 5 cm (Belesky and Fedders, 1997). Over the life-cycle of alfalfa, high mowing increased weed competition in subsequent growths and reduced alfalfa plant size more quickly than low mowing. While a low cut provides more yield, it also results in a lower quality feed. Indeed, Taylor and Rudman (1965) measured up to 15% reduction in growth rate of steers fed grass hay cut at 6.5 cm compared with grass cut at 10 cm. The cut closer to the ground contained more old growth and a higher proportion of less digestible fibre.

In Wisconsin, Wiersma and Wiederholt (2001) observed that a low mowing height of 5 cm increased alfalfa yield by as much as 25% compared to a higher 10 cm cutting height (11.0 vs. 8.8 t DM/ha). The more abundant crop had a slightly lower quality but overall dairy milk yield per unit area was 17% more (13.8 vs. 11.8 t milk/ha). More recently in New York State, Thomas (2007) also suggested that the conventional mowing height of 10 cm should be lowered to 5 cm for alfalfa-grass mixtures fed to dairy cows. He observed that the lower cut produced 13% more yield for a three-cut system than the higher cut (9.42 vs. 8.31 t DM/ha/yr). The quality was slightly lower (0.7 percentage unit less crude protein, 1.4 percentage unit more NDF). The higher quality

10-cm cut produced 3% more milk per unit forage but produced 10% less milk per unit field area. The author concluded that most farmers would prefer a 13% increase in yield at the price of a 3% loss in quality. The optimal mowing height might not be the same in all crops (grasses tend to regrow more slowly than legumes after a low cut) or environments, so local adjustments have to be validated.

When comparing hay systems using different mowers, Rees (1982) reported losses ranging between 2 and 14% (Barrington and Bruhn, 1970; Schechtner, 1977; Beckhoff et al., 1979; Dervedde and Honig 1979 cited by Rees, 1982). Rotz and Abrams (1988) reported losses for specific mowers between 1 to 3 %.

The way forage is spread on the ground after mowing will influence field drying. Rotz and Sprott (1984) observed that alfalfa left in a wide swath dried 20 to 30% faster than alfalfa left in a narrow windrow. Drying time was also reduced for alfalfa haylage in a wide swath compared to a narrow windrow (Kung et al., 2009). Grass left in a wide swath dried faster compared to grass in a narrow windrow, saving half a day of wilting time over three days when the crop was immature and under sunny conditions (Savoie et al., 1984). Another advantage with rapid drying is the preservation of nutrients such as non structural carbohydrates whose concentration remained 8% higher in fast drying swaths compared to slow drying windrows (Tremblay et al., 2011).

Conditioning and maceration

Mechanical conditioning applied at mowing or soon after can speed up the field drying process and reduce the risk of rained-on hay (George et al., 2004). Very intensive mechanical conditioning, usually referred to as maceration, will also accelerate drying but it can cause significant breakage loss when it is applied and also subsequently because the windrows are more susceptible to oxidation and leaching (Rotz et al., 1991; Savoie et al., 1993b). For example, total losses were 14.5% for macerated windrows and 3.5% for conventionally conditioned windrows after an 18-mm rainfall; they were 26.8 and 6.9%, respectively, after a 62-mm rainfall. With conventional conditioning, DM losses were reduced by increasing forward speed, or by reducing flail conditioner rotary speed (Macdonald and Clark, 1987).

Savoie et al. (1982) observed that roll conditioning and flail conditioning applied at mowing increased the field drying rate compared to no conditioning. Subsequent crimper conditioning applied to windrows also increased drying rate but caused greater DM losses than conditioning applied at mowing. Meanwhile, Rotz and Sprott (1984) noted that drying rates were not significantly different among cutterbar, flail, rotary disk and rotary drum mowers, and between roll and flail conditioners. However, shatter loss of leaves was 3 percentage points higher for the flail mower conditioner than for the roll conditioning devices. According to Shinnars et al. (1991), leaf loss associated with four different types of conditioning rolls was practically the same (5.2 to 5.9% of DM) while drying rates were also similar between roll types. Koegel et al. (1985a) observed greater loss in second and third alfalfa cuts (16.8%) than in first cut (8.5%). During the second and third cuts, a haying system based on a flail-type conditioner had more loss (18.6%) than a system using a fluted-roll conditioner (16.5%) but no important difference was observed in the drying rate. Desiccants such as potassium carbonate can also be added at the conditioning step in order to speed up drying (Akkharath et al., 1996). Such treatments increase the drying rate of alfalfa hay but they are seldom used because of their cost and the logistical problem of filling regularly a spraying tank during mowing operations.

Alfalfa hay produced after maceration under good field drying conditions demonstrated an increased digestibility of neutral detergent fibre (NDF) and acid detergent fibre (ADF), according to Agbossamey et al. (2000). However, when rain occurred during field wilting, losses were greater for macerated alfalfa compared to traditional windrows because of increased leaching of soluble nutrients (such as Ca and Mg) and increased respiration (Savoie et al., 1993a). In a study in Wisconsin, losses were less than 10%, and maximum drying rate was obtained with macerated mats less than 0.64 cm in thickness (Shinnars et al., 1985). In an experiment conducted by Savoie et al. (1993b), macerated alfalfa dried faster than conventional windrows with final moisture contents of 26.5 and 61.3%, respectively, after 28 h of field wilting. However, macerated forage had higher mechanical losses (7.8%) than conventional windrows (3.5%).

Windrow handling

Tedding and raking operations are necessary to aerate windrows and prepare them for subsequent collecting by a baler or a forage harvester. Tedders have a vigorous action of spreading and lifting up windrows and making them fluffy; they are designed to be used early in the drying process when the crop is wet and less sensitive to breakage (Macdonald and Clark, 1987). Rotz and Muck (1994) reported losses ranging between 1 and 3% for tedding which is similar to the 1 to 2% range reported by Rotz and Abrams (1988). Klinner et al. (1971, cited by Rees 1982) reported a greater range of tedding losses up to 8%. Dry matter losses are greater for legumes than for grass hay (Rotz and Muck, 1994). Based on laboratory results, tedding losses were six times greater for alfalfa than for grass hay; losses increased as moisture content decreased and could be over 20% for very dry legumes (Savoie, 1988). This explains why tedding is more commonly used in Europe where grass is dominant while in North America alfalfa is the more common hay species (Macdonald and Clark, 1987).

Rakes are used to displace the windrow and expose the wetter bottom portion for improved aeration and drying. The windrow is usually narrowed to facilitate pickup by the baler or forage harvester (Macdonald and Clark, 1987). Raking losses can be highly variable, ranging between 1 to 20% (Rotz and Muck, 1994), but they are typically within a range of 3 to 6% (Rotz and Abrams, 1988). Losses are influenced by moisture content and yield. According to Buckmaster (1993), relative raking losses are reduced when hay is wetter or yield is greater (1.4% less loss per 1000 kg/ha of yield increase). Raking losses may reduce the nutritive value of forages, especially legumes, because of the higher proportion of nutritious leaves that shatter compared to fibrous stems that remain in the windrow (Rotz and Abrams, 1988; Buckmaster et al., 1990). In an experiment by Rotz and Abrams (1988), measured losses were 3.5% for raking a full swath into a windrow, 0.8% for simply turning a windrow with a rake, 1.8% for picking up a windrow with a baler pickup and 1.1% within the baler. In the same experiment, losses of the highest quality material (leaves) were observed in the bale chamber. Buckmaster et al. (1990) estimated that the greatest monetary value of mechanical losses occurred during

raking, followed by baling, forage harvesting and mowing-conditioning, corresponding to relative losses of 7.1, 5.4, 4.1, and 2.2%, respectively.

Baling

According to Rotz and Muck (1994), typical DM losses at baling range between 2 and 10%. Honig (1980, cited by Rees 1982) reported losses at baling ranging between 4.4 and 11.1%. In a study by Koegel et al. (1985a), DM losses from a round baler with a fixed chamber (10.8%) were greater than losses from a small rectangular baler (2.8%) and a round baler with a variable chamber (3.8%). Chamber losses were less in a mid-size rectangular baler (0.7%) than in a small rectangular baler or a large round baler (1.6% each), according to Shinnars et al. (1996). Rotz and Muck (1994) reported chamber losses for small rectangular balers ranging between 1 to 3%. Rotz and Abrams (1988) measured losses of 1.8% at the baler pickup and 1.1% in the bale chamber of a small rectangular baler. Rotz (1995) reported baler losses at pick up between 1 and 3% based on other studies (Koegel et al., 1985a; McGechan, 1989; Shinnars et al., 1992).

A bottom-fed baler for small rectangular bales, tested by Shinnars et al. (1992), had slightly less pickup losses (1.1 vs. 1.3%), less bale chamber losses (2.3 vs. 2.7%) and more leaves retained than a side-fed baler.

Oxidative and leaching losses

When plant respiration occurs, non structural carbohydrates are metabolized into CO₂, water and heat by plant cells and epiphytic microorganisms (Rotz and Muck, 1994). These reactions cause DM disappearance. If the generated heat is not dissipated outside, it will accumulate inside the forage and may even, in some circumstances, cause self-ignition (Coblentz et al., 2010). Mechanical and oxidative losses are somewhat interlinked. Some mechanical treatments increase the susceptibility of crop components to respire or absorb rain water; hence these soluble cell constituents are more easily lost by leaching and oxidation afterwards. Table 1 presents a summary of reported values for mechanical losses during hay making operations.

Table 1: Dry matter losses as a function of hay making operations

Operation / Factor	Reported losses (% of DM)	Reference
Mowing	1 to 3% 2 to 4%	Rotz and Abrams, 1988 Honig, 1980
Mowing height	Mowing at 10 cm instead of 5 cm reduced yield by up to 38% Mowing at 10 cm instead of 5 cm reduced yield by 13%	Belesky and Fedders, 1997 Thomas, 2007
Tedding	1 to 3% up to 20% for very dry legumes up to 8%	Rotz and Muck, 1994 Savoie, 1988 Klinner et al., 1971
Raking	3 to 6%	Rotz and Abrams, 1988
Baling	1 to 3% for small rect. baler 1 to 3% at pick up 4 to 11%	Rotz and Muck, 1994 Koegel et al., 1985a Honig, 1980
Total mechanical losses (excluding height factor)	6 to 27% (average 16%)	Koegel et al., 1985a

Field management to control losses

Good management can contribute to reduce field losses during haymaking. In the usual sequence of operations, the first decision is to determine the proper time to start mowing. On the one hand, lactating dairy cows benefit from early cut when herbage has a high level of energy. On the other hand, dry cows, beef cows and horses are usually fed more mature herbage to correspond to their metabolic need for fibre. This means that alfalfa may be mowed early at the bud stage for dairy cows or later at the flowering stage for dry cows. With grasses, the proper stage of development at harvest may be the boot stage or the heading stage depending on the type of hay required. Anderson (1976, cited by Fonnebeck et al., 1986) estimated that DM digestibility of alfalfa was reduced by 0.3% per day of delay. Changes in fibre concentration and digestibility over time are also species-dependent and the rate of decline of digestibility increases with increasing growth temperature for all species (Thorvaldsson et al., 2007). The impact of DM digestibility loss will start early for dairy cows. However, this impact will appear later for animals

requiring a forage with more fibre and less energy. Table 2 lists a number of questions that should be addressed before starting the haymaking process.

Sequence of operations and timing

Mechanical losses will be greater when handling drier material. For example, over a five-day period, tedding was applied once daily. Loss due to tedding was 0.5% on the first day and increased to 4.5% on the last day of the experiment, with an average of 1.5% per handling operation (Overvest and Schukking, 1974 in McGechan, 1989).

Models have been developed to help farmers choose the proper timing for each field operation and thereby reduce losses caused by weather damage. One of the first models to predict field drying of hay was developed in Canada by Dyer and Brown (1977). It predicted drying based on potential evapotranspiration estimated from rainfall and dew. A multi-layer model based on heat and mass balance was developed by Tuzet et al. (1993). The model used weather data, biological characteristics of the plant and physical parameters of hay to predict moisture evaporation. Barr and Brown (1995) predicted drying with the Penman-Monteith equation based on solar radiation, air temperature, humidity, wind and rainfall. This model predicted DM and quality losses. Another model for hay drying was developed in Australia (Gupta et al., 1990) involving five sub-models: one for pasture growth, one for drying, one for losses (at cutting, during drying, raking, baling and losses in digestibility), one for the weather forecast and one for management. Models have to be adapted to local forage species, weather conditions and livestock feed requirements to provide proper advice to farm managers during haymaking.

Weather forecasts and decisions to carry out operations

Rain can leach soluble nutrients from forage and reduce digestibility (Collins, 1983; Rotz and Abrams, 1988). The level of loss will depend on forage moisture content, swath density and amount of rainfall (Rotz et al., 1991; Collins, 1983). Dernelde and Wilmschen (1969, cited by McGechan, 1989) measured 1% DM loss in runoff from hay

initially at 20% moisture; the amount of loss doubled when hay had previously been lacerated. When respiratory losses were included, total DM disappearance due to both oxidation and leaching was as high as 16% in laboratory experiments. Rain loss ranged between 3 to 34% of DM and averaged 11.2 % with alfalfa hay (Rotz and Abrams, 1988).

Farmers can delay harvest to avoid rain damage. Delaying harvest will normally result in a lower nutritive quality of the standing crop because it becomes more fibrous and less digestible as it matures (Conseil Québécois des Plantes Fourragères, 2002). For example, a one-week delay in mowing timothy after late heading in spring growth reduced the *in vitro* digestibility of DM (-7%) and NDF (-10%), and increased NDF concentration (+5%) according to Pelletier et al. (2008). However, losses due to rain may be greater than losses due to delayed harvest. In the case of coastal bermudagrass, Hart and Burton (1967) reported a 0.25% loss per day in digestibility when mowing was delayed between the third and eighth week of growth while a 2.5 cm rainfall caused 7 to 10% digestibility loss. According to this estimate, a harvest delay up to six weeks is preferable to the digestibility loss caused by up to 2.5 cm rainfall. In the case of alfalfa, Fonnesbeck et al. (1986) found that hay quality was reduced by rain to a greater extent than by a one week advance in maturity. Plant cell wall constituents increased from 38.1 to 41.6% in an extra week of maturity while they increased from 39.4 to 43.6% after a 20-mm rainfall. So the decision of delaying mowing when heavy rain (> 15 mm) is forecast appears to be sound for several forage species.

Handling rained-on windrows

Despite all precautions that may be taken such as delaying mowing when rain is forecast, some windrows are likely to receive a shower of rain occasionally (Fonnesbeck et al., 1982). Leaching losses are less important when the amount of rain is light or when rainfall occurs quickly after mowing (Smith and Brown, 1994). So it may be acceptable to mow even if some light showers are forecast, especially when good weather is expected afterwards and large areas of forage still need to be harvested. Leaving forage in a narrow windrow provides better protection against rainfall loss than leaving hay in a widely spread swath (Savoie et al., 1993a). The windrow will have to be spread out again

after rain to improve the drying process. Afterwards, the farmer will have to determine if the crop can still be salvaged as a moderate quality feed (e.g. for dry cows) or for non-feed uses (bedding). In any case, the crop must be removed to make way for the next growth.

Harvesting wet hay to reduce weather risk

Baling hay before it is completely dry, i.e. below 20% moisture, will reduce field DM loss compared to baling very dry hay which has become very brittle. Early baling will also reduce total field exposure time, field respiration loss and the risk of having rained-on hay (Rees, 1982). Different storage techniques such as barn ventilation and antifungal additives must be considered to conserve wetter hay.

Storage loss is largely influenced by moisture content (McGechan, 1990). The heating of bales in storage can cause further nutritive loss (Buckmaster et al., 1989, Collins et al., 1987). Heating usually increases with moisture and bale density (Buckmaster et al., 1989). In an experiment with alfalfa hay stored during 60 days, 89% of DM was recuperated from bales initially at 20% moisture and 84% of DM from bales initially at 30% moisture (Coblentz et al., 1996).

Moisture enhances the growth of heat-generating microorganisms. Temperatures as high as 77°C were measured in alfalfa-orchardgrass large round bales (Coblentz and Hoffman, 2009a). Maximum temperature was negatively correlated with DM recovery and *in vitro* true digestibility. The maximum DM loss measured during storage was 15% in bales that had high moisture contents (26.7 to 46.6%).

Table 2: Management points to consider in controlling field losses of hay.

Are weather forecasts available for the decision-making process?

When should forage be mowed with regards to its maturity?

At what time of the day should forage be mowed (i.e. morning or evening; Pelletier et al., 2010)?

What area should be mowed in a single day?

Should mowed forage be left in a narrow windrow or a large swath?

When should windrows be tedded?

When should windrows be raked?

At what moisture content should hay be baled?

What shape and density of bales are optimal?

What are the best machinery adjustments?

Is hay fed on the farm? If sold, what is the market (dairy, beef, horse, etc.)?

Storage options

Several storage options are available to the farmer. When hay is very dry at harvest, i.e. less than 15% moisture content, there is practically no mold growth (Couture et al. 2002). Hay will then conserve almost indefinitely as long as it is protected against re-humidification. When hay is stored in conditions above the mold-growth threshold, then storage losses will increase as a function of moisture, oxygen availability and micro-organism activity (McGeachan, 1990). One of three approaches can be followed to limit storage losses: 1) reducing moisture content quickly; 2) closing off the supply of oxygen; 3) destroying micro-organisms. Either approach will substantially inhibit biological reactions and minimize DM loss. Table 3 presents some of these options to reduce storage losses.

Additives to inhibit hay spoilage

Additives are sometimes used to inhibit microbial growth. Rectangular bales with moisture content as high as 28% have been preserved adequately with ammonia applied at a rate of 1.1 to 2.5% of total wet mass. Color characteristics of hay were improved when moisture content was below 25%. Large round bales either treated with ammonia or untreated and sealed with plastic film were preserved at much higher moisture contents, sometimes as high as 55 to 60% (Koegel et al., 1985b; Shinnars et al, 2009a). When hay is sealed with a plastic cover, moisture becomes almost irrelevant with respect to DM loss.

Storage losses were reduced in mid-size alfalfa bales (35 x 50 x 90 cm) that were treated with propionic acid without plastic film. The treatment was effective in bales having between 25 and 38% moisture content but the effects were not consistent as far as quality was concerned (Buckmaster and Heinrichs, 1993). Adding 1.3% of urea to alfalfa round bales at 26% moisture reduced temperature, non enzymatic browning, mold growth and chitin content. It increased NDF and ADF after a five-month storage period but there was no negative impact on forage digestion, milk yield and milk composition when the hay was fed to dairy cows (Alhadhrami et al., 1993).

Microbiological additives have also been used. The maximum temperature reached in baled alfalfa hay was reduced by 15°C when treated with an inoculant compared with untreated hay (Dulcet et al., 2006). The addition of exogenous fibrolytic enzymes such as endoglucanases and exoglucanases improved the degradability of NDF by up to 21% in alfalfa hay (Eun et al., 2007).

In barn ventilation or drying

Another approach to high moisture hay conservation is to remove excess moisture with in-barn drying or natural ventilation. Total DM loss during in-barn drying is usually less than 5% but can be as high as 15% under certain conditions (Segler, 1958 in Rees, 1982; Dijkstra, 1964 in Rees, 1982). Parker et al. (1992) successfully dried small rectangular alfalfa hay bales initially at 37% moisture with forced heated air. Bales were of adequate quality for dairy cattle but would not meet quality standards for horses because of the presence of some mold. Authors indicated the difficulty to monitor hay bale drying because of occasional non uniform airflow and gradual drying across the stack. Some bales had to be over-dried before all bales were dry enough for good conservation. When hay bales are ventilated with unheated ambient air, it is recommended to make low density bales, typically in the order of 100 to 140 kg DM/m³. Low density facilitates airflow across the bales and rapid removal of excess moisture (House and Stone 1988).

Wrapping bales

Harrigan and Rotz (1994) reported different levels of DM loss for alfalfa round bales stored outside: 9.6% with plastic cover, 16.3% with net wrap and 16.5% with twine. The control hay stored inside with twine had 6.0% DM loss. Losses in quality, as measured by ADF, NDF and acid detergent insoluble N generally followed the same order. Shinnars et al. (2009b) observed less DM loss from net-wrapped large round alfalfa bales compared to twine-wrapped bales stored outside. They also noted that avoiding direct ground contact of bales further reduced DM loss. Large round bales of alfalfa and brome grass had less loss when they were produced at a higher density (Russell et al., 1990). Loss during inside storage of round and rectangular bales was about half of the loss observed outside (Buckmaster et al., 1990). During three months of indoor storage, alfalfa hay in round and rectangular bales had similar DM losses but round bales had greater NDF at the end (Collins et al., 1987). Bales wrapped with plastic mesh had less NDF, less ADF, less acid insoluble N and less DM loss in the 30-cm outer layer than twine-wrapped bales (Russell et al., 1990). Feeding and handling losses were 25% with uncovered round bales stored outdoors, 14% with covered bales stored outdoors and 12% with uncovered bales stored indoors (Belyea et al., 1985, cited by Buckmaster et al., 1990).

Storage losses

Losses of DM during inside storage of hay usually range between 2 and 5% (Rotz and Abrams, 1988). For alfalfa hay stored outside during three months, Collins et al. (1987) reported 9.1% loss of DM and 13.6% loss in digestible DM. For similar bales stored inside, DM loss was 3.8% and digestible DM loss was 7.5%. Buckmaster et al. (1990) estimated that average DM loss during hay storage was 2.8% but the monetary value of those losses was greater because changes in quality contributed to reduced digestibility. Rotz and Abrams (1988) reported 4.5% DM loss on average for dry hay (11 to 20% moisture) and 10.9% DM loss for wet hay (25 to 34% moisture). Buckmaster and Heinrichs (1993) calculated that storage loss increased by 0.7% for each additional percent of moisture at baling. Concentrations of NDF, ADF, hemicellulose, cellulose, and

lignin increased with increasing maximum storage temperature (Coblentz and Hoffman, 2009b). Rotz and Muck (1994) estimated that crude protein was lost at a steady rate of about 0.25% units per month of storage. More recently, McCormick et al. (2011) observed that round bales of bahiagrass conserved as hay (16% moisture) had 12.8% DM loss when stored outside and 2.9% DM loss when stored inside. Forage quality expressed as net energy of lactation was initially 1.36 Mcal/kg at harvest; it deteriorated more in hay bales stored outside (1.17 Mcal/kg) than hay bales stored inside (1.32 Mcal/kg). This difference was reflected by a 7% lower milk production from dairy cows fed outside-stored hay compared to inside-stored hay (28.2 vs. 30.2 kg milk/day). These authors also evaluated bahiagrass baleage at 50% moisture content which had similar quality (1.32 Mcal/kg) to inside-stored hay but slightly lower (4%) milk response (29.0 kg milk/day).

Table 3: Management points to consider when storing hay bales.

Should additives be used? Which one, when, and at what rate should it be applied?

Is barn-ventilation or heated air drying available to conserve wet hay bales?

If not, how should bales be stored?

Is bale wrapping available to deal with wet bales?

During storage, what should be done if wet hay is exposed to air?

Is there a disposal system for used plastic wrap?

Cost of hay making

The cost of hay making can be viewed basically from three different perspectives: 1) hay is produced on the farm for internal use; 2) hay is produced on the farm for external use; 3) hay is produced outside the farm and purchased for internal use. When a farmer produces his own hay to feed his livestock, he can reduce the cost by increasing yield, increasing total area cultivated in forage, and decreasing both quantitative and qualitative losses. When a farmer buys hay from the outside, he has less control on costs which are influenced by the local market price fluctuations.

For example, in a free market like the United States, prices of hay vary considerably from one year to the other, and also seasonally. The yearly average price for hay (all types) in 2003/2004 was \$85.50/ton while in 2008/2009 it was \$152.00/ton, a record high (USDA, 2011a). Prices were back to \$108.00/ton in 2009/2010. When a farmer buys all his hay from the outside, he has to expect such variations. On many dairy farms in North America, hay has become a small input in the total forage ration (e.g. less than 10% hay is combined with more than 90% farm grown silage). In such cases, the farm would not be much affected by hay price fluctuations. However, if hay is a large part of the ration, it would be preferable to produce it on the farm to maintain greater independence with regards to hay price fluctuations.

When hay is produced on the farm, the total production cost will be sensitive to changes in the price of labour, energy, machinery, fertilizers and other inputs. In recent years, the price of several farm inputs has increased considerably. During the period from 2002 to 2008, prices paid in the United States for diesel increased from \$0.96/gallon to \$3.62/gallon (USDA, 2011b). The price for superphosphate increased from \$221/ton to \$800/ton (USDA, 2011c) and the price for nitrogen (44-46% urea) increased from \$191/ton to \$552/ton (USDA, 2011d). The production cost of hay on the farm has therefore increased markedly. Farmers producing their own hay have some management options to control such input price increases. They can consider legume-grass mixtures or crop rotations to reduce N-fertilizer costs. They can increase forage yields with better seeds or increase total cultivated area in forages to reduce fixed and variable costs. In the end, the total cost of hay making will depend on the quantity and quality of forage harvested and stored, and the resources required to feed it to livestock or the income that may be generated from selling some hay on the market.

Conclusion

Hay systems can be subject to important losses in the field and during storage. Mechanical field losses occur at mowing, conditioning, windrow handling and baling. Under good management practices, mowing-conditioning can cause dry matter (DM) losses within 1 to 3% of total yield; windrow handling losses due to tedding and raking

are typically within 3 to 6%; baling losses are within 2 to 11% depending on baler type and dryness of crop at the time of pickup. Therefore, total DM losses due to mechanical treatments may vary between 6 and 20%. Meanwhile, non-mechanical or invisible losses occur as a result of normal plant oxidation and leaching when rainfall occurs on the exposed windrows. These invisible losses typically range between 2 and 10%; they will be very low when hay dries quickly under sunny conditions but they will increase when hay dries slowly in the field under occasional rain. Once in storage, hay may lose additional DM due to respiration and mold growth. Storage losses have been observed to range between 5 and 10%, depending on moisture, storage time, and whether stored inside or outside.

Since most losses come from the highly nutritive leaves and the easily oxidized sugars (i.e. the non structural carbohydrates), the nutritive value of forage is also reduced during the hay making process. When large amounts of rain are forecast, it is better to postpone mowing to avoid important leaching losses even if a mowing delay implies harvesting a slightly more mature and less digestible crop. When local soil conditions allow mowing forage close to the ground, a low cut (5 cm above the ground) is preferable to a high cut (10 cm above the ground) because of higher harvested yields (10 to 30% over several cuttings) and only a small reduction of feed quality due to increased fibre.

Stable conservation of hay requires low moisture content, ideally below 15% to avoid any mold development. If hay is harvested at a higher moisture content, various options should be considered: the use of antifungal additives, in-barn ventilation of hay bales or wrapping bales to ensure an anaerobic environment. Overall, field losses are usually greater than storage losses in haymaking. Losses can be reduced by a variety of management decisions. These include the time to start mowing, the use of weather forecasts to minimize the risk of rain during field wilting, the type of windrow handling, the time to bale, the use of additives to inhibit mold growth, barn drying to lower moisture content or the use of plastic wrap to create an anaerobic seal.

Acknowledgements

The authors thank Agriculture and Agri-Food Canada for the long-term support for R&D related to forage and biomass.

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Near Infrared reflectance spectroscopy for management and utilization of forages

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Abstract

Rapid on-farm forage analysis with near infrared spectroscopy (NIRS) is changing the way producers manage and utilize forages. NIRS moisture prediction has been demonstrated to accurately predict forage moisture on the harvester where other technologies (*viz.* conductive, capacitive, microwave) have struggled with the heterogeneous nature of these biological materials. Furthermore, NIRS has demonstrated its ability to move the information beyond moisture to the inclusion of forage compositional factors opening the possibility to use NIRS for plant nutrient management and precision diet composition.

Introduction

Ensiling forage allows producers to provide a season-independent feedstuff of consistent quality. The success of ensiling is due to the development of an anaerobic environment that promotes fermentation. Fermentation depends on many factors, including crop moisture, pH, presence of certain microorganisms and available fermentable sugars (Barnes et al., 2003).

Water content is a major factor influencing successful fermentation. Thus, the focus of research has been to aid the forage producer in the prediction of crop moisture. Moisture prediction guides decisions regarding harvest and storage of forage crops, minimizes production costs and maximizes forage quality. Management decisions influenced by moisture level include deciding optimum harvest time, determining targeted use of silage, choosing storage method, and optimizing harvester setup and site-specific crop management. In the early 1970s, USDA research scientist Karl Norris identified the capabilities of Near Infrared Reflectance Spectroscopy (NIRS) and developed statistical methods to identify constituents in agricultural products (Fahey et al., 1994). Presently, NIRS is used to determine the quality of agricultural products through prediction of the concentration of desired constituents. These analyses are conducted world-wide in laboratories linked together with calibration networks.

The technology used on today's farms owes its success to the same physical and chemical theory that enables laboratories to provide accurate constituent information of

agricultural products. However, until recently the measurements made by an NIR spectrometer were sensitive to environmental and physical factors, including sample presentation, temperature, and humidity. Advancements in NIRS hardware and statistical methods afford a more robust NIRS model. These new robust models lend themselves to successful integration into mobile applications (Paul, 2003) which will result in both moisture and constituent information in real-time, offered in a way that may be addressed spatially for integration into modern, site-specific farming methods.

Theory

NIRS is based on selective absorption of light energy in the near infrared region of the electromagnetic spectrum (EMS). The near infrared region is defined as the wavelengths of light between 780 and 2500 nm. Near infrared light energy is selectively absorbed based upon the molecular makeup of the material and the frequency of the light energy. Molecular absorption of light energy in the NIR region is primarily due to stretching, bending, or deformation of X-H bonds where X is carbon, nitrogen, or oxygen (Fahey et al., 1994). These bonds are particularly useful in determining the composition of organic materials such as forages.

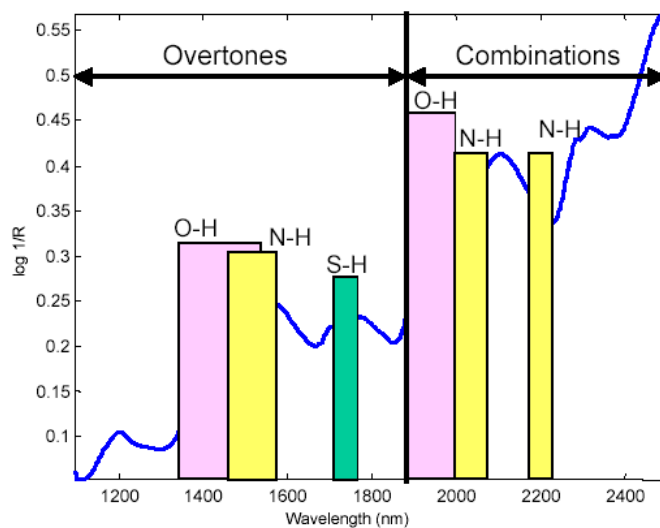


Figure *Erro! Nenhum texto com o estilo especificado foi encontrado no documento.:* Overtone and combination absorption bands for X-H bonds (Bertrand, 2002).

The near infrared region encompasses these combination and overtone bands, providing rich spectral information that can be related to molecular composition. Energy in the near infrared region is not as strongly absorbed as in the mid infrared region, enabling

measurement of light absorption indirectly by light reflection. The relationship between absorbed and reflected light is given by the Beer-Lambert Law which states that the amount of light energy diffusely reflected is nearly proportional to the light absorbed. Here absorbance is described as the quotient of the incident light I_0 and reflected light I_R . The relationship between light reflected and light absorbed is the principle relationship governing reflectance spectroscopy and the near infrared (NIR) spectrometer (Figure 2).

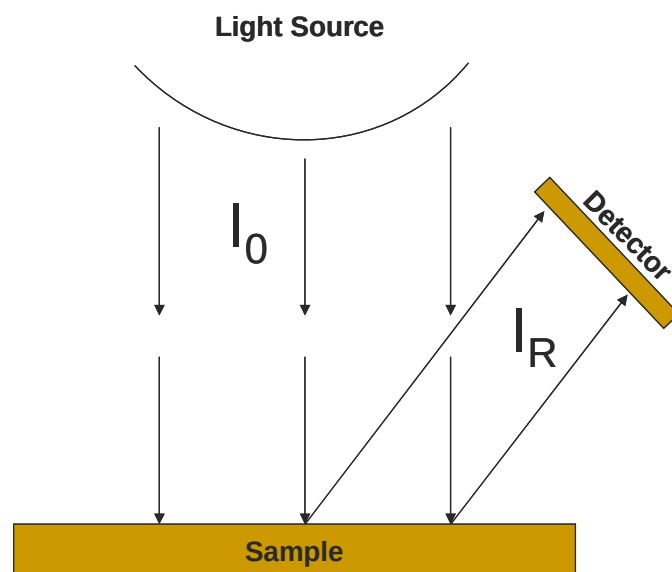


Figure 2: Basic reflectance spectrometer layout.

In this basic spectrometer, there is a light source that provides a stable light intensity to interact with the sample I_0 . Some of the light from the source is absorbed, transmitted, scattered by the sample surface, reflected, and diffusely reflected. The diffusely reflected light I_R energy is measured at some angle with respect to the source by the detector.

The method of measuring individual wavelengths of light is dependent on the type of spectrometer utilized. There are two main methods. In the first, sequential instruments, commonly used in forage testing laboratories to measure the wavelengths of light, are indexed by a filter wheel that is located at either the light source or the detector (Figure 3). These instruments filter polychromatic light into monochromatic light so that a known wavelength of light only exists at the detector. This information is then detected by a lead sulfide (PbS) detector and light intensity is recorded for each wavelength.

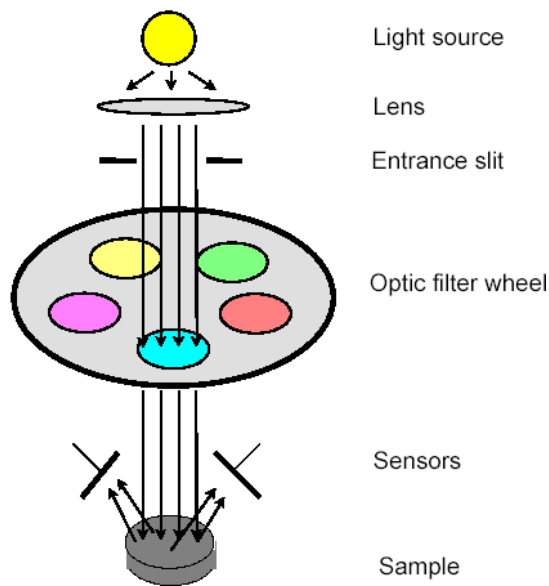


Figure 3: Sequential NIR spectrometer (Bertrand, 2002).

The second method commonly used for ... involves a multichannel spectrometer which measures all detectable wavelengths of light simultaneously (Figure 4). In this spectrometer, polychromatic light illuminates the sample and is partitioned into its component wavelengths by a monochromator, or grating. The wavelengths are then presented to a photodiode array which simultaneously measures the intensity corresponding to each wavelength of light.

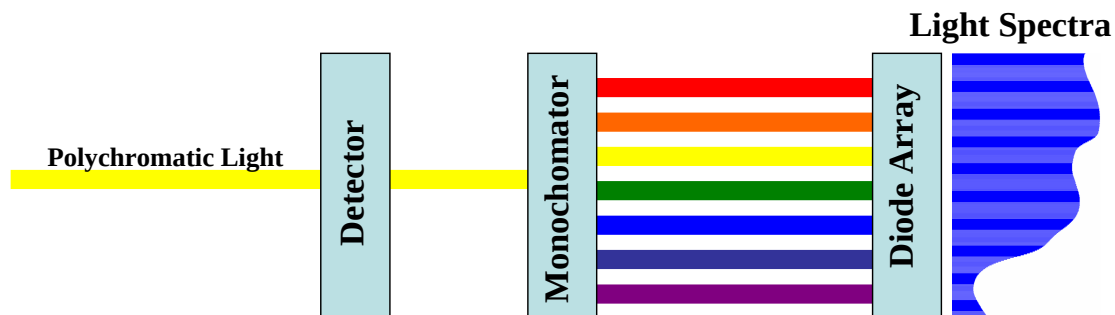


Figure 4: Diode array spectrometer.

The detectable wavelength range of the spectrometer is dependent on the material properties of the photosensitive diodes. Typical diode array materials include silicon (Si), lead sulfide (PbS), and indium gallium arsenic (InGaAs). The resolution of the spectrometer is a function of the size of the diode array and the detectable wavelength range. Unlike sequential instruments, diode array spectrometers have no moving parts. These spectrometers produce spectra nearly instantaneously and are very robust, lending them to be useful for on-harvester applications (Paul, 2003).

In summary, it can be shown that the molecular composition of a material can be determined by the type of light energy absorbed. This light energy is only a function of the wavelength of the light. Light is absorbed if the energy is equal to a molecular energy level of the molecule, which is a function of the bond strength and atomic mass of the atoms that make up the molecule. This phenomenon provides the basis for NIRS as absorbed wavelengths of light can be indirectly measured as the intensity of the wavelengths reflected. These reflection spectra contain a great deal of information regarding the molecular composition of the material being measured.

Applications

NIRS for yield-mapping and documentation

Early farming techniques were small scale and the labor of working the soil, planting and maintaining proper nutrition for the crop was completed manually. This intimate contact between the farmer and his crop enabled him to spatially identify crop needs, allowing him to meet these needs on a site-specific basis. In the late 1800s this intimacy was altered by the advent of modern farm equipment. Farm machinery greatly increased farm productivity, but the close relationship that once existed between the crop and the farmer was lost. The farmer now managed the crop on a field-by-field basis, greatly reducing the resolution when determining and meeting a specific plant's needs (Kuhar et al., 1997). Modern technology has sought to remedy this challenge associated with large scale farming. Two major developments can be credited with increasing the producer's field resolution: the miniaturization of modern computing technology and the deployment of the NAVISTAR satellite array by the U.S. Department of Defense. NAVISTAR provided a global positioning system (GPS) for military and civilian use. Mobile computers and GPS technology enabled producers to link agronomic and yield data spatially, enabling site-specific prescription of nutrients and soil amendments.

This technology has been adapted to aid in production of many types of crops such as vegetables, citrus, cereal grains, and forages. In forages, however, one key component has been missing. Forage crop yield is usually expressed in terms of kg DM/ha as any variation in moisture that may exist in harvested crop would skew yield results when considering yield on a wet basis. Correcting for moisture in forages has been challenging because it requires a method of rapidly determining moisture content of a non-uniform crop stream with a very short residence time in the harvester.

A technology for rapid determination of moisture in forages would enable yield-mapping technologies to be used in management of forage crops. Yield maps could be used in conjunction with site-specific technologies in application of soil amendments, fertilizers, and pesticides to determine the resulting yield response for these management decisions. Yield maps in multiple cutting systems would enable the producer to modify inputs affecting crops, thus allowing the farmer to monitor yield response as soon as 30 days from application. Yield improvement would not be the only factor increasing profitability as site-specific application of fertilizer and pesticides could lead to lower usage, resulting in lower input costs and environmental impact.

Having precise yield information would also provide the producer with an accurate inventory of the harvested crop. This information would allow ration formulation based on forage inventory. The yield information system would provide the producer accurate records, enabling forage production costs and inputs to be closely managed. For a cash crop operation, precise recordkeeping would not only provide the same management and inventory benefits as for a dairy producer but would also provide information about his product, allowing him to demand premiums for high quality forage.

NIRS for storage of forage.

Forage availability does not always correspond to animal needs. To overcome this problem, modern agriculture has developed methods of storing forage. The storage of forage has provided a consistent food supply through non-growing seasons. Storing forage as an ensiled material is highly dependent on creating an anaerobic environment that promotes fermentation. The success of this storage method is dependent on many factors, including moisture, density, oxygen available, pH, and presence of certain microorganisms.

Moisture is one of the primary factors affecting the storage of forage as moisture content at harvest directly affects dry matter losses during storage (Figure 5).

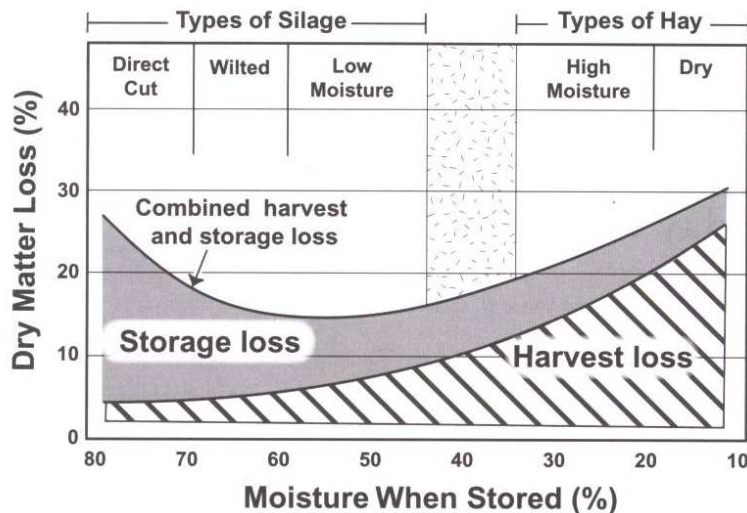


Figure 5: Forage losses as a function of moisture content at harvest (Barnes et al., 2003).

Silages high in moisture also increase the development of clostridia and other undesirable bacteria. These organisms produce butyric acid and amines that reduce animal intake and increase dry matter losses (Figure 6). Properly wilting forage will minimize the amount of fermentable carbohydrates required to raise the acidity. Raising the acidity is essential to the preservation of the forage and the restriction of the development of undesirable microorganisms. In extremely high-moisture forage, nutrients can also be carried away by water seepage, or effluent, as the forage is compressed in the storage structure (Barnes et al., 2003).

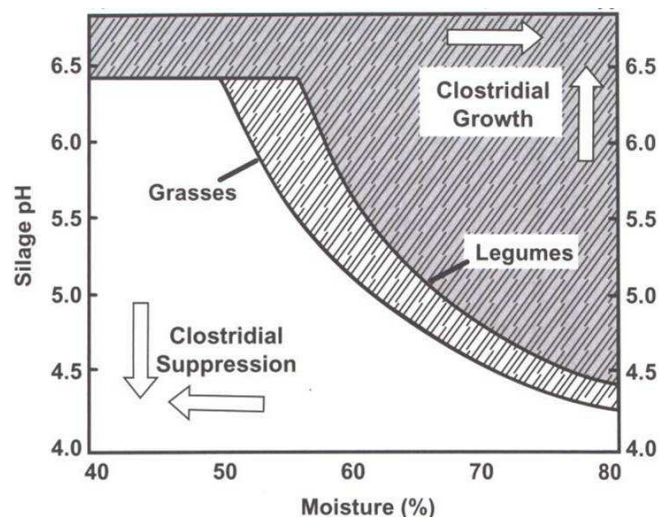


Figure 6: Unfavorable bacterial growth as a function of moisture content in ensiled forage (Barnes et al., 2003).

Alternatively, if forage moistures are too low, losses may occur as the result of low forage density in storage. Low forage density promotes aerobic respiration, yielding bacterial

and mold growth. This process increases the temperature of the forage and is known as heating. The heating of forage uses sugars and reduces protein digestibility. In severe cases, spontaneous combustion may result.

There are many factors that prevent producers from storing forage at the proper moisture. The producer cannot control some factors such as weather and ambient drying conditions. Other factors could be controlled if certain tools existed, such as an instrument that efficiently measures forage moisture. A rapid and accurate method of determining forage moisture would enable a producer to make storage decisions based upon current conditions and impending weather.

A rapid means of predicting forage moisture could be possible through near infrared reflectance spectroscopy (NIRS). NIRS would aid the producer by monitoring current harvest conditions, allowing him to switch locations or to mix fields based upon moisture. Having an accurate dry matter (DM) mass flow rate through the harvester could aid in applying the proper amount of forage amendments and inoculants, potentially reducing the costs associated with these treatments. This tool could greatly enhance the producer's ability to provide optimal conditions when ensiling forage.

NIRS in feeding of forage

If accurate records of feed inventory are to be maintained, then accurate removal rates for stored forages must be included in the assessment. The NIR sensor in stationary mode would allow for the rapid determination of forage moisture content as it is removed from storage. This would enable the producer to compare removal rates to storage inventories from previously recorded yield data.

TMR rations are balanced considering the amount of dry nutrients present in forages. However, forages are fed based upon their weight when removed from storage. This method assumes a uniform moisture content in the forage removed for that particular feeding or week of feeding, depending on the frequency of DM correction. However, DM in corn silage has been found to vary five to six percentage units in consecutive weeks (Holter, 1983). Variations are especially large in horizontal silos (Mertens and Berzaghi, 2009).

In 2004, Stone conducted a study to investigate DM variation in horizontal silos (Stone, 2004). Stone's research included sampling nine haylage and eleven corn silage bunker silos in central New York. He found that typical variations in the haylage bunkers were so great that a feeder could deliver an entirely different ration during the same day if care was not taken when loading feed. He concluded that not only does DM need to be

assessed frequently, but care must be taken when sampling to ensure the bunker is accurately represented.

During a precise feeding experiment, Mertens and Berzaghi (2009) measured the daily variation in silage composition for both an alfalfa and corn silage bunker (Figure 7). Although the bunkers were mechanically defaced, thereby reducing variation, substantial daily variation was observed. Mertens and Berzaghi attribute abrupt changes in DM to rain or snow precipitation that contaminated the silage before mixing and feeding.

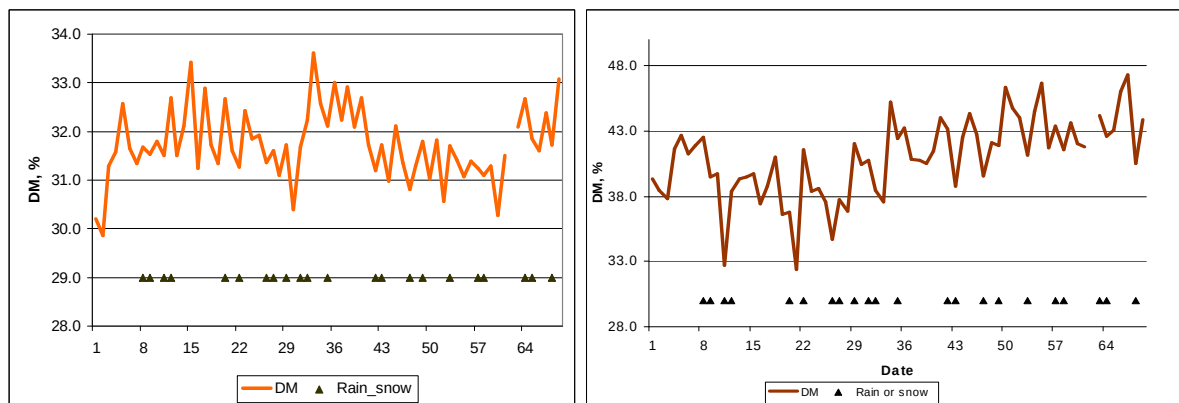


Figure 7: Variation in horizontal silage dry matter over a two-month feed-out period (Mertens and Berzaghi, 2009).

It is recommended that forages fed in total mixed ration (TMR) with less than 75% DM be corrected for DM content each week as variability in stored forage moisture content can result in unbalanced rations if not considered in ration formulation (Linn, 1995). A ten percent increase in forage DM content would result in a 0.1 lb decrease in crude protein intake. Crude protein intake is directly proportional to milk protein levels (Linn, 1995). Low DM content can also lead to off-feed and acidosis problems (Nordlund, 2003).

Current tools for analysis of forage moisture require drying the forage and determining the moisture content gravimetrically (Oetzel et al., 1993). These methods are time consuming and cumbersome. It has been found that few farmers practice these methods of DM determination but rather use animal intake at the bunk to adjust forage concentrations in the TMR (Nordlund, 2003). A stationary NIR sensor would provide a producer a rapid method of correcting his ration for DM each feeding.

Our research – on-harvester prediction of moisture via NIRS

Water content is a major factor influencing successful fermentation when ensiling forage. Thus, our research focused on the investigation of NIRS technology to meet the challenge of predicting moisture in real time on the forage harvester (Digman, 2006; Digman and Shinnars, 2008). The feasibility of this technology was investigated through calibration development and verification. The research goal was to develop a moisture calibration that will be insensitive to the variability expected in mobile forage harvesting. Moisture prediction will guide decisions regarding harvest and storage of forage crops, minimizing production costs and maximizing forage quality.

Spectra and moisture reference samples were collected throughout the harvest season in 2004 and 2005 at various field locations at the University of Wisconsin - Arlington Agricultural Research Station, Marshfield Agricultural Research Station, Dairy Forage Research Center Farm (USDA-ARS), and several dairies in southwest Wisconsin and California for the development of static (in-lab) and dynamic (on-harvester) moisture calibrations (Figure 8). These locations provided much variability in ambient, harvesting and agronomic conditions. Data were also obtained from other researchers in Arizona, Bavaria and Zweibrücken, Germany.



Figure 8: Prototype NIRS sensor mounted on forage harvester spout.

The following two criteria were used when comparing individual calibrations or the effectiveness of certain math pre-treatments. Math pre-treatments are transformations of the spectra used to remove non-constituent spectral information. Each calibration was ranked first by the number of principal components (PCs) as identified by the software program used to develop the calibration (Unscrambler). The fewer PCs, the less spectral information required to build the calibration. A calibration with fewer PCs will exhibit more robustness

when predicting future data, as it is less likely to be over-fit to the data used for calibration development. The second criterion for calibration performance was root mean standard error of prediction (RMSEP) or cross-validation (RMSECV). The goal was to obtain a RMSEP(CV) of less than two moisture percentage points.

Moisture prediction models for whole-plant-corn-silage (WPCS) developed using static data had a RMSECV of 1.12% using five PCs while a calibration developed using dynamic data had a RMSECV of 3.28% using four PCs. Alfalfa validation results were slightly worse with RMSECVs of 2.50% using four PCs and 3.74% using three PCs for models using static and dynamic data, respectively.

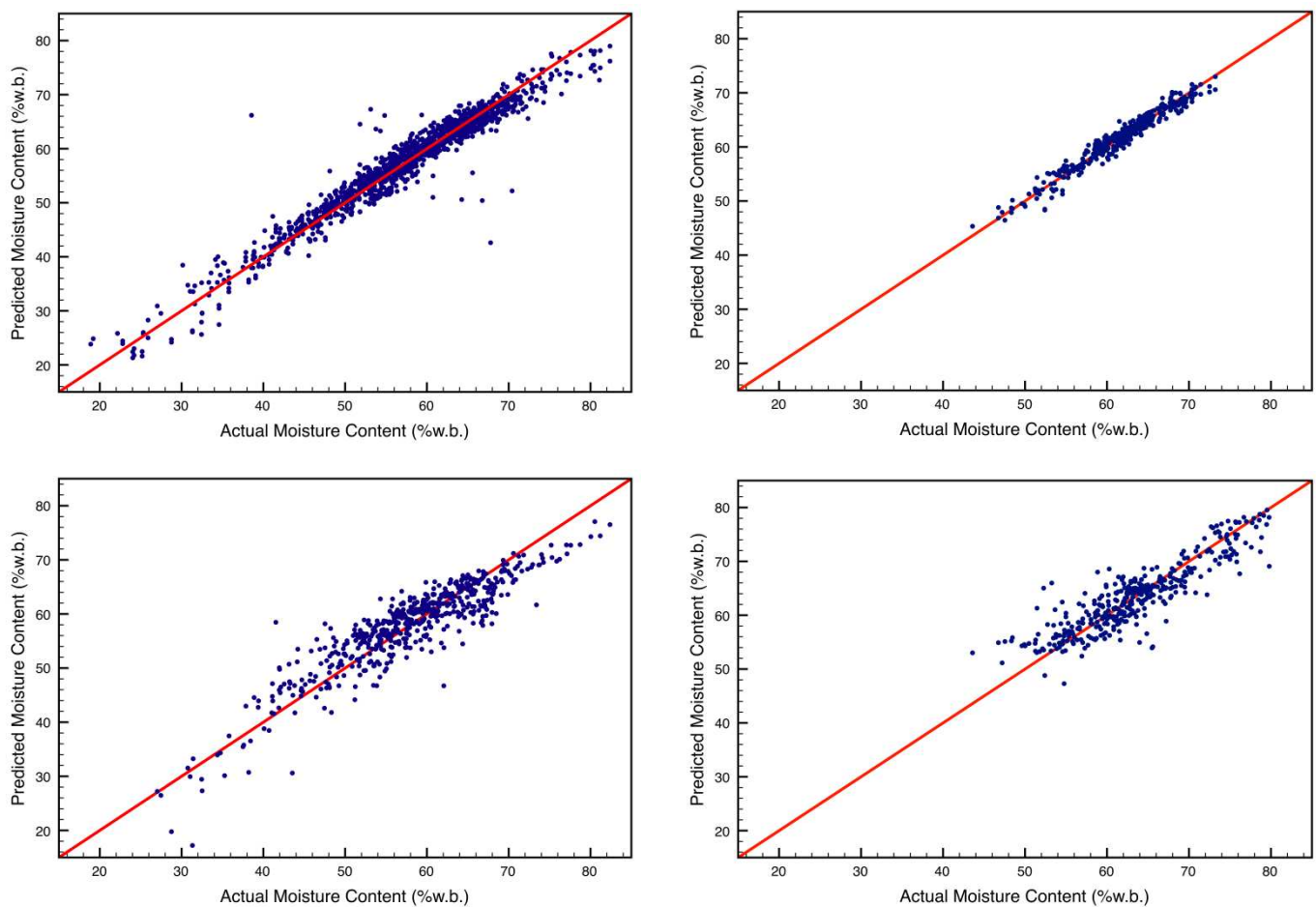


Figure 9: Calibration performance depicted as predicted vs. actual moisture content (%w.b.) for best performing models including alfalfa laboratory (top left), whole plant corn silage laboratory (top right), alfalfa field (bottom left) and whole plant corn silage field (bottom right).

Predicting dynamic data with a model developed using static data would greatly reduce overhead associated with collecting dynamic calibration data and would increase the mobility of the data collection effort because the harvester would not be needed. Dynamic data were predicted with calibrations developed using static data with similar performance as defined by root mean standard error (RMSE). In models with similar RMSEs, more spectral information was required to obtain similar measures of calibration effectiveness as evidenced by the larger number of PCs required for these models. A static alfalfa model predicted dynamic data with a RMSEP of 3.41% using 4 PCs compared to the dynamic model's RMSECV of 3.74% using 3 PCs. Similar results were found with WPCS models.

The ability to predict moisture independent from the crop would greatly reduce the amount of data that would be necessary to support predicting moisture on a forage harvester. When predicting data independent from the crop, it was evident that models developed with alfalfa data were more successful in predicting WPCS moisture than models developed with WPCS were at predicting alfalfa moisture. This is likely due the larger variability represented by the alfalfa dataset.

Understanding the influence that spectrometer hardware has on a calibration's ability to predict moisture should be considered when spectrometer hardware is upgraded in the future. Three sensors were used in this research, a Corona 45 (C45), and two Corona prototypes (CP04 and CP05). Principal component analysis showed the Corona 45 (C45) and 2004 Corona prototype (CP04) produced similar spectra while the spectra from the 2005 Corona prototype (CP05) was slightly different than the other two sensors. This did translate into better calibration transferability between the CP04 and C45 than the CP04 to the CP05 when comparing RMSEPs and PCs for prediction of moisture on a validation dataset. From this experiment it does appear that calibrations can be transferred between spectrometers depending on the spectrometer hardware and math pre-treatment method.

The question of regional calibration stability was addressed through comparisons of data collected in WI, CA, AZ and Bavaria, Germany. The WI developed WPCS dynamic calibration performed quite well on the Bavarian WPCS dataset with an RMSEP of 3.58%. However, poor performance was seen on data collected in the western U.S. with RMSEPs ranging from 11.71% and 4.97% for AZ and CA WPCS data, respectively.

Two fractional factorial experiments were conducted to investigate calibration robustness. First, static calibration robustness due to moisture content, mean particle size (MPS) and temperature was investigated. It was found that moisture prediction performance was variable, depending on the math pre-treatment scheme employed. Two math pre-

treatment schemes were found to not only provide the lowest RMSEPs but were also insensitive to the influence of the factors explored.

Next, dynamic calibration robustness was investigated using a fractional factorial experiment evaluating MPS, lens angle, and position on spout (POS). Moving the sensor to the aft location of the spout greatly reduced the reflection intensities recorded by the spectrometer. This phenomenon lead the POS factor to be the most significant factor identified in this investigation. Math pre-treatment did lower the RMSEPs but did not successfully eliminate the effect of the POS factor.

In 2005, a small dataset was collected to determine the feasibility of developing a calibration for predicting MPS. The results of this investigation demonstrated a MPS calibration is feasible both statically and dynamically with RMSECVs of 2.1 mm using 4 PCs and 2.1 mm using 6 PCs, respectively.

Although not part of our work, Kormann and Flohr evaluated three NIR spectrometers for measurement of forage moisture on a commercial forage harvester. They explored the feasibility to extend on-harvester NIR capabilities to predict protein and starch with SECVs of .28% and 1.62% respectively (Kormann and Flohr, 2002). This work demonstrates that predicting diet composition and harvest nutrient removal is possible with on-farm NIRS. Forage composition prediction will provide the producer essential data to precisely manage nutrient cycling from the field to the animal reducing production costs and the environmental impact.

Conclusions

Moisture prediction models for whole-plant-corn-silage (WPCS) developed using laboratory data had a RMSECV of 1.1% using five PCs while a calibration developed using field data had a RMSECV of 3.3% using four PCs. Alfalfa validation results were slightly worse with RMSECVs of 2.5% using four PCs and 3.7% using three PCs for models using laboratory and field data, respectively. Models developed with laboratory data could predict forage moisture from spectra collected in the field with about the same level of performance (RMSEP) as a model developed with field data. However, the models developed with laboratory data required more spectral information to obtain similar measures of calibration effectiveness. When predicting data independent of crop, it was evident that models developed with alfalfa data were more successful in predicting WPCS moisture than models developed with WPCS were at predicting alfalfa moisture. This is likely due the larger variability represented by the alfalfa dataset.

Acknowledgements

This research was partially sponsored by the University of Wisconsin College of Agriculture and Life Sciences, John Deere Ag Management Solutions, John Deere Ottumwa Works, John Deere Technical Center, John Deere Werke Zwëibruken, and CAMO Software. This research could not have been completed without the assistance of the staff of the Arlington Agricultural Research Station, United States Dairy Forage Research Center Farm (USDA-ARS), and Marshfield Agricultural Research Station.

Addendum

Since the publication of this research two commercial NIRS products have been marketed for forage management (Table 1). The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

Table 1. Commercially available NIRS for on-farm forage management

Product	Company	Headquarters
AgriNIR	dinamica generale srl	Mantova, Italy
HarvestLab	Deere & Company	Moline, Illinois, U.S.A.

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Custom Services for Forage Conservation Services

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1. Introduction

In Brazil, specialization in the beef and dairy cattle industry has led to significant growth in sales of related products and services. Until recently, the hay market has been the only reference for the forage conservation segment, and even then data is only available for a few elite herds of animals or for critical situations when forage is insufficient for herd production. Currently, custom services or product purchasing including large volumes from specialized producers is a good alternative for ranchers. The process of outsourcing can begin with services related to crop implantation, fertilization (receiving the chemical fertilizer in the field or periodically distributing organic fertilizer) and all phytosanitation management, as well as transportation and storage processes involved in forage harvest.

The producer has the advantage of specializing property use, which focuses their efforts and investment into the area in which they are strongest, resulting in better crop quality, a quicker harvest, lower investment in machinery, higher feed quality and especially more careful planning of their activities (Pereira et al., 2008).

2. Custom services for forage in Brazil

According to Pereira & Barros (2004), custom services were first used by the milk volume partnership system during the mid 90's in the Campos Gerais region of Paraná state. This program primarily involved milk producers from the Batavo and Castrolanda cooperatives. It arose from beef and dairy cattle producers need to grow or at least continue in an area that was undergoing great changes. Some of these changes can be summarized as follows:

a) Small dairy producers: Areas destined for milk production were reduced due to family divisions, and acquisition of new areas was not viable due to rising property values. Production increases were needed to enable new investments or even to pay old ones off in addition to stimulating the market by offering differential payment for greater volumes of milk delivered. The high cost and relatively low number of hours forage equipment is utilized during the year impede acquisition of new machines. In addition, the need for consistent milk production throughout the year and the offer of adequate feed for more productive animals make the use of conserved forage fundamental.

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b) Large milk producers: need to invest in forage equipment (greater fixed cost) for large scale silage production, high maintenance costs of equipment, production of high quality silage, and dairy and agricultural integration (integrated planning of activities).

c) Farmers: need to find new income options, to integrate this activity in their planning or even offer services with their machinery (reduced fixed costs), to amortize part of the cost of winter crops that were seeded as cover for direct planting.

Nowadays custom services, has become increasingly common in different regions of Brazil. This practice has been stimulated by significant growth in the beef and dairy cattle industry. It should be stressed that custom forage conservation production is not only associated with self-propelled forage harvesters, but also with all forms that involve cash payment and/or exchange of services for the use of machinery, equipment and vehicles during production and harvesting forage destined for conservation.

Kruppa (2009) in a joint study with one of the largest dairy coops in Rio Grande do Sul, the Santa Clara Coop, reported that 62% of producers (municipal or private) that customized the harvesting of corn or sorghum for silage, 21% utilized group owned equipment and only 17% owned their own machinery. Private harvesting equipment was primarily one row choppers with some two-row choppers. In the Campos Gerais region of Paraná state, it is estimated that more than 70% of the corn for silage is harvested by custom self-propelled forage harvesters.

In 2010, approximately 7,000 with one or two-row harvesters were sold in Brazil (information gathered from manufacturers). Together, the two leading brands of self-propelled harvesters in Brazil sold over 100 machines over the last three years.

3. Forage crops and custom harvesting

Currently, harvesting corn for silage is the most common type of custom service. The fact that corn silage provides high yields dry matter (DM) per area with a high energy concentration make it an indispensable ingredient in most dairy herds, as well as much of the beef cattle feedlots.

Marketing research studies in Brazil estimate the area of corn grown for silage to be approximately 980,000 hectares at 2009/2010 summer harvest and about 450,000 hectares for the winter harvest, the area of corn grown for silage is estimated to represent about 14 to 18% of all the certified corn seed sold in the country.

Cultivation of winter corn has shown great technological advances over the last few years. Until the mid 90's, the off-season harvest occupied a little over 1.5 million hectares, representing a modest 10% of all domestic corn production. Today winter corn accounts for more than 35% of all domestic corn production, occupying more than 4.5 million hectares with an average yield of 3500

kg/ha. The area of winter corn planted has increased by more than 3 million hectares over the last 15 years, and average yield has practically doubled over this same period (Seleme, 2011).

Some technical aspects were decisive in increasing the productivity during professionalization of the off-season. Among these are the following: selecting and seeding early-maturing soybean cultivars seeking to anticipate off season planting and minimize some effects of stress, such as drought in Mato Grosso, Goiás and São Paulo as well as thermal inversion and frosts that occur in Paraná and southern Mato Grosso do Sul; selecting hybrids suitable for the planting season by combining different hybrids with complementary characteristics of yield potential, early maturity and defense; adapting the plant population so that it presents the best overall results in a final population ranging between 55,000 and 62,000 plants/ha; reducing the space between rows from 90 to 45 cm in order to improve the spatial distribution of plants in the field seeking to increase exposure to solar radiation and reduce water evaporation from the soil by closing the culture more quickly (Seleme, 2011).

Thus, it is increasingly evident that agility in harvesting corn for silage is a key factor in guaranteeing the quality of the produced silage. High temperatures in the summer accelerate culture maturity, and when associated with frequent rain, harvest is more difficult and often delayed. On the other hand, the off season harvest should be quick given the early culture cycle and the higher risks of loss due to disease or frost. Another important factor is that, off season crops, or even summer ones, seeded with reduced space between the rows, require appropriate cutting platforms with rotating disks that are independent of the orientation of the plant line. Regarding this aspect, only self-propelled harvesters have a high performance cutting system even in sloped areas.

Sorghum is the second most common culture in area intended for silage production in Brazil. It is mostly planted during the off season. Despite its greater resistance to pests and disease, sorghum is at risk for hydric stress and frost; consequently, it faces the same challenges to accelerate harvest as winter corn crops.

4. Silage production costs

Tables 1 and 2 present the production costs of two corn silage crop yields. Winter corn yield presents productivity of 25 tons/ha, while a summer crop presents productivity of 50 tons/ha. Fertilizer costs are in agreement with the extraction of nutrients for crop yields without considering any residual fertility. For respective productivities of 25 and 50 t/ha for winter and summer crops, machine costs for seeding and cultivating represent 7 and 8% of total costs, while harvesting costs involving cutting, transportation and packing represent 22% and 20%.

Table 3 presents production costs of off season sorghum silage in which machine costs for seeding and cultivation represent 13% and harvesting 27% of total costs. To produce oat haylage, machine costs in seeding and cultivation represent 15% and harvesting 23% of total costs (Table 4).

Table 1 – Production cost of corn silage crop yield of 25 tons/hectare.

Production of fresh forage	25000 kg/ha			
Dry matter (%)	30			
Production of forage dry matter	7500 kg/ha			
Annual return of capital	8.75 %			
Annual interest loan(bank loan)	8.75 %			
Financial cycle	5 months			
Items	R\$ /unit	quantity/ha	R\$/ha	%
Seeds			436.7	1
			6	6
P 30R50	286.09 /bag	1.30	371.9	1
Crop Star	162.10 /L	0.40	2	4
			64.84	2
Fertilizers			498.4	1
			9	9
NPK 10-20-20 + Zn	0.95 /kg	300	285.1	1
			5	1
			189.8	
Urea	0.79 /kg	240	4	7
Potassium Chloride	0.96 /kg	0	0.00	0
Simple Superphosphate	0.52 /kg	0	0.00	0
Limestone	0.02 /kg	500	7.90	0
Fertilizer transportation	15.00 /ton	1.04	15.60	1
Herbicides			89.36	3
Insecticides			59.87	2
Fungicides			37.92	1
Machinery			210.5	8

			0	
Sprayer – 2000L	10.00 /ha	4.00	40.00	1
Dual-disk fertilizer spreader	9.00 /ha	2.00	18.00	1
Seeder or – 8 rows (40 cm)	75.00 /ha	1.00	75.00	3
Manure spreader	72.00 /ha	1.00	72.00	3
Limestone spreader – 5000 kg	22.00 /ha	0.25	5.50	0
			1332.	5
Direct Costs			90	0
Financing Cost			48.60	2
			480.2	1
Administration			5	8
			528.8	2
Indirect Costs			5	0
Harvesting (self-propelled harvester)	320.00 R\$/h	0.9	288.00	1
			0	1
			126.0	
Transportation	35.00 R\$/h	3.6	0	5
Packing	50.00 R\$/h	0.9	45.00	2
Labor	41.00 R\$/day	0.2	8.20	0
Plastic Film	1.27 R\$/m2	25	31.75	1
Bunker Silo	2.85 R\$/m3	30	85.50	3
			113.9	
Diesel fuel	1.89 R\$/L	60.3	7	4
			698.4	2
Silage			2	6
			115.2	
Interest on inventory	19.20 R\$/month	6	1	
		Silage	With	Without
			2,675.	1,861.
Total costs	R\$/ha		3	7
Dry matter cost	R\$/kg		0.357	0.248
Fresh forage cost	R\$/kg		0.107	0.074

Costs estimated for 2010/2011 harvest. Harvested with self-propelled harvesters (6m platform) and transported with dump trucks (cost approximately R\$ 35/hour for the forager).

Source: Fundação ABC (2011).

Table 2 – Production cost of corn silage – crop yield of 50 tons/hectare

Production of fresh forage	50000 kg/ha			
Dry matter (%)	30			
Production of forage dry matter	15000 kg/ha			
Annual return of capital	8.75 %			
Annual interest loan(bank loan)	8.75 %			
Financial cycle	5 months			
Items	R\$ /unit	quantity/ha	R\$/ha	%
Seeds			436.7	1
			6	5
P 30R50	286.09 / bag	1.30	371.9	1
Crop Star	162.10 /L	0.40	64.84	2
Fertilizers			666.4	2
			8	3
NPK 10-20-20 + Zn	0.95 /kg	300	285.1	1
			5	0
Urea	0.79 /kg	240	189.8	
			4	7
Potassium chloride	0.96 /kg	158	151.9	
			3	5
Simple superphosphate	0.52 /kg	26	13.32	0
Limestone	0.02 /kg	500	7.90	0
Fertilizer transportation	15.00 /ton	1.22	18.35	1
Herbicides			89.36	3
Insecticides			59.87	2

Fungicides			37.92	1
Machinery			210.50	7
Sprayer – 2000L	10.00 /ha	4.00	40.00	1
Dual-disk fertilizer spreader	9.00 /ha	2.00	18.00	1
Seeder or – 8 rows (40 cm)	75.00 /ha	1.00	75.00	3
Manure spreader	72.00 /ha	1.00	72.00	2
Limestone spreader – 5000 kg	22.00 /ha	0.25	5.50	0
Direct Costs			1500.89	52
Financing Cost			54.72	2
Administration			480.25	17
Indirect Costs			534.97	19
Harvesting (self-propelled harvester)	320.00 R\$/h	0.9	288.00	10
Transportation	35.00 R\$/h	3.6	126.00	4
Packing	50.00 R\$/h	0.9	45.00	2
Labor	41.00 R\$/day	0.2	8.20	0
Plastic Film	1.27 R\$/m2	50	63.50	2
Bunker Silo	2.85 R\$/m3	30	85.50	3
Diesel fuel	1.89 R\$/l	60.3	113.97	4
Silage			730.17	25
Interest on Inventory	20.75 R\$/month	6	124.47	
		Silage	With	Without
Total costs	R\$/ha		2,890.5	2,035.8
Dry matter cost	R\$/kg		0.193	0.136

Fresh forage cost	R\$/kg	0.058	0.041

Costs estimated for 2010/2011 harvest. Harvested with auto-propelled forager (6m platform) and transported with dump trucks (cost approximately R\$ 35/hour for the forager).

Source: Fundação ABC (2011).

Table 3 – Production cost of off season sorghum silage – Crop yield of 30 tons/hectare.

Production of fresh forage	30000 kg/ha			
Dry matter (%)	30			
Production of forage dry matter	9000 kg/ha			
Annual Return of capital	8.75 %			
Annual interest loan(bank loan)	8.75 %			
Financial cycle	4 months			
Items	R\$ /unit	quantity/ha	R\$/ha	%
Seeds			197.83	4
AG 2005E	14.92 /kg	10.00	149.20	1
Crop Star	162.10 /L	0.30	48.63	3
Fertilizers			418.90	3
Herbicides			46.42	3
Insecticides			40.90	3
Machinery			186.00	3
Sprayer – 2000L	10.00 /ha	3.00	30.00	2
Dual-disk fertilizer spreader	9.00 /ha	1.00	9.00	1
Seeder or – 8 rows (40 cm)	75.00 /ha	1.00	75.00	5
Manure spreader	72.00 /ha	1.00	72.00	5
Limestone spreader - 5000 kg	22.00 /ha	0.00	0.00	0
Direct Costs			890.05	3
Financing Cost			25.96	2

Administration			0.00	0
Indirect Costs			25.96	2
Harvesting (self-propelled harvester)	320.00 R\$/h	0.6	192.00	4
Transportation	35.00 R\$/h	2.4	84.00	6
Packing	50.00 R\$/h	0.6	30.00	2
Labor	41.00 R\$/day	0.2	8.20	1
Plastic Film	1.27 R\$/m2	30	38.10	3
Diesel fuel	1.89 R\$/l	40.2	75.98	5
Silage			428.28	30
Interest on inventory	10.08 R\$/month	6	60.49	
		Silage	With	Without
			1,404.	916.0
Total Cost	R\$/ha		78	1
Dry matter cost	R\$/kg		0.156	0.102
Fresh forage cost	R\$/kg		0.047	0.031

Costs estimated for 2010/2011 harvest. Harvested with auto-propelled forager (6m platform) and transported with dump trucks (cost approximately R\$ 35/hour for the forager).

Source: Fundação ABC (2011).

Table 4 – Production cost of oat haylage.

Number of cuts	1 Cut			
Dry matter yield	4000 kg/ha			
Harvesting stage	Initial Panicle Emission			
Annual return on capital	8.75 %			
Annual interest loan (bank loan)	8.75 %			
Financial cycle	5 months			
Ítems	R\$ /unit	quantity/ha	R\$/ha	%
Seeds			101.64	9
Common black	1.09 /kg	80	87.20	8
Cruiser 350 FS	180.50 /L	0.08	14.44	1

Fertilizers			441.61	40
Herbicides			25.62	2
Machinery			161.50	15
Sprayer – 2000L	10.00 /ha	2.00	20.00	2
Dual-disk fertilizer spreader	9.00 /ha	1.00	9.00	1
Planter – 19 rows	55.00 /ha	1.00	55.00	5
Manure spreader	72.00 /ha	1.00	72.00	7
Limestone spreader – 5000 kg	22.00 /ha	0.25	5.50	0
Direct Costs			730.37	66
Financing Costs			26.63	2
Administration			0.00	0
Indirect Costs			26.63	2
Mower conditioner	107.00 R\$/h	0.5	53.50	5
Hay tedder	29.00 R\$/h	0.5	14.50	1
Hay rake	32.00 R\$/h	0.5	16.00	1
Collection	320.00 R\$/h	0.3	96.00	9
Transportation	35.00 R\$/h	0.9	31.50	3
Packing	50.00 R\$/h	0.3	15.00	1
Labor	41.00 R\$/day	0.2	8.20	1
Plastic Film	1.27 R\$/m2	25	31.75	3
Diesel fuel	1.89 R\$/l	17.7	33.45	3
Silage			299.90	27
Interest on Inventory				
	7.93 R\$/month	6	47.56	
		Silage	With	Without
			1,104.4	
Total Costs	R\$/ha		6	757.00
Dry Matter Cost	R\$/kg		0.276	0.189

Costs estimated for 2010/2011 harvest. Harvested with auto-propelled forager (6m platform) and transported with dump trucks (cost approximately R\$ 35/hour for the forager).

Source: Fundação ABC (2011).

Table 5 presents operational costs of machinery and forage equipment as a reference price for services and production costs in the Campos Gerais region. Note that fuel cost, which is often considered a primary cost component, did not exceed 50% of total costs of tractor hours. Depreciation of forage equipment was considered to be 40% of the hourly cost.

As for the value charged by companies hired to harvest with auto-propelled foragers, the mean value charged in the region was about R\$ 420 to R\$ 460. Due to the large supply of this service in the region, its cost has decreased over the last few years. Companies offset investments in machinery and equipment by offering services in other regions or states. The harvest season begins with cutting corn from December (early planting) to July in off season areas (from western Paraná to the central western region of Brazil).

Table 5 – Machinery and equipment costs for conserved forage production.

Equipment	Cost Components – R\$/hour					Total/hour	Total/ha.
	Depret.	Insurance	Interest	Maint.	Comb.		
Tractor 70- 90cv 4x4	6.90	0.80	6.10	6.50	18.90	39.20	-
Tractor 90 –110cv 4x4	8.60	1.00	7.50	8.10	24.60	49.80	-
John Deere7350/New Holland 9050	89.50	10.00	72.70	76.70	104.00	352.90	90.80 ¹
Single row forage harvester	6.10	0.20	3.30	5.50	-	15.10	107.50 ¹
Dual row forage harvester*	12.20	0.40	6.50	10.80	-	29.90	100.00 ¹
Harvester (cas./silt)	8.40	0.30	4.50	7.50	-	20.70	36.90
4-wheel trailer	2.00	0.60	1.30	1.00	-	4.00	-
Mower Conditioner	27.90	1.00	14.90	18.60	-	62.50	55.90
Tedder – 3m	4.80	0.20	2.60	2.70	-	10.10	29.80
Baler - rectangular	7.00	0.30	4.60	4.40	-	16.40	5.50
Baler – cylindrical	14.60	0.70	9.60	9.10	-	33.90	6.90

Source: Fundação ABC (2011). ¹ For corn silage; *Attached to a tractor with 110 cv. Remaining pull type equipment attached to tractors with 90cv.

5. Impact of harvest efficiency on conserved forage quality

A comparison of harvest costs per area of corn crop for silage production (Table 5) verified that harvest with self-propelled forager costs approximately 15% less than a single row harvester and 9% less than one with two rows. However, the primary benefit to harvesting with self-propelled machines is the possibility of obtaining better quality forage.

The ideal moment to harvesting the corn, expressed by the dry matter content of the whole plant and the maturity stage of the kernel, has a significant impact on silage quality. This point occurs when the plant accumulates the greatest quantity of dry matter with high nutritional quality. In general, it happens when the kernel attain the hard-floury stage, and the whole plant reach 32 to 38% of dry matter. Meanwhile, the difficulty in harvesting within this ideal range is evident if one considers problems such as frequent rain and accelerated maturity in the summer and precociousness and risk of loss due to disease or frost during the off season.

Forms to lessen these risks basically consist of good agricultural planning to create the widest harvest window possible for cutting associated with practices such as combining hybrids with different cycles with staggered seeding so that the ideal moments for cutting appear in a sequenced way. A second phase includes all the steps in silage such as cutting, transportation and packing in a quick efficient manner which will certainly have a positive effect on the quality of the silage produced.

As few producers consider these variables during crop planning for silage production, the most common practice is to anticipate the harvest. Research conducted on the 2010 harvest in Carambeí, PR with the P30F36 hybrid indicate that early cutting significantly reduces the quality of corn silage, and consequently, its potential to be transformed into the milk or meat (Table 6).

Table 6. Field productivity and nutritional quality of corn silage at different harvesting periods

	Dry matter content at harvesting			
	27%	31%	35%	39%
DM yield, kg/ha	14,680	16,180	17,660	21,050
NDF, %	53.7	49.1	46.6	41.2
TDN, %	67.6	68.3	66.5	70.2

Milk, kg/ton of DM	1,358	1,394	1,362	1,484
*Milk kg/ha	19,930	22,552	24,045	31,238
*Meat kg/ha	1,900	2,159	2,171	3,042

Source: Pereira et al. (2010) – ongoing study. *estimates

Studies made by Marquardt (2007) and Kruppa (2009) on silage quality in two Brazilian regions indicate that early cutting of corn for silage is the most common error made regardless of the form of harvest. Even in regions with elevated use of auto-propelled harvesters, denominated region A, more than 40% of the sample evaluated presented dry matter values below 30%, while in regions that mainly use single row harvesters, denominated region B, this occurs in more than 50% of the samples (Table 7).

Table 7 – Dry matter content of silage samples collected from two Brazilian regions.

Dry matter%	Region A (% samples)		Region B (% samples)
	2006	2007	2008
20 - 25	4	6	-
25 - 30	41	40	58
30 - 35	34	33	36
35 - 40	21	21	6

Adapted from Marquardt (2007) and Kruppa (2009).

Corn harvesting with dry matter content higher than 35-37% associated with greater participation of forage grains demand greater efficiency in the quality of cutting and kernel processing. Removal of air from the silo mass is fundamental to reduce the respiration process and increase the temperature, whose main consequence is the energy losses as heat. Thus, uniform chopping of the particles allows packing to be more intense, increasing the silage density and the storage capacity of the silo, which significantly reduces forage losses.

Furthermore, it is possible to produce good quality silage with single or double row harvesters. Aware of the lower harvest efficiency, the farmer should have a good seeding strategy for a staggered harvest, daily equipment maintenance and division of forage stored in lower

capacity silos that can be closed more quickly during ensiling and larger portions removed during the silage feedout.

6. Final considerations

Custom harvesting services is a very interesting option that has grown significantly in Brazil. From a financial point of view, the advantage for the producer who hires these services is reduced investment in acquisitions as well as lower depreciation costs and maintenance.

Large scale harvesters (self-propelled) have greater operational efficiency, allowing for a quicker harvesting within the ideal time for cutting, less time for filling and closing the silo and, eventually greater availability of tractors for packing, which all contribute to the quality of the silage produced. However, producers do not take advantage of a significant part of these benefits. This fact could be due to lack of a planting strategy, unavailability of machinery at the time of harvest or even a lack of knowledge of the machine's resources.

Use of single or dual row forage harvesters does not impede the production of high quality silage as long as some seeding strategy and silage plan are involved.

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MODELS TO ESTIMATE THE NUTRITIVE VALUE AND INTAKE OF CONSERVED FORAGES

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Introduction

Ruminant animals have a unique ability to produce high quality human food from resources (pastures, forages, by-products) that otherwise cannot be utilised for food production. This is based on the symbiosis between rumen microbes and host animal: the host provides ideal conditions for anaerobic fermentation in the rumen and the microbes produce volatile fatty acids (VFA) and microbial cells that are the main energy and protein sources for the host animal. Rumen microbes can ferment both fibre and non-fibre carbohydrates (NFC), whereas mammalian enzymes can digest only NFC. However, microbial fermentation in the rumen does not take place without losses, since part of the digestible energy is lost as methane and fermentation heat, and microbial cells are not completely digestible in the animal.

Variation in nutritive value of forages is much greater than that of concentrate feeds. Therefore, an accurate and precise determination of the nutritive value of forages is required for optimal utilization of farm-produced forages in ruminant livestock. Determination of forage feeding value is also important in attempts to minimize the impact of ruminant livestock production systems on the environment. Most emphasis in research has focused on the determination of forage digestibility/energy concentration and protein value. However, the intake potential has a much greater influence on nutrient intake than forage nutrient concentrations. Intake potential can be considered as an intrinsic characteristic of forages at the time of harvest, but the changes in forage composition during conservation can markedly influence the extent to which this potential is realized. The intake potential of forage is usually closely associated with its nutritive value emphasizing the importance of accurate estimation of forage digestibility.

The methods available for the evaluation of the nutritive value include chemical analysis, *in vitro* digestibility with rumen bacterial inoculum or enzymes, *in situ* incubations in nylon bags

in the rumen and near infrared reflectance spectroscopy (NIRS). With ensiled forages both the extent and type of in-silo fermentation affect intake potential stressing the importance of the analysis of silage fermentation characteristics. In addition to the analysis of feed parameters, appropriate models are required to predict nutritive value and intake potential from these parameters.

The objectives of this paper are to discuss the models and methods used in the evaluation of the nutritive value and intake of conserved forages. A special emphasis is focused on the digestibility determination due to its fundamental role in determining both energy and protein value of forages as well as intake potential.

Digestibility

Faecal energy is a greater and more variable loss of feed gross energy than methane and urinary energy, and therefore accurate determination of digestibility is a prerequisite in the determination of feed metabolizable energy (ME) concentration and of forage/diet intake potential. Much effort has been directed toward developing empirical regression equations that relate various chemical components to digestibility. These attempts have not been very successful because of the lack of real causal relationships, and large interspecies and environmental effects on the relationship between composition and digestibility (Van Soest 1994). Within year and forage species the relationship between chemical parameters and digestibility are usually reasonably good, but in global regression analysis over years and forage types the relationships were poor and residual standard deviation (RSD) was not markedly less than the standard deviation (SD) of digestibility in the data (Huhtanen et al., 2006a). Because forage indigestible NDF (iNDF) fraction in cell wall is attributable to cross-linking between lignin and hemicellulose when plants mature (Van Soest, 1994), attempts have been made to predict digestibility and iNDF from lignin concentration in DM or NDF (see Traxler et al., 1998). Despite this biological relevance, it has not been successful due to relatively high proportional errors in lignin analyses, as well as differences between forage types in lignin to iNDF ratio (Huhtanen et al., 2006). Within forage type permanganate lignin was clearly better predictor of digestibility than fibre fractions and CP, but RSD was greater than with biological methods.

Definitions

NDF (g/kg DM) = neutral detergent fibre. NDF represents the cell wall fraction of feed that be digested only by microbial enzymes. Pectins and β -glucans are also plant cell wall, but they are completely digested in ruminants. Digestibility of NDF is highly variable.

NDS (g/kg DM) = neutral detergent solubles. NDS can be digested by both mammalian and microbial enzymes. Originally NDS was defined as DM – NDF, but because ash does not provide any energy to the animals, organic matter (OM) – NDF could nutritionally be a more appropriate definition for NDS. NDS is considered as a uniform nutritional entity, i.e. it has a constant (0.98) true digestibility (Van Soest, 1994). There is indication that some differences exist in the true digestibility of NDS between forage types (Huhtanen et al., 2006).

iNDF (g/kg DM) = indigestible NDF. Digestibility of iNDF is by definition constant (zero) and therefore iNDF is a uniform nutritional entity. It is an intrinsic feed character and it is usually determined a long-term *in situ* incubation in the rumen (Huhtanen et al., 1994) or *in vitro* (Van Soest et al., 2004). Nylon bags of small pore size (6 – 17 μ m) is used to avoid particle losses and long incubation time to ensure that maximum extent of digestion is obtained.

pdNDF (g/kg DM) = potentially digestible NDF = NDF – iNDF. Digestibility of pdNDF is variable, but variation is much less variable than total NDF digestibility. Typically the digestibility of pdNDF is approximately 0.85 in sheep fed forages at maintenance level (Huhtanen et al., 2006a) and 0.75 in dairy cows fed mixed grass silage based diets (Nousiainen et al., 2009).

uNDF (g/kg DM) = undigested NDF = faecal NDF. uNDF is a fraction of DM that is excreted in faeces. Because pdNDF is not completely digested in the animal within the residence time in the digestive tract, uNDF is always greater than iNDF. In the literature terminology can be confusing, since iNDF is sometimes used to denote uNDF.

updNDF (g/kg DM) = undigested pdNDF = uNDF – iNDF. This fraction represents the variable loss of potentially digestible OM in faeces, since faecal NDS is mainly of metabolic and endogenous origin and represents obligatory loss of potentially digestible OM.

NDFD (fraction of unity) = NDF digestibility = $(\text{NDF} - \text{uNDF}) / \text{NDF}$.

pNDFD (fraction of unity) = potential extent of NDFD = $\text{pdNDF} / \text{NDF} = (\text{NDF} - \text{iNDF}) / \text{NDF}$

pdNDFD (fraction of unity) = pdNDF digestibility = $(\text{pdNDF} - \text{uNDF}) / \text{pdNDF} = [(\text{NDF} - \text{iNDF}) - \text{uNDF}] / (\text{NDF} - \text{iNDF})$. Digestibility of pdNDF is always higher than that of NDF. Note that pdNDFD is not the same as pNDFD.

dNDF (g/kg DM) = digested NDF = $\text{NDFD} \times \text{NDF} = \text{pdNDFD} \times \text{pdNDF} = \text{NDF} - \text{uNDF}$. Note that dNDF is smaller than pdNDF, since pdNDF is not completely digested.

Digestible NDS (digestible NDS) = $a + b \times \text{NDS}$ (b = true digestibility ~ 0.98 , a = metabolic faecal OM ~ 100 g/kg DMI). Metabolic faecal OM consists of undigested microbial cells synthesized in the rumen, microbial cells produced in the hind-gut, sloughed epithelial cells and digestive enzymes that are not reabsorbed.

D-value (g/kg DM) = concentration of digestible OM = $\text{dNDF} + \text{dNDS}$;

Biological methods

Owing to the constraints in routine measurements of *in vivo* digestibility, several *in vitro* laboratory methods have been used for estimating forage digestibility. The two-stage rumen fluid *in vitro* technique developed by Tilley and Terry (1963) and Goering and Van Soest (1970) are the most widely used methods. Both techniques are based on the incubation of forage samples in rumen fluid for 48 h followed by incubation in pepsin-HCl (Tilley and Terry, 1963) or extraction with neutral detergent (Goering and Van Soest, 1970). Tilley and Terry (1963) demonstrated a close correlation between DM digestibility (DMD) determined *in vivo* and *in vitro* and reported that the values determined based on *in vitro* incubations were almost the same as DMD determined in sheep.

Due to difficulties in obtaining rumen fluid in commercial laboratories, standardization of the *in vitro* incubation system, variation in the activity of rumen fluid and increasing concern over the use of surgically modified animals, enzymatic *in vitro* procedures for the determination of forage digestibility have been developed and evaluated (Jones and Theodorou, 2000). The cellulase method differs from measurements of *in vivo* digestion in at least two key aspects: no endogenous matter is produced such that solubility reflects true rather than apparent digestibility,

and the capacity of commercial enzymes to degrade cell wall carbohydrates is lower than that of rumen microbes (McQueen and Van Soest, 1975; Nousiainen, 2004). Therefore regression equations are required to predict organic matter digestibility (OMD) from cellulase solubility. In a recent evaluation based on 86 forage samples of known *in vivo* digestibility measured in sheep the coefficient of determination (R^2) was 0.80 and RSD 0.025 units (Huhtanen *et al.*, 2006a). Because the relationship between pepsin-cellulase solubility (OMS) and *in vivo* OMD was highly dependent on forage type, using a forage specific correction equation was found to increase the R^2 of this relationship to 0.93 associated with a decrease in RSD to 0.0152. Recent study (Jančík *et al.*, 2011) indicates that the relationship between OMS and OMD may also vary between the species of primary growth grass.

Determination of iNDF concentration either by extended *in vitro* or *in situ* incubation divides forage OM into three components: NDS, iNDF and pdNDF. Two of these fractions (NDS, iNDF) are nutritionally uniform entities that have a constant digestibility; NDS is (almost) completely digestible and iNDF by definition is indigestible. The close relationship between iNDF determined by 12-d *in situ* incubations in nylon bags of small pore size and *in vivo* OMD was first demonstrated by Nousiainen *et al.* (2003) and later confirmed by a larger number of samples of different forage types (Huhtanen *et al.* 2006a). In the later study the relationship between iNDF and *in vivo* OMD was more uniform for iNDF compared with the OMS (0.019 vs. 0.025). For the prediction of D-value the following simple regression model was derived from this data:

$$\text{D-value (g/kg DM)} = 891 \pm 18.5 - 1.19 \pm 0.043 \times \text{iNDF} - 0.09 \pm 0.021 \times \text{pdNDF} - 1.02 \pm 0.14 \times \text{Ash (n=86, } R^2 = 0.91, \text{ RSD} = 15.7)$$

All parameters were statistically significant ($P < 0.001$) and biologically meaningful. The deviation of the intercept from 1000 (by 109 g/kg) is biologically equivalent to the metabolic faecal OM output. The coefficient -0.09 for pdNDF indicates that maximum digestibility of forage pdNDF was 0.91, whereas the coefficient < -1.0 for iNDF suggests a decrease in pdNDF digestibility with increased iNDF concentration. An advantage of iNDF is that it can be successfully calibrated for NIRS (Nousiainen *et al.*, 2004)

Combining the data from a recent study by Krizsan *et al.* (2011) and Huhtanen *et al.* (2006a) resulted in total 128 forages. Also in the combined dataset iNDF predicted *in vivo* OMD more precisely than OMS ($R^2 = 0.87$ vs. 0.78; RSD = 0.0216 vs. 0.0278). Greater RSD in the

combined dataset may be due to more heterogeneous set of samples or greater RSD of *in vivo* OMD. In the study of Krizsan et al. (2011) iNDF tended to under-predict OMD of lucerne samples and over-predict OMD of straw samples, especially treated straw.

Sources of RSD

It is often believed that prediction errors of OMD by the *in vitro* methods are entirely due to the inaccuracy of the *in vitro* methods. However, there is also random variation in determination of *in vivo* OMD. Van Soest (1994) stated that SD between the animals in carefully conducted digestibility experiments is 0.02 units, i.e. standard error (SE) of OMD estimates in typical digestibility trials with 4 sheep per feed would be 0.01. The mean SE in digestibility trials conducted according to Latin square was smaller (0.007; Nousiainen, 2004), probably because animal variation is excluded from the residual. Unless the number of sheep in digestibility trials is increased above four, 0.007 – 0.010 units could be considered as the minimum RSD of OMD prediction by the laboratory methods. This excludes sampling errors, random variation in the laboratory methods and true variation in the relationship between laboratory and *in vivo* estimates of OMD. In our datasets SD of replicate samples was 5 g/kg for iNDF and OMS methods, and 9.5 – 11.0 g/kg for the *in vitro* methods using 48 or 96 h incubation in rumen fluid. Sampling error (4 samples per feed) for OMS was 8 g/kg. Smaller SD for the iNDF than rumen fluid *in vitro* methods rumen is probably due to the longer incubation period.

A Monte Carlo simulation study was conducted to evaluate feasible levels of RSD in predicting OMD by the laboratory methods. The dataset (n = 86 forages) of Huhtanen et al. (2006a) was used to develop synthetic data by using the relationship between OMS and OMD estimated by a simple regression analysis (RSD = 0). The following sources of errors (SE; g/kg) were assumed for OMS: sampling error 4 ($8 / \sqrt{4}$) and error of duplicate OMS analysis 3.5 ($4 / \sqrt{2}$) ; and for OMD: random error of Latin Square (4×4) digestibility trial 0.007 and random error of group of four sheep (SD = 0.020 $\rightarrow$ SE = 0.010). Mean RSD of OMD prediction in 1 000 simulations was 0.0126 units that is smaller than observed RSD within forage type (0.0152). Excluding the effect of sheep group RSD reduced to 0.0082 that is smaller than observed RSD adjusted for the random study effect (0.0106). Greater observed values could result from the true variation in the relationship between OMS and OMD. However, it should be noted that both OMS and OMD were analyzed in the same laboratory. When the datasets are

compiled from different laboratories, RSD is likely to increase because greater between than within laboratory variation in determination *in vitro* digestibility and also because of between laboratory differences in the *in vivo* digestibility (Cochran and Galyean, 1994).

Mechanistic models

Models, which are based on fractionating feed components into entities that behave uniformly, can be considered mechanistic. Van Soest (1967) developed a comprehensive system of feed analysis and its application to forages. He divided the feed into NDS fraction that is essentially completely available, but its digestibility is apparently incomplete and variable because of faecal endogenous and microbial non-cell wall material. The second fraction corresponds to the plant cell wall and its availability is controlled by structural features that link cellulose, hemicellulose and lignin together. Cell wall fraction is not uniform between forages. Goering and Van Soest (1970) presented a summative model to describe availability of forage DM:

$$dDM = 0.98 \times NDS + NDFD \times NDF - M$$
, where dDM = digestible DM, NDFD = coefficient of NDF digestibility, and M = microbial and endogenous faecal output. Theoretically this model is sound, but generally NDFD is not known. Conrad et al. (1984) developed this model further by dividing feeds into NDS and pdNDF. They applied surface area law (mass raised to power 0.67) to calculate NDF that is covered by lignin, and this proportion was multiplied by lignin-free NDF to obtain an estimate of the pdNDF. Weiss et al. (1992) revised the Conrad et al. (1984) model and it was adopted by NRC (2001) to estimate total digestible nutrients (TDN). In the revised model NDS fraction was divided into CP, fatty acids (FA) and NFC. The NFC fraction is calculated as difference (OM – CP – Fat – NDF). Neutral detergent fibre in the equation is corrected for NDF-bound CP (NDICP) to avoid double subtraction; NDICP is also included in total CP concentration.

The model was evaluated with data from digestibility trials with sheep fed at the maintenance level conducted in Finland (Huhtanen et al., 2006a). The data included 77 silages (primary and regrowth timothy-meadow fescue grass, red clover, and whole crop barley or wheat). Lignin was analysed as permanganate lignin (NRC model use ADL = acid detergent lignin). Concentration of NDICP was assumed to be 0.15 of total CP (Rinne et al., 1997). During in-silo fermentation a large proportion of NDICP is degraded (Jones et al., 1992; Rinne et al., 1997) and the proportion of NDICP is less than in dried forages. Truly digestible NDF is

$$\text{tdNDF} = 0.75 \times (\text{NDF} - \text{NDICP} - \text{Lignin}) \times [1 - (\text{Lignin}/(\text{NDF} - \text{NDICP}))^{0.667}].$$

Truly digestible CP (tdCP) is calculated as $\text{tdCP} = \text{CP} \times \exp(-1.2 \times \text{ADICP}/\text{CP})$, where ADICP is acid detergent insoluble protein. An empirical equation was derived by regressing iNDF concentration against proportion of ADICP of CP:

$$\text{ADICP (g/kg CP)} = 0.256 \times \text{iNDF (g/kg DM)} + 6.7 \quad (N = 36; R^2 = 0.76, \text{RMSE} = 5.0).$$

The samples were from the dataset used in the model evaluation.

A FA concentration of 20 g/kg DM was assumed. Variation in forage FA concentration is small and mainly related to the maturity at harvest (Advidsson, 2009). The effects of possible deviations from 20 on TDN would be minimal, since the model assumed complete digestibility for FA and 0.98 for NFC; a deviation of 10 g/kg DM only corresponds to 0.2 g/kg DM in digestible OM and higher TDN of FA would influence similarly on predicted TDN and *in vivo* TDN.

The model clearly underestimated tdNDF (Figure 1). The RSD was 57 g/kg and the mean bias (mean observed - mean predicted) was 44 (SE = 3.8; $P < 0.001$). The model under-predicted especially early harvested primary growth grass silages, whereas tdNDF of whole-crop silages was over-predicted. Under-prediction can partly be related to using permanganate lignin instead of ADL, since ADL concentrations are usually approximately 0.80 of that of permanganate lignin. However, using a relative value of 0.80 of determined lignin concentration decreased mean bias only from 44 to 32 g/kg DM and the range of residual did not markedly reduce (164 vs. 160 g/kg DM).

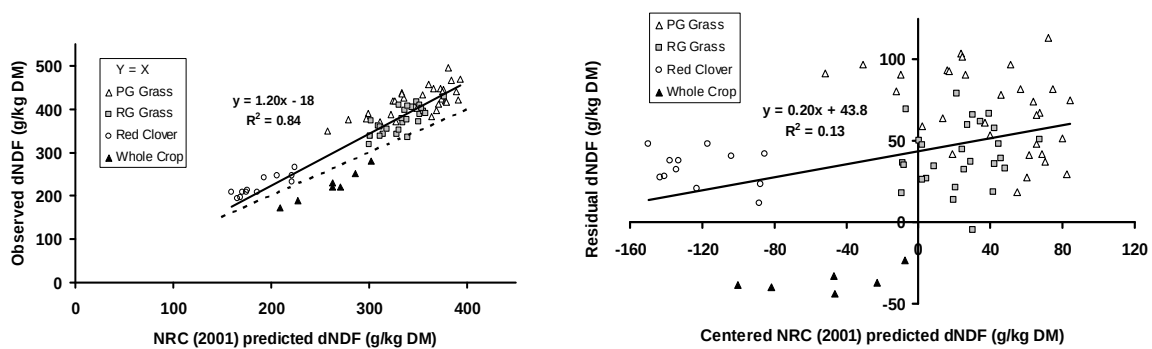


Figure 1. Observed against predicted (NRC, 2001) digestible NDF (dNDF) concentration (left) and the plot of residuals against centered predicted values of dNDF (right). Values were centered by subtracting mean dNDF from each data point.

The main reason for the poor performance of NRC (2001) model for predicting tdNDF is an assumption of a constant digestibility of 0.75 for pdNDF. This value is too low, since in the current dataset even the total NDFD exceeded 0.75 for 18 silages out of 77 silages. The observed mean value of pdNDFD *in vivo* (0.85; SD = 0.057) was clearly higher than the default value of the NRC (2001) model. Weiss et al. (1992) used a single compartment rumen model by Waldo et al. (1972) in deriving the value 0.75 for the pdNDFD. However, using the same mean rumen retention time ($1 / (0.02/\text{h}) = 50 \text{ h}$) and digestion rate (0.076/h) in a two-compartment rumen model (Allen and Mertens, 1988) gives a similar value (0.87) to the current *in vivo* data. On average, the NRC (2001) model predicted pdNDF reasonably accurately (mean bias -17 g/kg; $P < 0.01$) and precisely ($R^2 = 0.90$) relative to the *in situ* pdNDF. However, the mean bias was highly variable between forage type ranging from 7 (primary growth grass) to -51 (whole crops). Interestingly, the surface area lignin model did not predict observed iNDF concentration more precisely than the linear model (R^2 0.50 vs. 0.50). Residual SD of tdNDF was acceptable (20.1) when analysed with the GLM procedure of SAS using forage type as a class variable, but the slope (0.82) was different from 1.0 ($P < 0.05$) and the forage type effects were highly significant.

Mean bias in TDN was smaller than in tdNDF (18 vs. 44 g/kg DM), but random variation was large as indicated by relatively low R^2 (Figure 2). The residuals varied markedly between the forage types. *In vivo* TDN was calculated by adding $1.25 \times \text{FA}$ (20 g/kg DM) to correspond the NRC (2001) TDN equation. The mean bias was significant ($P < 0.001$). Metabolic TDN (70 g/kg DM) in NRC (2001) was determined by Weiss et al. (1992). This value is clearly lower than the observed faecal output of NDS (105 g/kg DM), especially considering that metabolic fat component was multiplied by 2.25. On OM basis NRC (2001) faecal metabolic output would be 62-64 g/kg DM. Indigestible components of CP and NFC accounted for 10.5 g leaving about 30 g/kg DM difference in faecal metabolic output. Part of this can be attributed to indigestible lipid components, but also to the metabolic output of CP and NFC which were greater (39 and 38 g/kg DM) than the values (27 and 31 g/kg DM) determined by Weiss et al. (1992).

Although the errors in tdNDF and tdNDS partly counterbalanced, RSD for the NRC (2001) model remained rather high (41 g/kg DM), and even root mean squared error (RMSE) that describe random variation across the regression line was relatively high (37 g/kg DM). A simple bivariate empirical regression model ($\text{TDN} = \text{iNDF} + \text{Ash}$) was much more precise (RMSE = 17.8) than the NRC (2011) model in predicting TDN. The residual of the NRC (2001)

model was strongly ($R^2 = 0.61$) and negatively related to iNDF/NDF ratio, whereas the correlation to the corresponding Lignin/NDF ratio was poor (Figure 3). This indicates that the Lignin/NDF ratio does not describe accurately the digestibility of pdNDF fraction. This is not very surprising, since both permanganate lignin (Buxton and Russell, 1988) and ADL (Jung et al., 1997) had a stronger influence on the NDFD of grass than on the NDFD of legumes.

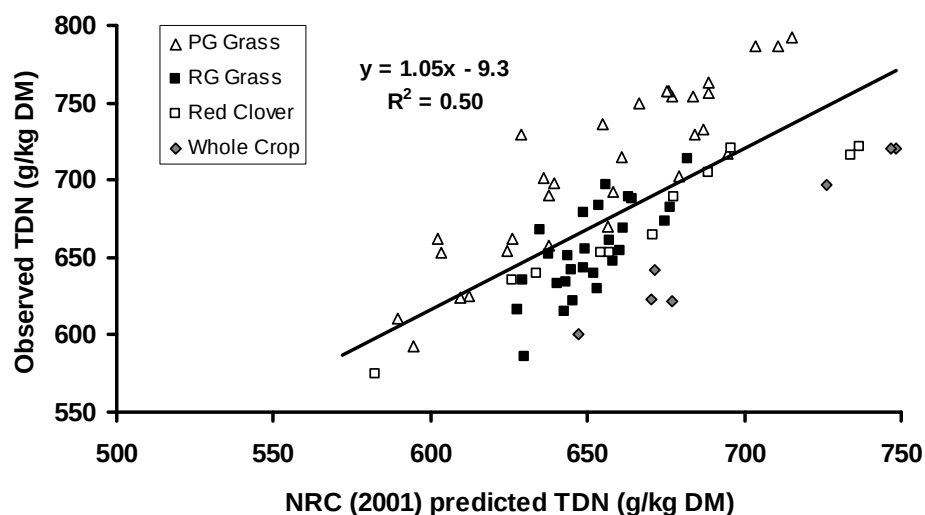


Figure 2. Observed against predicted (NRC, 2001) TDN concentration.

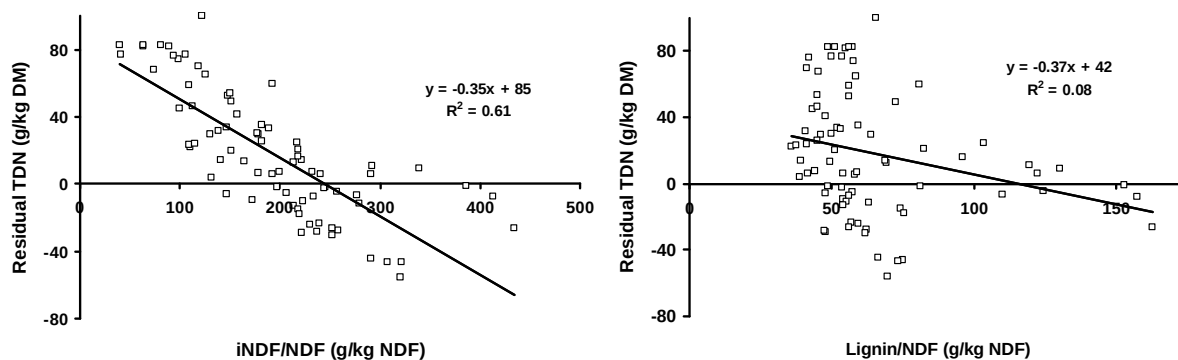


Figure 3. Relationships between iNDF/NDF and Lignin/NDF ratios to residual (observed – predicted) TDN.

Simplification of the NRC (2001) equation by using total NDF with NDICP correction and uniting NDS fraction (true digestibility = 0.98) only marginally increased RSD of tdNDF (44). It can be concluded that the major problems of the NRC (2001) TDN model are poor ability

of lignin to predict iNDF, constant and too low pdNDFD and underestimation of faecal metabolic TDN, even though the latter partly counterbalance the errors in tdNDF.

Mertens (2002) derived a simple equation in which dNDF is a linear function of NDF and lignin:
$$\text{dNDF} = a \times \text{NDF (g kg}^{-1} \text{ DM)} + b \times \text{Lignin (g kg}^{-1} \text{ DM)},$$

where a and b can be estimated by regression. Constant a is the digestibility coefficient of pdNDF and constant b is the proportion of NDF protected by lignin. This equation has no intercept, i.e. neither endogenous nor microbial excretion of NDF. Huhtanen et al. (2006a) modified this model by replacing NDF with pdNDF and lignin with iNDF and estimated parameter values by Excel Solver. The simplified model of Mertens (2002) described dNDF as precisely as the surface area law, but the precision was markedly improved when pdNDF and iNDF were used in the model instead of NDF and lignin (RSD of D-value within a forage type 23.0 vs. 13.8 g/kg DM).

Mechanistic dynamic models

Waldo et al. (1972) developed a model that incorporates digestion and passage to estimate digestibility:

$$\text{Digestibility (D)} = k_d / (k_d + k_p),$$

where k_d = digestion rate (1/h) and k_p = passage rate (1/h). This model is not adequate for NDF, since it does not take into account that only part of NDF is potentially digestible. For pdNDF the model can be described as:

$$D = \text{pdNDF} \times k_d / (k_d + k_p)$$

The single compartment model indicates that digestibility is competition between digestion and passage; the faster the digestion rate in relation to passage rate the higher digestibility will be. Mertens (1993) concluded that assuming the rumen as a single compartment is not an adequate mathematical or biological representation of rumen functions. Mean rumen retention time (MRRT) in sheep fed at maintenance is approximately 50 h and its reciprocal passage rate 1/50 h = 0.02/h. Average *in vivo* pdNDFD of different forages ($n = 86$) was 0.85. To achieve this digestibility with a passage rate of 0.02, digestion rate should be 0.113 – an unrealistically high value. Digestion rate should be 0.18 to reach a pdNDFD of 0.90, which is not an unusual value for early harvested grass. Alternatively, using a realistic value of 0.06 for digestion rate, the passage rate should be only 0.0106 (MRRT = 94 h) to achieve pdNDFD of 0.85. With a

digestion rate of 0.08 MRRT should be 112 h to achieve a pdNDFD of 0.90 in a single compartment system. These examples clearly demonstrate that the single compartment rumen model is inadequate in predicting pdNDFD accurately with realistic parameter values. Despite this, in the literature this is the most commonly used model to describe NDF digestion or protein degradation (with protein an immediately degraded component is included in the model).

Allen and Mertens (1988) derived a model that includes selective retention of feed particles in the rumen:

$$D = \text{pdNDFD} \times (k_d / (k_d + k_r) [1 + k_r / (k_d + k_p)]), \text{ where}$$

k_d = digestion rate (1/h), k_r = rate of release from the rumen non-escapable pool to the escapable pool (1/h) and k_p = passage rate of feed particles from escapable pool (1/h). Parameter k_r describes selective retention of particles in the rumen, i.e. the rate in which recently ingested feed particles become eligible for passage. These processes include particle size reduction as a result of chewing and increase in specific gravity as a result of reduced fermentation activity occurring simultaneously with time after ingestion. Using the values of 0.06 for digestion rate and 50 h MRRT (20 + 30 h) for non-escapable and escapable pools) pdNDFD would be 0.84, whereas with the simple model it would be 0.75. Two-pool model predicts higher pdNDFD with same MRRT, because recently after ingestion the particles become gradually eligible for passage but digestion takes place also in the non-escapable pool. The effect of digestion lag on the digestibility is less with the two-pool model compared with the one-pool model as the particles are not available for passage during the lag time. Allen and Mertens (1988) showed that the lag time will not affect digestibility, if there is a similar lag time for passage as for digestion.

Selective retention of feed particles in the rumen has been demonstrated by various techniques. The passage rate of feed particles from the rumen is inversely related to particle size (Poppi et al., 1980; Huhtanen et al., 2007a). Selective retention of feed particles in the rumen is also evident from duodenal marker excretion curves (Ellis et al., 1993; Huhtanen and Hristov, 2001; Lund, 2002), which clearly indicated an ascending phase. This challenges the interpretation of the rumen as a single compartment system, in which the probability of particles to escape is a random process. Also different particle size distribution in ruminal digesta and faeces demonstrates the mechanism of selective retention in the rumen.

Dynamic mechanistic models are excellent tools in understanding the mechanisms of fibre digestion, but to be useful in practical feed evaluation the predictions of feeding values

should be at least as accurate and precise as those with the empirical models based on *in vitro* and/or NIRS techniques. In addition to the theoretically correct model structure, accurate and precise parameter estimates are required to produce accurate estimates of feeding values. In the following methodologies to determine the parameter values for NDF digestion model are discussed.

Determination of model parameters

Potential NDF digestibility (pNDFD)

The concentration of iNDF was the most important parameter determining diet digestibility according to the Nordic Dairy Cow Model Karoline (Danfaer et al., 2006). Close empirical relationship between forage iNDF concentration and OMD also point out the importance to determine pNDFD. Potential NDFD can be described an intrinsic character of feed It should be determined in optimal conditions when only the plant characteristics – not e.g. the *in vitro* system - limit the digestion. Whether iNDF is determined either by *in vitro* or *in situ* incubations the animals should be fed a diet that ensures optimal environment for fibre digestion in the rumen.

The concentration of iNDF and pNDFD can be determined either as the asymptotic value of indigestible residue or digestibility by non-linear models or by long term incubations *in vitro* (Van Soest et al., 2004) or *in situ* (Huhtanen et al., 1994). Mertens (1993) recommended that at least three observations (time points) for each parameter to be estimated in the model. Most digestion models like a simple first-order model with discrete lag time have three parameters, indicating that nine time points are needed. However, in published literature the number of fermentation times seldom meets the minimum number, probably because of labour demand.

Long term *in vitro* or *in situ* incubations determine pNDFD and iNDF directly. The authors experience is that the variation in iNDF concentration between the animals and between replicates within animal is small and smaller than SD of *in vivo* OMD (see Page 6). In contrast, in the literature RSD is usually greater for potential DMD and NDFD digestion than for *in vivo* DMD/OMD and NDFD when *in situ* data determined using 5 – 6 fermentation times up to 72 – 96 h. In a Monte Carlo simulation study using the observed SD of NDF disappearance at six different fermentation times between 0 and 96 h (pNDFD = 0.80, k_d = 0.04/h and NDF = 550 g/kg DM) SD of calculated iNDF concentration was 11.7 g/kg DM. Increasing the number of

time points to nine decreased SD to 10.4 g/kg DM. It could be more beneficial to include time points beyond 96 h rather than increase them between 0 – 96 h. In addition, one long term incubation is less labour intensive than time series of *in vitro* or *in situ* incubations. *In vitro* study (Van Soest et al., 2004) clearly demonstrated that digestible NDF pool can be divided into rapidly and slowly digestible fractions. Consequently, it is possible that *in vitro* and especially *in situ* techniques can underestimate pNDFD unless fermentation times longer than 96 h are used. Rinne et al. (2002) found that the difference between observed pNDFD in 12-d *in situ* incubation and asymptotic NDFD derived from fermentation times up to 96 h increased with advancing maturity of grass. Small variability in iNDF concentration with long term *in situ* incubation strongly supports this method is estimating pNDFD for mechanistic models.

Attempts to predict digestion kinetic parameters from chemical composition have been met with variable success. A close linear relationship between lignin and iNDF contents for a diverse population of forage samples was reported by Smith et al. (1972) and Mertens (1973). In the dataset of Huhtanen et al. (2006a) lignin concentration was moderately correlated to iNDF ($R^2 = 0.50$), but prediction errors of iNDF (28.4) and pNDFD (0.065) were relatively high. A large part of variability resulted from between forage type variation in lignin to iNDF ratio as indicated by much smaller (18.0 and 0.031) prediction errors within forage type. In a recent study with tropical forages (Gomes et al., 2011) lignin analysed by various methods was significantly correlated with iNDF concentration, but the prediction errors were relatively high (58.7 – 87.3 g/kg NDF). It can be concluded that although lignin certainly plays a role in the cell wall degradation, and consequently is closely correlated with iNDF concentration, measurements of lignin cannot be universally used for the determination of iNDF concentration and pNDFD.

Digestion rate (k_d)

Digestion rate of pdNDF is an intrinsic character of feed and as pNDFD, it should be determined in conditions when only plant characteristics restrict digestion. The *in situ* method is the most commonly used method to estimate digestion rate of pdNDF. Several excellent reviews (Mertens, 1993; Stern et al., 1997; Nozière and Michalet-Doreau, 2000) have been published that provide a detailed insight into the sources of variation and methodological aspects of the procedure.

Estimates of NDFD derived from the rumen models using *in situ* data are seldom validated against *in vivo* data. Usually the values for ruminal NDFD calculated from the *in situ* kinetic data are low compared to the observed or expected *in vivo* NDFD. Archimède (1992) used *in situ* digestion kinetic data to predict ruminal NDFD estimated from duodenal flow (cited by Nozière and Michalet-Dureau 2000). The model clearly underestimated ruminal NDFD. The slope between predicted and observed values was 1.00 and the proportion of variance accounted for was relatively high ($R^2 = 0.65$). Underestimation of *in vivo* digestibility was suggested to be due to the underestimation of digestion rate by the *in situ* technique. The use of single compartment rumen model could be one reason for the underestimation of *in vivo* NDFD from the *in situ* data, especially if the passage rate estimates are only based on descending phase of marker excretion curve. Post-ruminal digestion has been suggested to explain under-prediction of NDFD by models based on the *in situ* data. However, the contribution of post-ruminal digestion to the total NDF digestion of typical dairy cow diets is marginal (see Huhtanen et al., 2010a).

Digestion rates determined by *in situ* incubation have been lower than values derived from rumen evacuation in studies conducted in different laboratories (Aitchison et al., 1986; Tamminga et al., 1989; Rinne et al., 2002). These findings suggest that normal microbial colonization of samples within the bags was not achieved and/or that conditions in the bag were sub-optimal. Meyer and Mackie (1986) showed that the bacterial population inside the bags was smaller than in the surrounding digesta, particularly for the cellulolytic bacterial populations. Lower fibrolytic activities in particle-associated microbes in bag residues than in rumen digesta (Huhtanen and Khalili, 1992; Nozière and Michalet-Doreau, 1996) is consistent with lower microbial numbers within the bags. Recently Jančík et al. (2011) reported significantly slower digestion rates of pdNDF with the *in situ* method compared with incubations of isolated NDF with automated *in vitro* gas production system. The correlation between the methods was reasonably good, but both the mean and slope bias were significant (Figure 4). Digestion rates estimated by a gravimetric *in vitro* method were higher than those determined *in situ* (Bossen et al., 2005), which also suggests that the *in vitro* techniques may allow a more accurate estimation of the true intrinsic k_d than the *in situ* technique.

The problem with determination of the digestion rate of pdNDF has been difficulties in validating the data. Comparing the accuracy and precision of two *in vitro* methods or *in vitro* vs. *in situ* almost worthless unless the model predicted values are compared with *in vivo* data.

Huhtanen et al. (2006b) proposed a method estimating *in vivo* k_d from the *in vivo* digestibility of pdNDF by solving the equation of Allen and Mertens (1988):

$$k_d = [-(k_p + k_r) + [(k_p + k_r)^2 + 4Dk_rk_p/(1 - D)]^{0.5}] / 2,$$

where D = *in vivo* pdNDFD and other parameters as described before. To estimate the rate of digestion indirectly by this method, an estimate of total mean retention time in the fermentation compartments and the distribution of the residence time between the two compartments are required. We have used an estimate of 50 h for the MRRT that is distributed as 40:60 between the two compartments, i.e. values of 0.05 and 0.033/h are used for k_r and k_p , respectively.

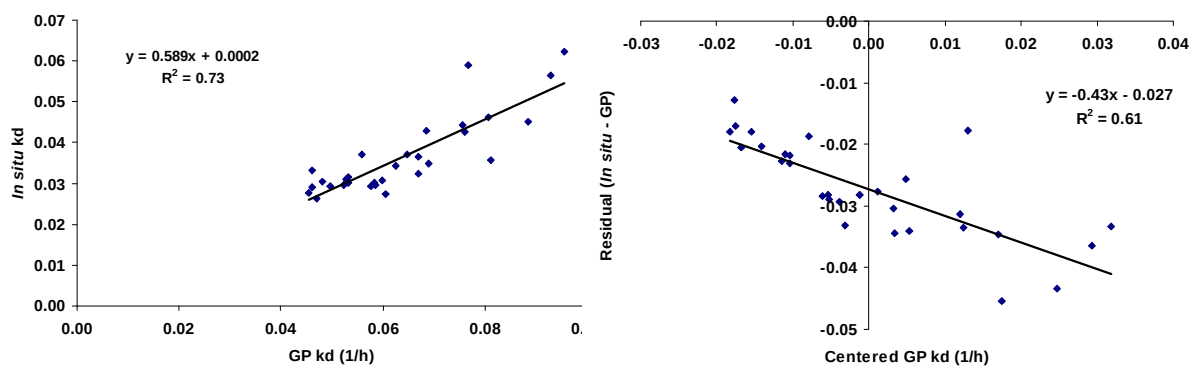


Figure 4. Relationship between pdNDF digestion rate determined by *in vitro* gas production (GP) method or *in situ* (left) and residual analysis (right). The data points with GP method are centered by subtracting the mean value of GP data from each data point. From Jančík et al. (2011).

Huhtanen et al. (2008a) validated this model by using 15 primary growth silages, of which *in vivo* digestibility was determined in sheep, iNDF by 12-d *in situ* incubation and k_d of isolated NDF by *in vitro* gas production technique. The two-pool Gompertz model was fitted to gas production data, ruminal pdNDFD was simulated by a two-compartment dynamic rumen model and first-order k_d determined by solving the previous equation. *In vivo* NDFD and k_d were predicted accurate and precisely (NDFD: $R^2 = 0.99$, RSD = 13 g/kg; k_d : $R^2 = 0.86$; RSD = 0.005/h) providing support for the *in vitro* gas production system to determining kinetic data.

Most of the new models are non-first-order models, i.e., the digestion rate is variable during fermentation. A limitation of the non-first-order kinetic parameters is that they cannot be used in steady-state rumen models. Moreover, the coefficients in gas production models may be difficult to interpret, because the models with several parameters do not permit straight forward

comparisons among different feeds (Pitt et al., 1999). To overcome these problems, Pitt et al. (1999) derived equations to estimate the effective first-order k_d from non-first-order models assuming that the rumen is one-compartment system with random passage of particles. Huhtanen et al. (2008b) extended this approach by including selective retention. A number of one- two- and three-compartment models were fitted to gas production data and pNDFD in the two-compartment rumen system was simulated. Interestingly, parameters from rather simple models such as first-order exponential with discrete lag predicted *in vivo* NDFD and k_d as accurately and precisely as more complicated models with 6 – 9 parameters despite the latter gave much better fit of gas production data. This was probably because the residuals between observed and predicted gas data was mainly during early fermentation, when the particles still remain in the rumen. Anyway, non-first-order digestion rates must be converted to single first-order rates for steady-state rumen models.

As for pNDFD there has also been interest to predict k_d from chemical composition of the diet. These attempts have not been very successful; within a feed type there can be reasonable correlation between lignin and k_d , but overall relationship is poor. In the dataset of Huhtanen et al. (2006a) comprising 86 forages (primary and regrowth grass, red clover and whole crop) the correlation between lignin and k_d derived from *in vivo* data was not significant. For a homogenous set of forages such as primary growth grass from the same field in the same year (Jančík et al., 2011) the logarithmic relationships between ADL and k_d determined by *in situ* or automated *in vitro* gas production system were moderately high ($R^2 = 0.61$ and 0.67), and even higher between iNDF and k_d (0.75 and 0.90 , respectively).

Passage rate

In addition to parameters for pNDFD and k_d , mechanistic dynamic models require estimates of passage kinetic parameters. Passage rate is influenced by intrinsic feed characteristic and extrinsic animal and diet factors. Details of the models, methodology and interpretation of passage kinetics are described in reviewed in detail by Ellis et al. (1994) and Huhtanen et al. (2006b). For predictions of digestibility at the maintenance level of feeding a constant value of passage kinetic parameters may be used. Our experience is that using 50 h MRTT divided as 40:60 between rumen non-escapable and escapable pools and k_d determined by the gas production technique gives NDFD estimates that are similar to observed *in vivo* values. Using an

appropriate rumen model incorporating selective retention is much more important than small differences in MRRT; for example, 34 and 50 h MRRT result in the same NDFD with one-pool and two-pool models ($k_d = 0.06/\text{h}$). Marker kinetic data indicate that the release of feed particles is a time-dependent process (e.g. Ellis et al., 1994); the rate of release of feed from non-escapable to escapable pool increases with time. In steady-state rumen models this process can be described by dividing the non-escapable pool (20 h) into two pools (10 + 10 h) in the case of gamma2 time-dependency. Model simulations (Huhtanen et al., 2006b) suggest that ruminal NDFD would increase about 0.02 units by including time-dependency in the first rumen compartment. Distribution of MRRT between the two rumen pools will also influence the simulated NDFD estimates but within the range from 30:70 to 70:30 the effect is not more than 0.01 units.

Passage rate is influenced by diet composition and feed intake in addition to intrinsic feed characteristics. In a recent meta-analysis (Krizsan et al., 2010) NDF intake (in g/kg of BW) was the most important dietary factor influencing the passage rate of iNDF estimated by rumen evacuation technique. Interestingly, observed iNDF passage rates were much slower than those predicted by NRC (2001) and the Cornell Net Carbohydrate and Protein System (CNCPS; Fox, 2004). The reason for the high passage rate estimates of these models is because the estimates are based on the passage data derived from the descending phase of marker excretion curve, and the retention in the non-escapable pool is ignored.

Depression in digestibility with increased intake can be predicted either by empirical models as described by NRC (2001) and Huhtanen et al. (2009) or by mechanistic models (e.g. Danfær et al., 2006). The NRC (2001) model predicts much greater depression in digestibility with increased intake than the model of Huhtanen et al (2009), probably because of the differences in diet composition in the data from which the models were derived. Using the passage rate estimates of Krizsan et al. (2010) in a two-pool rumen model predicted accurately *in vivo* pdNDFD and depression in NDFD with increased feed intake. This provides evidence of the accuracy of passage data determined by rumen evacuation technique, whereas to account for *in vivo* pdNDFD with passage rate values of the NRC and CNCPS had required unrealistically high k_d – even with the two-pool rumen model.

Forage protein value

The primary function of feed protein in the diet is to provide the ruminants with absorbed amino acids (AA), often denoted as metabolizable protein (MP) in the form of α -amino nitrogen. The MP requirement of ruminants is met from two sources: microbial protein synthesized in the rumen and feed protein that escapes microbial degradation in the rumen. Determination of the protein value of ruminant diets is complex, because the dietary supply of AA is modified both quantitatively and qualitatively by microbial fermentation in the fore-stomachs before digestion in the small intestine. The main emphasis in feed protein evaluation has been the determination of effective protein degradability in the rumen (EPD) and intestinal digestibility of rumen undegraded protein. Protein degradability has most commonly been determined by ruminal *in situ* incubations and digestibility of undegraded feed protein by the mobile nylon bag method. The merits and demerits of these methodologies have been discussed in several excellent reviews (Broderick, 1994; Noziere and Michalet-Doreau, 2000; Hvelplund and Weisbjerg, 2000). However, the data derived using these methods have seldom been validated against *in vivo* measurements of post ruminal nutrient flow or milk protein yield responses.

In meta-analysis of large datasets from North-American and North European production experiments marginal milk protein yield responses to increased microbial MP were 5-fold compared to feed MP (Huhtanen and Hristov, 2009). This is consistent with the reports of the regression coefficient (0.77) between predicted (NRC, 2001) and observed post-ruminal feed protein flow (Broderick et al., 2010) indicating that the true differences in EPD for diets of variable composition are smaller than predicted. On the other hand, the corresponding regression coefficient for microbial N flow was 1.26 suggesting that the contribution of microbial protein is greater than predicted.

The inconsistency in production and flow data between microbial and feed MP can partly relate to methodological problems of the *in situ* technique in determining EPD: *In situ* determinations of protein degradation are flawed because of several inherent problems, including 1) the assumption that proteins, peptides, and amino acids in the soluble fraction are completely degraded in the rumen, 2) escape of small feed particles from the bags without being degraded, 3) the physical restriction of feeds within the bag from microbial interaction and digestion, 4) the imprecision to quantify the microbial contamination of the undigested residues, and 5) incorrect

kinetic models used to calculate protein degradability in the rumen (Broderick and Cochran, 2000; Huhtanen and Hristov, 2009; Broderick et al., 2010).

Various experimental techniques have demonstrated an escape of soluble non-ammonia N (SNAN). Depending on the kinetic model, 13-15% of SNAN fraction escaped ruminal degradation, when a single dose of intrinsically ¹⁵N labelled silage buffer-soluble N was given to dairy cows (Ahvenjärvi et al., 2007). According to generally used Ørskov and McDonald (1979) model SNAN is completely degradable, since it disappears at 0-h washing from the nylon bags. The proportion of SNAN of silage N had no influence on milk protein yield in meta-analysis of the data (253 diets) from milk production trials, when the concentration of silage MP was computed using a constant EPD irrespective of the proportion of soluble N (Huhtanen et al., 2008c). The only variable within a was silage fermentation quality (acids, ammonia N and soluble N). In a meta-analysis based 547 diets conducted to evaluate the effects of different forage variables on milk protein yield (Huhtanen et al., 2010b) forage DM intake and D-value (concentration of digestible OM) were the most important forage parameters influencing milk protein yield (Table 1). Ammonia N had a negative effect on milk protein yield, but the effect of SNAN was not significant. Calculated marginal efficiency of the utilization of incremental CP from forage was only 3.7%. This analysis clearly demonstrates that forage intake potential and OMD are much more important determinants of production responses than CP concentration or degradability.

Table 1. The best-fit mixed model regression equation of milk protein yield responses to forage variables (RMSE adjusted for random study effect = 16.0)

Effect	Unit	Estimate	Error	P-Value	SD ¹	Response per SD unit
Intercept		-310	46.8	<0.001		
CMP ²	kg/d	790	66.7	<.0001		
CMP × CMP	kd/d	-192	38.7	<.0001		
Forage DMI ³	kg/d	27.7	1.42	<0.001	1.53	42.4
D-Value ⁴	g/kg DM	0.490	0.069	<0.001	40.9	20.1
Forage CP	g/kg DM	0.417	0.114	<0.001	22.4	9.3
Ammonia N	g/kg N	-0.217	0.067	0.001	21.5	4.7

¹ SD = standard deviation

² CMP = Concentrate metabolizable protein

³ DMI = dry matter intake

⁴ D-value = Digestible organic matter in dry matter

Theoretically, forage rumen degradable protein (RDP) can be calculated using the following model:

$$\text{RDP} = \text{CP} \times \text{ISP} \times (k_d\text{-ISP} / ((k_d\text{-ISP} + k_r) [1 + k_r / (k_d + k_p)] + \text{CP} \times \text{SNAN} \times ((k_d\text{-ISP} / (k_d\text{-SNAN} + k_l) + \text{CP} \times \text{Ammonia N},$$

where ISP = proportion of insoluble CP, $k_d\text{-ISP}$ = digestion rate of ISP, k_r = rate of release of ISP from non-escapable to escapable pool, k_p = rate of passage of ISP from escapable pool, SNAN = proportion of soluble NAN, $k_d\text{-SNAN}$ = digestion rate of SNAN, k_l = liquid passage rate and Ammonia N = proportion of ammonia N of total N. This model takes into account selective retention of feed particles and escape of SNAN in the liquid phase. According to model simulations the effects of CP solubility on RDP and EPD are much smaller than the Ørskov and McDonald (1979) model predicts. However, it is questionable if the current experimental methodologies are sufficiently accurate to justify more complicated models. Evidence from the meta-analysis of the data from milk production trials suggests that marginal, if any, improvements in forage protein evaluation can be expected from using more complicated methodologies and models compared with using constant values for both ruminal CP degradability and intestinal digestibility of undegraded protein. Considering inaccuracies of the current methods, the benefits of determination forage protein degradability in practical ration formulation is questionable.

According to models by Huhtanen and Hristov (2009), marginal milk protein yield responses increased MP supply derived from reduced ruminal protein degradability (constant energy and CP intake) was only 6 – 8%. This corresponds to about 1 g/d increase in milk protein yield per 1 %-unit decrease in forage EPD (forage dry matter intake (DMI) = 12 kg/d and CP 150 g/kg DM). The action of polyphenol oxidase in red clover polymerizes with proteins (Jones et al., 1995) and reduces proteolysis (Hatfield and Muck, 1999). Reduced proteolysis of red clover silage has increased protein flow from the rumen (Dewhurst et al., 2003; Vanhatalo et al., 2009). However, incremental protein supply to the small intestine was not utilized for milk protein synthesis in these studies. One reason for the poor efficiency of incremental protein flow with diets based on red clover silage could be low intestinal digestibility of undegraded protein. In the dataset of Huhtanen et al. (2006a) faecal CP output in sheep was much higher for red clover than primary growth grass silages (56 vs. 39 g/kg DMI; n = 19 vs. 33).

Forage intake

Accurate prediction of DMI has a central role in the low cost ration formulation, since most of the variation in total nutrient intake is related to differences in intake rather than digestibility. However, energy intake, DMI and digestibility are interrelated, due to the close relationship between diet digestibility and DMI. Regulation of feed intake in ruminants involves multiple mechanisms related to dietary and animal factors that are poorly understood (Mertens, 1994). Despite extensive effort over the past 30-40 years, no generally accepted intake model has been developed. Limited success in this field is, at least in part, due to the complicated interactions between the animal and diet characteristics, and the difficulties in distinguishing and quantifying these factors.

Predicting actual intake is difficult due to strong animal effects that can mask differences in the intake potential of forages, especially if individual animal data and simple fixed regression models are used. To overcome these problems Huhtanen et al. (2002) proposed relative silage DMI index (SDMI index) derived from a mixed model regression analysis using treatment means data. The model included D-value, total acid and ammonia N concentration. The model was extended (Huhtanen et al., 2007b) to include additional factors [DM concentration, harvest: primary vs. regrowth, NDF concentration and forage type (grass, legume, whole crop)]. Mertens (1994) proposed a conceptual model of intake regulation, in which NDF was used as a factor to regulate the intake of fill limited diets. However, in the study of Huhtanen et al. (2007b) digestibility (D-value) was a much better predictor of DMI than NDF concentration. It seems that in addition to NDF concentration its digestibility and passage, and probably also particle breakdown characteristics influence intake. Bivariate model with iNDF and pdNDF predicted DMI as well as D-value. Negative coefficient of iNDF was much higher than that of pdNDF indicating the slower turnover of rumen iNDF pool. Increased NDF concentration at a constant D-value had a small negative effect on intake reflecting increased bulkiness of feed.

Reductions in intake of silage induced by fermentation end products have often been evaluated in comparisons with either grass or hay diets. However, the strong negative effect of silage total acid concentration on silage DMI (Huhtanen et al., 2007b) suggests that extent of in-silo fermentation rather than preservation method *per se* influence silage intake. Krizsan et al. (2007) addressed the intake reductions in growing cattle primarily to two patterns of in-silo fermentation; extensive lactic acid and secondary fermentation of the grass were negatively

related to silage DMI. Reductions were between 6 and 32% compared with the silage giving the highest intake. Moreover, a nine percentage unit increase in intake was observed of silage displaying restricted fermentation compared to hay of equal maturity produced from the same standing crop (Krizsan and Randby, 2007). Silage fermentation products provide less energy than WSC to the rumen microorganisms; microbial protein synthesis in the rumen can be reduced in cows fed grass silage-based diets (Jaakkola et al., 2006) and impact the ratio of amino acids to energy available at tissue level. An imbalance between amino acids and energy could explain a reduction in intake of extensively fermented silages, in line with positive intake responses to supplementary protein in cows fed grass silage-based diets (Oldham, 1984; Huhtanen et al., 2008d). The lower intake of secondarily fermented silages is most likely a consequence of reduced palatability, i.e. smaller meals and a lower frequency of meals. Attempts to isolate specific fermentation products involved in controlling silage intake have failed to pinpoint any particular product as responsible for reducing silage intake. Modelling the effect of fermentation end products on intake should rather be restricted to assess the relative importance among different fermentation quality parameters and at what concentrations they are detrimental to intake.

Silage DM concentration had a quadratic effect on DM intake with the maximum at 350 – 400 g DM/kg (Huhtanen et al. 2007b). Feeding mixtures of grass and red clover silages or grass and whole-crop cereal silages demonstrated positive associative effects on intake; i.e. the intake of mixtures was greater than the mean of both forages when fed separately. Intake of regrowth silage was 0.44 kg/d lower than that of primary growth silage even when other factors (D-value, fermentation, DM and NDF concentration) were taken into account. The reasons for this are unclear, but could be related to increases in the amount of dead material in offered silages.

In the British Feed into Milk system forage intake potential is predicted by NIRS (Keady et al., 2004a). Calibration was developed by feeding 136 farm silages to growing cattle (Agnew et al., 2001). NIRS analysis takes into account different factors influencing intake without specifying them. It is an attractive alternative that is inexpensive to use, but producing and maintaining calibration dataset is expensive. Relative prediction error of the NIRS model (7.6% residual variation) was greater than residual variation within a study for the SDMI index system (3.1%).

Because the total DMI is strongly influenced by the amount and composition of supplementary feed, the SDMI index was extended to total DMI (TDMI) index (Huhtanen et al., 2008d). The following factors are included in the TDMI-index: linear and quadratic effects of the amount and CP concentration of the concentrate, linear effects NDF and fat concentration of the concentrate and interaction between the amount of concentrate and SDMI index. The model predicts greater depressions in silage DMI with increased concentrate supplementation for high intake potential silages. Prediction error of the model within a study was 0.37 kg/d.

Although the TDMI index model predicts the differences in intake potential between the diets within a study, it is not useful alone to predict actual DMI due to strong animal influence. Many intake models use milk yield (MY) as an input variable (Vadiveloo and Holmes, 1979; Dulphy et al., 1989; NRC, 2001; Keady et al., 2004b), but the usefulness of such models in ration formulation may be questioned, as the final output (MY) is used to predict input (feed intake). These models are primarily useful in predicting intake required to sustain a given level of MY, as stated by Keady et al. (2004b). It should be noted that MY is not known at the time of prediction (Ingvarsen, 1994). To overcome this problem animal and diet factors were integrated by using standardised energy corrected milk yield (sECM) instead of actual observed yield in the model (Huhtanen et al., 2011). The idea is to standardize the current yield according to days in milk, the intake potential (TDMI-index) and MP concentration of the current diet, i.e. how much the cows would produce on given day (150) with a standard diet. Residual MSE adjusted for the random study was 0.36 kg/d and RMSE with simple regression model 0.91. An advantage of this model is that information is available at the time ration formulation, not only retrospectively as is the case with models using observed yield.

Conclusions

Accurate and precise prediction of OMD or D-value is of fundamental importance in forage evaluation. This is not only because of digestibility is closely related to the ME concentration of forage, but also because forage digestibility is the main determinant of intake potential and synthesis of microbial protein that is the major source of MP in forages. Empirical models based on chemical composition are usually not accurate enough for practical feed evaluation due to lack of causal relationships between composition and digestibility. Within forage types and limited datasets these models may be acceptable, but over a wider range of forage types and

environmental conditions the accuracy of predictions is not acceptable for practical feed evaluation and ration formulation.

In vitro methods usually predict OMD more accurately than empirical equations based on statistical relationships between chemical composition and digestibility. Even though values determined *in vitro* may approach digestibility coefficients *in vivo*, it is important to calibrate *in vitro* methods using *in vivo* data to derive reliable prediction equations. Generally there are no consistent differences in the performance of methods based on rumen fluid or commercial enzymes. However, most of the comparisons between the methods are based on narrow range of forage types. Our experience is that the relationship between cellulase solubility and OMD varies between forage types, and even between grass species from the same harvest. Determination of iNDF concentration by a long term *in situ* incubation in the rumen is a promising tool for predicting OMD by an empirical regression; iNDF (or pNDFD) is also an essential parameter of dynamic models. Because of long incubation times (12 d) the variability between the replicates is small (SD = 5 g/kg), much less than in digestibility trials in sheep (0.010 – 0.02 digestibility units). The relationship between iNDF and OMD is more uniform than that between cellulase solubility and OMD.

Mechanistic dynamic models of ruminal NDF digestion are excellent tools to understand mechanisms of fibre digestion, but whether they are useful in practical feed evaluation is questionable at the moment. Except for pNDFD, the estimation of the feed specific values of model parameters (digestion rate, MRRT, distribution of MRRT between the two compartments, variation in faecal metabolic OM) is less accurate than the current *in vitro* / NIRS methods in predicting OMD. For practical feed evaluation NIRS can be an excellent tool, but the success depends on the quality of calibration. Good OMD calibrations need a large number and a wide range of samples. Because *in vivo* calibration is not a practical option, the reference *in vitro* method should properly be calibrated against *in vivo* method.

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Particle size effects of forages on the ensiling process and animal performance

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Introduction

Depending on the ensiling method, forages are chopped to various degrees prior to ensiling. Chopping of the plant material influences the ensiling process, and has direct and indirect effects on performance of the animals consuming the silages. The objective of this paper is to elucidate the basis of these effects on ruminant nutrition and review the results from experiments of this topic with main emphasis on dairy cows.

The theoretical benefits from shorter chop length on silage preservation are clear (McDonald et al., 1991). In practical silage making, chop length is confounded with other factors such as rupturing of plant cells, and degree of compaction and sealing after ensiling. Preserving forages precision-chopped in well compacted and sealed bunker silos provides sound foundation for good anaerobic fermentation and minimal losses. Short chop length is however not a prerequisite for good fermentation quality as longer particle size forages also have good ensiling quality when silage raw material is of good quality and ensiling techniques are correctly applied. Good fermentation quality of silage is reflected in high feed intake and productivity of animals and low risks of contamination of animal products with potential feed originated micro-organisms such as *Listeria* or spores of butyric acid bacteria.

Forage particle size also affects animal performance. The effect of diet particle size on dairy cow digestion and performance has been extensively studied. Feed intake and subsequently animal productivity often increase in response to smaller feed particle size, but sometimes these positive effects can be compromised by lack of adequate physical structure in the diet and decreased rumen pH. The main drivers for the research have been to prevent milk fat depression and subacute ruminal acidosis (SARA).

The ample availability of low price feed components such as cereal grains and by-products provide large amounts of easily fermentable carbohydrates into ruminant diets. In many areas globally, the main task of ration formulation is to maximize the intake of easily fermentable carbohydrates without posing a risk to animal health, welfare and longevity. This is

somehow controversial, because the main evolutionary benefit from ruminants is their ability to use fiber as a source of nutrients. This discrepancy creates need to study forage particle size and other factors contributing to the maintenance of normal rumen function.

Defining and determining particle size is equivocal. Several methods including dry or wet sieving according to variable protocols, microscopic image analysis or laser diffraction have been used for particle size determination (Kennedy, 2005), although physically effective fiber (see Mertens, 1997; Kononoff et al., 2003a) is widely used in scientific literature. The different methods are typically not interchangeable, which complicates the interpretation of various experimental results. In the current review, no attempts were made to standardize the particle sizes reported in various articles, but they are used as reported in the original source. The term “particle size” has been used to replace the variable terms in literature such as particle length or theoretical length of cut.

Effect of forage particle size on silage preservation

Chopping and other physical damage such as maceration, bruising, and mincing improve substrate availability to lactic acid bacteria, contribute to more effective compaction and oxygen removal and thus result in fast homofermentative lactic acid fermentation of the plant material (McDonald et al., 1991; Pauly et al., 1999; McEniry et al., 2008). Chopping may also facilitate an even dispersion of additives within the forage material.

There are several different technical solutions available for silage making resulting in significant differences in the chop length of the final forage. Precision-chopping results in the shortest chop length (typically <35 mm), while big balers and forage wagons can conserve forage without any chopping. Typically big balers and forage wagons are equipped with cutters which cut the forage to a variable degree. Crop characteristics, harvesting methods and machine settings will affect the mean particle size and variability achieved (Shinners, 2003). Forage material prepared by any method includes an array of particles of different sizes, and it can be described as a curve (Figure 1). The particle size may also be affected by the driving speed as Suokannas and Nysand (2008) reported a clear reduction in particle length, when the driving speed of a forage wagon equipped with cutters was increased.

Changes in chop length in practical silage making are typically confounded with other changes including the effect of forage dry matter (DM) concentration (forage ensiled in bunker

silos has typically lower DM concentration than in big bales), level of cell rupturing and qualities of the storage (type of silo/clamp, bale etc. including differences in compaction/density and air infiltration). The mechanical effects of harvesting machinery on forage material can be divided into reduction of chop length, which improves compaction and obtaining homogeneous forage mass, and rupturing forage cells, which releases cell saps providing substrates to lactic acid bacteria. Pauly et al. (1999) measured the electric conductivity of water extracts from long, cut and precision-chopped grass, and found a 4-fold increase in precision-chopped vs. the other treatments indicating greater release of cell saps in precision-chopped material.

Decreasing forage particle size at harvest increases energy consumption of the machinery (Shinners, 2003). Suokannas and Nysand (2008) reported a 65 % higher energy consumption for the cutting function of a precision-chopper compared to that of loader wagons. On the other hand, decreasing forage particle size at harvest may facilitate total mixed ration (TMR) preparation by reducing the power requirements, and loading and mixing time. Basically the same phenomenon can be realized when animals are consuming the feed – eating time is typically shortened when finely chopped feeds are given to animals (see section on feeding behavior).

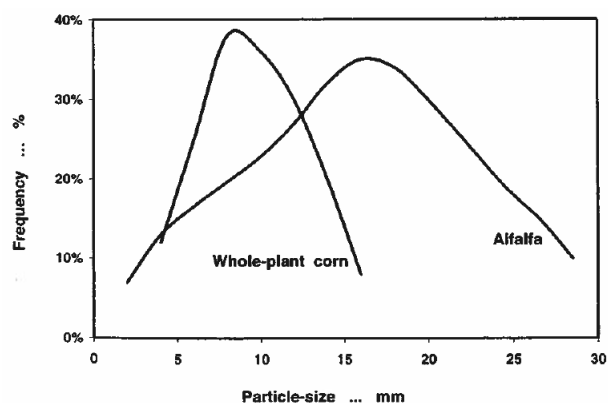


Figure 1. The same theoretical length of cut (10 mm) produces variable mean and variation in particle size depending on forage crop (Shinners, 2003).

Grass silages

In a review by Haigh (1998) including 21 experiments in UK, grass silages produced by flail harvester, forage wagon, double-chopper or precision-chopper were compared. The shorter

the chop length in the harvesting system, the better the preservation quality and animal performance as precision-chop silages ranked typically first and those harvested with forage wagon last. Similarly, Petit et al. (1993) reported highest DM intake and milk production in precision-chopped silage followed by silages harvested using forage wagon or big baler. In Scandinavia there is a long tradition in producing high quality grass silages using direct-cut flail-harvested silages with ca. 200 g/kg DM concentration with the aid of using relatively high levels of formic acid (4 l/per ton fresh grass) in additives. Other methods such as wilted precision-chopped and big-baled silages have however taken over nowadays with important benefits in capacity compared with flail-harvesters.

Forages conserved in big bales or picked up by forage wagons may be in the long form without reduction in particle size, but currently it is common to include cutters to them in order to increase silage density, and facilitate the use of silages at the feeding phase (Shinners, 2003). It has not been very easy to show experimentally improvements in bale silage fermentation quality in response to decreased chop length (Borreani & Tabacco, 2006; Müller, 2009). Addition of knives into forage wagons did also not improve the fermentation quality of grass silage in the experiment of Pauly and Lingvall (1999), although grass chop length was markedly decreased. The chop length effects were also minimal in the experiment of Paziani et al. (2005) using tropical Tanzania grass as the raw material.

McEniry et al. (2007) demonstrated that DM concentration and air infiltration had greater effects on grass silage fermentation quality and losses than chopping or compaction. In a later study (McEniry et al., 2008), unchopped and precision-chopped ryegrass were compared in laboratory silos with faster fermentation and improved final fermentation quality of precision-chopped vs. unchopped silage. Pauly et al. (1999) reported faster onset of fermentation and less heterogeneous forage mass of precision-chopped vs. unchopped or cut silage in laboratory silos, which resulted in a better preservation of precision-chopped silage (Figure 2). If moist silages are ensiled, chopping will increase effluent losses (McDonald et al., 1991; McEniry et al., 2008).

Precision-chopped silages seem to have an advantage compared to unchopped grass or grass cut by big balers or forage wagons. The faster onset of fermentation is probably related to the cell ruptures and not solely to particle size. Further, the storage conditions in bunker silos, which is the most typical storage method for precision-chopped silage, are typically more favorable (higher density, less aerobic exposure than in big bales). The particle size *per se*

probably has a small independent positive effect on the ensiling process, but it is not an absolute prerequisite for good fermentation quality as good quality silages can be produced by all typically used methods, when appropriate ensiling methods are used. The method chosen on a particular farm must fulfill the economic and other management needs, which may override the differences between the biological ensiling characteristics of the methods (Muck and O’Kiely, 2002).

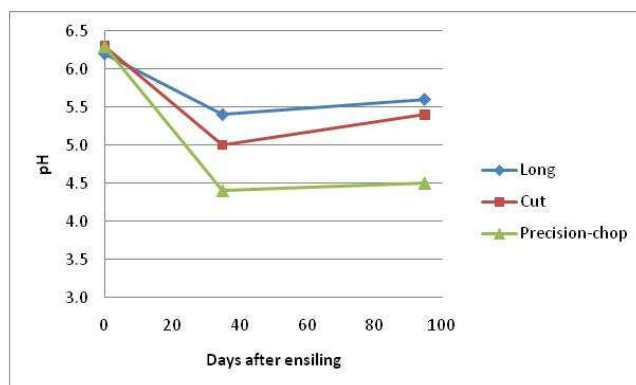


Figure 2. The development of grass pH after ensiling in the study of Pauly et al. (1999) demonstrate the benefits from precision-chopped vs. cut or long material. The average particle lengths were 30, 40 and 230 mm, respectively.

Whole crop cereal silages Corn (maize) silage is an important raw material for preparing whole crop cereal silage. It is typically harvested with a self-propelled chopper directly from the standing crop equipped with a head for chopping corn. This method produces rather short chop. Several studies have evaluated the effects of chop length and other mechanical treatments on corn silage and benefits in ensiling quality have been achieved with more aggressive treatments (Johnsson et al., 2002, Hara & Tanigawa, 2010), although differences have not always been observed particularly if also the control treatment has been well preserved [e.g. Fernandez et al., 2004; Cooke & Bernard, 2005; Yang and Beauchemin, 2006 (barley silage)].

The preferable harvesting method for small grain cereals is using a chopper, which harvest directly the standing crop. Sometimes cereals like barley or wheat are also ensiled in big bales, although first mowing and then picking up often increases the kernel losses to unacceptable levels. The compaction of the bales may also be poor and hard stems may penetrate the plastic thus leading to air infiltration into the bales. Ohlsson (1998) reported benefits in bale

density and fermentation quality, when barley was cut using a precutter before ensiling in round bales, but simultaneously the losses of kernels increased.

Aerobic stability Aerobic stability describes the time that silage remains stable (typically measured as a maximum of 2 °C rise compared to the ambient temperature) when exposed to oxygen at feedout. Aerobic deterioration may cause large DM losses and reduced animal performance (McDonald et al., 1991; Kung, 2005)

The risk factors for poor aerobic stability are contamination of the crop with aerobic microbes and air infiltration into the silage mass enabling survival and growth of aerobic microbes. Porosity of compacted plant material contributes to gas flow and air infiltration rate with consequent increases in DM losses due to aerobic deterioration as emphasized by Holmes and Bolsen (2009). Porosity increases with increasing DM concentration of forage and it could be expected to decrease in response to shorter chop length and subsequent more effective compaction. McEniry et al. (2007) found no effect of grass chop length on aerobic stability, while in the later study (McEniry et al., 2008) precision-chopping decreased the time to temperature rise $>2^{\circ}\text{C}$ compared to unchopped grass. Both long and cut silages prepared by Müller (2009) remained stable for the 5-day observation period.

Effect of particle size on animal performance

Indirect effects of feed particle size on animal performance The various experimental situations require consideration because particle size and fermentation quality of silages may be confounded. The data set of Haigh (1998) is a good example of this: Precision-chopped silages gave best animal production results and had the shortest chop length, but were superior also in preservation quality.

If only the effect of particle size is to be evaluated, the particle size of experimental feeds needs to be manipulated just prior to feeding so that chopping has not influenced the preservation of the silage. Sometimes the treatments may also have other differences if e.g. treatments have resulted in variable shredding losses in the field so that the nutritional composition and digestibility differ between treatments.

Determination of particle size and physical structure of the diet In order to produce benefits in practical ration formulation, the system describing the physical effectiveness of diet must have close correlation to animal performance, and the values for farm feeds need to be readily accessible. De Brabander et al. (1999) recognized the challenge in defining the physical structure but presented the following description of it: “The extent to which a feedstuff, through its content and properties of the carbohydrates, contributes to an optimum and stable rumen function”. Particle size is one of the factors contributing to the physical structure.

Several systems have been developed to assist in formulating diets adequate in physical structure. Sudweeks et al. (1981) developed a roughage value index based on mean diameter of the feed determined by sieving, DM intake and fiber concentration. The index values range from ≈ 10 min/kg for concentrate feeds to up to ≈ 100 min/kg for low quality forages, and a minimum value of ≈ 30 min/kg for the diet was recommended to maintain milk fat concentration at normal levels.

Mertens (1997) introduced physically effective neutral detergent fiber (peNDF), which is based on neutral detergent fiber (NDF) concentration and the relative effectiveness of NDF in promoting chewing activity. The peNDF is measured by dry sieving so that NDF concentration of particles retained on a 1.18 mm sieve is considered physically effective, and the Penn State Particle Separator is widely used in measuring it (Kononoff et al., 2003a). The peNDF is presented in proportional values (0 to 1) providing a more consistent measure than the roughage value index, which is also influenced by animal factors. Mertens (1997) listed that on top of particle size, other factors such as DM intake, particle shape, fragility, moisture, type of preservation, and ratio of eating time to rumination time contribute to the physical effectiveness of fiber.

De Brabander et al. (1999) examined the required physical structure of various dietary components. Particle size of grass silages did not have an effect on the critical amount of roughage required in the diet to maintain normal rumen functions, whereas for corn silage, increasing particle size increased the structural value, probably because of the smaller average particle size in corn silages. Fiber (as NDF or crude fiber), effective fiber measures or feeding behavior did not produce consistent relationships with the amount of roughage needed to maintain the normal rumen functions. De Brabander et al. (1999) proposed a derived unit, which takes into account both feed characteristics (e.g. concentration of fiber, particle size and acidotic

effect) together with feeding management (i.e., number of concentrate meals per day) in order to formulate dairy cow rations for good rumen function. Nørgaard et al. (2010) presented the Nordic structure evaluation systems for dairy cows, where particle size also is considered.

Feed intake and animal performance

Increased feed intake is often observed, when forage particle size decreases (e.g. Kononoff et al., 2003b; Tafaj et al., 2007; Zebeli et al., 2009). The effect has been shown also in experiments, where the forage has been chopped to variable lengths just prior to feeding to avoid the confounding effect of chop length on fermentation quality. In the meta-analysis of Tafaj et al. (2007), the DM intake increased by 80 g/day when forage particle length decreased by 1 mm. Production responses could not be demonstrated, but decreasing particle size decreased rumen pH, and chewing and rumination time.

In situations where physical limitation is more important (grass rather than corn silage based diets, low proportion of concentrate in the diet), decreasing forage particle size is more likely to increase feed intake. The results from Castle et al. (1979) demonstrate a greater intake response to chopping of silage when silage was fed as a sole feed compared to a situation when also concentrate was included in the diet, which is explained by the decreased physical limitation of the diet including concentrates.

Increased feed intake typically results in increased production of the ruminants. However, if it is accompanied by decreased fiber digestibility, benefits from increased intake may partially or totally be lost (see later section on fiber digestion).

Feeding behavior

During ingestive mastication, feed particles are reduced in size and lubricated with saliva to prepare the bolus for swallowing. During rumination, saliva production is stimulated, and particles are further decreased in size and their relative specific gravity increases, which are essential characteristics in facilitating their passage from rumen to the lower digestive tract (Kennedy, 2005).

Decreasing particle size typically decreases chewing time during eating and/or ruminating, and subsequently total chewing time (Allen, 1997; Couderc et al., 2006; Yang & Beauchemin, 2006, 2009; Tafaj et al., 2007, Figure 3), although the relationship was not very

clear in the dataset of De Brabander (1999). The changes in eating behavior are not always reflected in changes in rumen pH and animal performance.

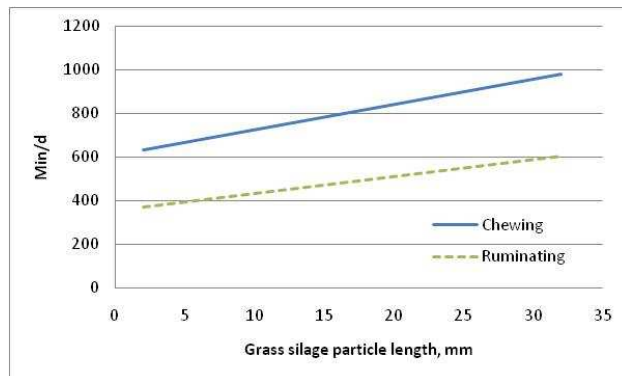


Figure 3. Increasing particle length linearly increased chewing and rumination times of dairy cows fed grass silage based TMR in the meta-analysis of Tafaj et al. (2007). Total chewing time increased by 11.7 min and rumination time by 7.6 min per 1 mm increase in grass silage particle size. The particle size effect on feeding behavior was not significant when corn silage based TMR was fed.

In theory, increased mastication may be considered as a loss of energy for the cow, which is compensated by less mechanical work required in feed preparation. Long chewing times may at least theoretically also compromise feed intake, because cows need to allocate part of their time for resting. Mertens (1997) proposed a maximum chewing time of 1000 min per day. There is a large variation in chewing times [average 668 min/d with a range of 364 to 962 in the data set of Allen (1997), and average 694 min/d with a range of 424 to 969 in the data set of Tafaj et al. (2007)]. Teller et al. (1993) presented a mean chewing time of 948 ± 45 min/day for 16 silage-based diets and depicted that the total number of jaw movements during eating and ruminating is approximately 60 000 per day. They proposed that genetically determined physical characteristics for efficient forage ingestion and chewing should be considered in animal breeding programs.

The effect of particle size on chewing time seems to be curvilinear. Allen (1997) studied the relationship between particle size and daily chewing time using data from 10 dairy cow experiments and found that a particle size threshold occurred at approximately 3 mm sieve, above which forage particle size did not further increase chewing time. Similarly, dramatic

changes in chewing time were achieved when reduction in grass particle size <3 mm was used (Tafaj et al., 2007). According to De Brabander et al. (1999) particle size did not affect the structure value of normal grass silages in practice with a particle size above 20 mm.

Particle size may affect the proportion of rumination in total chewing time. Allen (1997) reported that the proportion of rumination in total chewing time was 0.60 with a range from 0.43 to 0.74. The proportion of rumination time decreased when long hay vs. chopped forage was fed and when fiber concentration of forage increased, as these factors increased chewing during eating, although in absolute terms, also time spent ruminating increased. The equations of Tafaj et al. (2007) show practically no trend in the proportion of rumination in total chewing time (0.59 and 0.61 for diets with forage particle lengths of 2 and 32 mm, respectively; Figure 3). Reduced eating time could be considered beneficial in situations where feed bunk place is limited. Fast eating could particularly benefit animals low in social hierarchy, which may have problems in accessing the feed bunk.

There is large variation in the feeding behavior between cows. Almost half of the variance in feeding behavior, and 0.21 of critical roughage value could be explained by differences between cows in the data set of De Brabander et al. (1999). Currently there are equipments available that under practical farming situations register the chewing time of cows. Probably the best use of such equipments is to detect deviations from normal behavior rather than indicating adequate physical structure in the diet.

The salivary secretion rates during resting, eating and ruminating were estimated by Cassida and Stokes (1986) to be 0.15, 0.18 and 0.27 liters per min. emphasizing the role of rumination in buffering rumen pH. This is also depicted by the clearly visible increases in rumen pH during rumination bouts (Allen, 1997; Figure 4). Beauchemin et al. (2008) measured salivary secretion during eating of four clearly distinct forages (no direct comparison of particle size) and found similar rate of saliva secretion during eating (0.21 kg/min). The rate of chopped barley straw eating was slower than that of the other forages (barley silage, alfalfa silage and long alfalfa hay) so that per rate of DM intake, it was mostly salivated, but when expressed per kg NDF, alfalfa hay was mostly salivated. The rate of saliva production per kg pelleted concentrate DM consumed was only one fourth of that of the average of the four forages (Beauchemin et al., 2008). Faster eating rate of chopped forages would decrease their salivation during eating, but the changes on total saliva production are probably more related to the time spent ruminating.

Teller et al. (1993) speculated that longer eating time may restrict the time available for rumination, which – because of the greater rate of saliva production during ruminating compared to eating – might predispose to low ruminal pH.

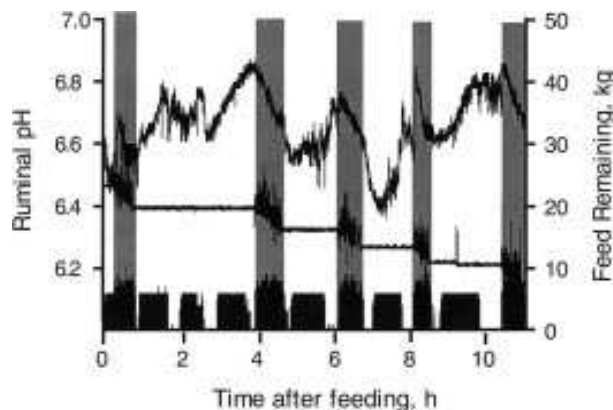


Figure 4. Continuous monitoring of a dairy cow feeding behavior and rumen pH show the clear decrease in rumen pH caused by ingestion of new feed. An increase in rumen pH during rumination is also clearly visible in response to salivary secretion, and absorption and passage of volatile fatty acids from the rumen. The top line represents rumen pH, the middle line the amount of feed left, and the bottom line the chewing activity. The vertical shaded bars indicate the meals (Allen, 1997).

Forage particle size affects feeding behavior and increasing particle size increases chewing time of cattle, which would, at least to some extent, increase saliva production and buffering effect in the rumen. The effects on rumen pH may however be limited, because other important factors also contribute to rumen pH regulation such as rate and extent of substrate fermentation in the rumen, and rate of absorption of acids produced in the rumen. Increasing the proportion of concentrate in the diet may also affect feeding behavior with very fast eating rates compared to forages. According to De Brabander et al. (1999), chewing time is not an ideal standard for describing physical structure of variable feeds. Tafaj et al. (2007) felt that the critical particle size of diet to stimulate chewing and rumination processes adequately may be smaller than often has been assumed.

Rumen pH

Dairy cows are at great risk of low ruminal pH, because of their high energy demand, and attempts to fulfill it by using highly digestible high concentrate diets for them. Other ruminant animal groups may also suffer from SARA, particularly if concentrate feed prices are low resulting in large quantities of easily fermentable carbohydrates in their diet. Chewing stimulates saliva production and increases the buffering capacity in the rumen. The role of excessive concentration of easily fermentable carbohydrates in the diet plays however a greater role in increasing the risk of SARA (Allen, 1997; Krause & Oetzel, 2006). If decreasing particle size increases feed intake, the total fermentable mass into the rumen and thus the production of acids thereof will increase and potentially cause a greater risk of rumen acidosis. With increasing DM intakes, a higher requirement for physical structure in the diet is needed due to greater production of fermentation acids.

The complicated interactions in dairy cow diet digestion were brought up by Krause and Oetzel (2006): Very long particle size of forages may also increase the risk of SARA by allowing the cows to select the more fermentable feed and leaving the fibrous long particles uneaten. Decreased selection against fiber with decreasing particle size was demonstrated in the experiments of Konoff et al. (2003) and Zebeli et al. (2009).

Allen (1997) concluded that although peNDF has an important role in stimulating saliva flow to neutralize fermentation acids, differences in rate and extent of organic matter fermented in the rumen seem to be the most important single factor affecting ruminal pH. Mertens (1997) showed a positive relationship between peNDF concentration and ruminal pH (Figure 5), but he also noted that peNDF and easily fermentable neutral detergent soluble fractions of feeds are negatively correlated. Although forage particle size may not be as important factor in causing SARA as the amount of easily fermentable carbohydrates fed, in a border-line situation it may be of importance.

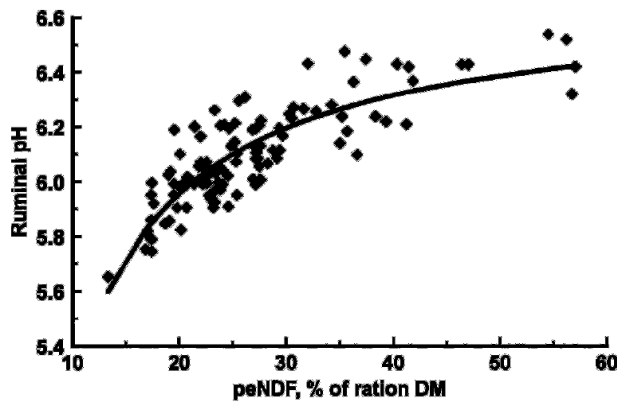


Figure 5. Mertens (1997) found a relationship between physically effective NDF concentration in the diet and ruminal pH.

A number of different background effects are likely to substantially modify the effects observed in individual experiments. In the data set of Tafaj et al. (2007), the effects of particle size were more profound on grass than on corn silage based diets. In a further analysis from the same group (Zebeli et al., 2008), on top of peNDF also ruminally degradable starch and DM intake affected ruminal pH, and the latest review (Zebeli et al., 2010) confirmed the important interactions between peNDF and ruminally digestible starch. Calculating the acidogenic value for the concentrate feeds could be used in ration formulation on top of peNDF to prevent SARA (Rustomo et al., 2006).

Forage NDF or peNDF concentration in the diet seems to be a better indicator of healthy rumen environment than total diet NDF concentration (Zebeli et al., 2008; Yang & Beauchemin, 2009). The results of Yang and Beauchemin (2009) illustrate that increasing alfalfa silage particle size can only marginally reduce the risk of SARA while increasing the forage-to-concentrate ratio is more efficient (Figure 6). Further, Onetti et al. (2003) could not alleviate the negative effects of tallow supplementation in dairy cow diets by increasing the chop length of corn silage.

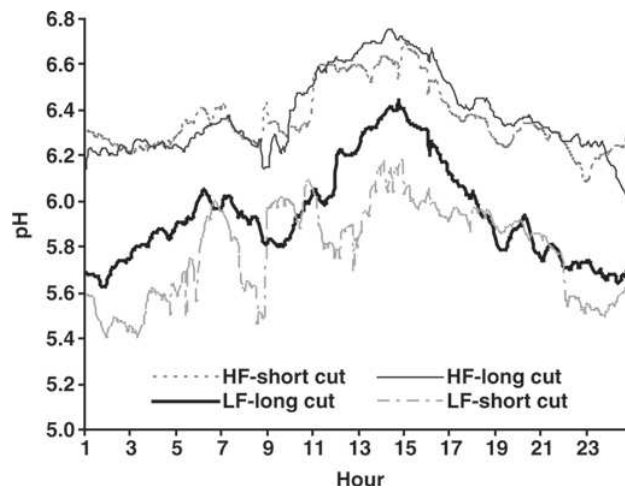


Figure 6. The ruminal pH measurements from Yang and Beauchemin (2009) demonstrate that diet fermentability (here manipulated by forage-to-concentrate ratio; HF = high forage and LF = low forage) has greater influence on ruminal pH than alfalfa silage particle size.

Grasses are more efficient than corn in maintaining stable rumen function as De Brabander et al. (1999) determined the critical values of roughage required in the diet to maintain normal rumen functions to be 205 g/kg for grasses and 348 g/kg for corn silages. This can be attributed to the higher fiber and lower easily fermentable carbohydrate concentrations in grasses compared to corn silage. Different types of grasses may also differ in physical effectiveness. Grasses typically have higher fiber concentration, but lower indigestible fiber (iNDF, determined as the residue after prolonged rumen incubation in nylon bag) concentration than forage legumes (Figure 7), and the high iNDF concentration of legumes might improve their structural value compared to grasses (Kuoppala et al., 2009). Further, the variation within forage types is also substantial. A 3-week delay in grass silage harvesting caused clear differences in NDF (486 vs. 654 g/kg DM) and iNDF (48 vs. 124 g/kg DM) concentrations, and in ruminal pH (5.99 vs. 6.29) of cows consuming similar concentrate allowance (Rinne et al., 2002). The role of iNDF in describing the physical effectiveness of ruminant feeds warrants further evaluation.

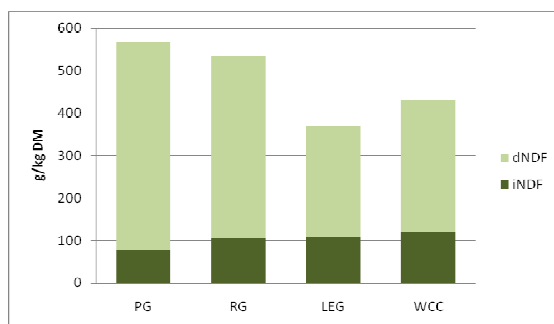


Figure 7. Primary growth grass (PG), regrowth grass (RG), legumes (LEG) and whole crop cereals (WCC) ensiled differ in the total fiber concentration [digestible NDF (dNDF) + indigestible NDF (iNDF)] as well as in the proportion of iNDF in total fiber (Huhtanen et al., 2006).

De Brabander et al. (1999) noted large differences in cows in the critical value of roughage needed in the diet to maintain normal rumen function. Thus relatively large safety margins are needed in practical diet formulation. Maybe with continuous technological development it will in future be possible to use individual rumen pH detectors placed in rumen of dairy cows also in practice, while the technology is currently available for experimentation (see e.g. Figures 4 and 6).

Digestion kinetics and fiber digestion

Rumen digestion is a dynamic process, where the digestibility of nutrients depends on the rate of digestion relative to the rate of passage (Allen & Mertens, 1988). Increasing passage rate and/or decreasing digestion rate will lead to decreased fiber digestion. Reduced particle size can decrease diet digestibility by increasing feed intake, which has been shown to decrease fiber digestibility (Huhtanen et al., 2009). Increased feed intake may further contribute to increased digesta passage rate, and decrease rumen pH, which both contribute to reduced fiber digestion. Tafaj et al. (2007) found a linear decrease in rumen pH and NDF digestibility with decreasing particle size (Figure 8).

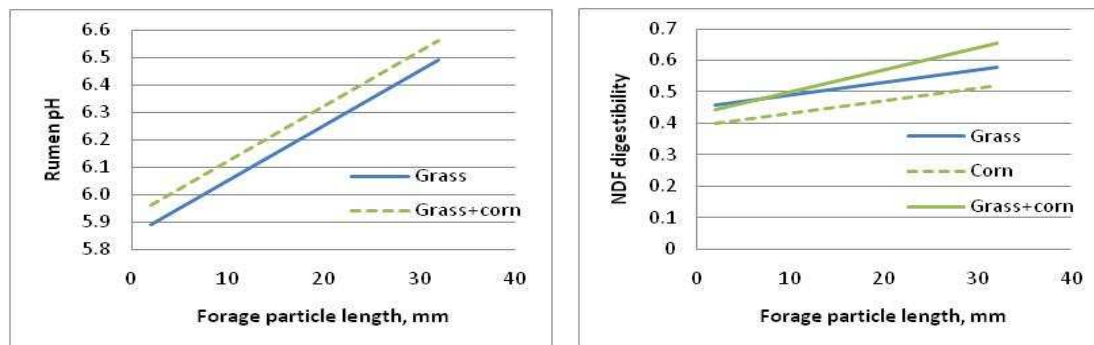


Figure 8. Increasing forage particle length linearly increased rumen pH and fiber digestibility in dairy cows (Tafaj et al., 2007).

Particle size has a role in determining the probability of particle escape from the rumen, but as majority of rumen particles is smaller than 1-2 mm, other factors such as specific gravity/buoyancy are also involved (Kennedy, 2005). Chewing and particularly ruminating contribute importantly to increasing the “escapability” of the forage particles by expelling the air pockets from feed particles.

The average particle size in the rumen is small compared to that in the diet. Differences in mean ruminal particle size have typically not been found when rations varying in forage particle size have been fed (Shaver et al., 1988; Tafaj et al., 2007). This indicates that the regulatory mechanisms of the ruminant on ruminal digesta particle size are more important than the particle size of the diet fed. In the meta-analysis of Tafaj et al. (2007), forage particle size did not affect ruminal passage rate and digesta mass suggesting that particle size reduction was not a rate-limiting step for particulate passage.

Fecal particle size can be used as a tool to measure the particle size of digesta leaving the rumen. Reducing the chop length of forage has increased the fecal particle size (Shaver et al., 1988; Teller et al., 1993; Rustas et al., 2010). Although this seems controversial, it may be explained by the reduced chewing time accompanied with reduced particle size of forage fed, and shows that the particle size decrease is more efficient when long vs. short chop forage is fed.

Effect of corn silage particle size on starch digestibility

When corn silage is prepared from a relatively mature crop with well developed cobs, grain processing is typically used to improve starch digestibility. Mertens and Ferreira (2005)

described a method to analyze corn silage particle size with the aim to give an estimate of the kernel fragmentation and subsequent starch digestion.

Johnson et al. (2003) used macro nylon bags to incubate corn silages in the rumen of dairy cows to find out the effects of chop length and mechanical processing on the rate and extent of digestion in 5 experiments. There was some variability in the results, but mechanical processing improved DM and starch disappearance from the nylon bags more clearly than reducing chop length. Interactions were found so that the effects of mechanical processing were greater when corn silage was harvested at a later stage of maturity. Cooke and Bernard (2005) also found increased starch digestibility in dairy cows in response to more aggressive kernel processing, particularly when longer theoretical length of cut was used. Shorter chop length increased starch digestibility in barley silage harvested at dough-stage of maturity, but not earlier at heading-stage (Rustas et al., 2010).

Adding coarse forages to the rations

One method of increasing physical structure in dairy cow diets (typically TMR) is to add coarsely chopped high fiber hay or straw in small quantities into the recipe. This would improve rumen environment by stimulating chewing and saliva production, but also by substituting more fermentable components with less fermentable ones, and by decreasing feed intake.

Couderc et al. (2006) reported increased DM intake, when coarse chop corn silage was supplemented with long hay, but when fine chop corn silage was fed, DM intake decreased linearly in response to long hay addition. In many cases feed intake and subsequent production in beef cattle (Alende et al., 2009) and dairy cows (Ferris et al., 2000; Humphries et al., 2010) has decreased in response to addition of low quality forage in the diet. Adding coarse forage in the ration may be able to prevent clinical problems related to low rumen pH, and coarse forage additions can be used as “first aid” in such situations to maintain normal rumen function. In long term, other feeding strategies may however be more economically profitable.

Reducing forage particle size during preparation of TMR

The key points in the TMR quality from normal rumen function point of view are fiber concentration, particle size, and rate and extent of organic matter fermentation. They are mainly determined by the recipe of the TMR, and the qualities of the individual components. However,

further processing of the diet also occurs at the time of TMR preparation in the mixer. The technical properties of the mixer and time of mixing will affect the final physical structure of the TMR produced. Humphries et al. (2010) reported smaller average particle size of vertical vs. horizontal mixer (11.7 vs. 14.2 mm) when identical ration components were used, and feed intake and milk production increased in cows consuming the smaller particle size diet.

Reduced feed selection is usually considered an important benefit of shorter chop length in TMR (Krause & Oetzel, 2006). Because cows usually select for the more digestible parts of the feed, reduced selection can be considered positive with regard to stable rumen function.

Conclusions

Based on the literature reviewed, the effects of forage particle size *per se* on both ensiling quality and rumen function are relatively small. Certain benefits from short particle size can be obtained in silage preservation, particularly when comparing ensiling technologies using precision-chopped vs. long grass, but good quality silage can also be obtained from long chop if good silage making practices are applied. Using diets with too low or too high physical structure will compromise the production of cattle, but it should be noted that too low physical structure will lead to animal health and welfare problems and decrease the longevity, while too high physical structure will decrease level of production without compromising animal health. The proportions of total fiber vs. easily fermentable carbohydrates in the diet may be more important than forage particle size when practical forage processing methods are used.

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MARKETING OF CONSERVED FORAGES BASED UPON QUALITY ATTRIBUTES

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ABSTRACT

The quality of Forages is one of the key ingredients for top animal performance, whether for milk or meat production. Unfortunately, forages are typically much more variable in quality than grains or concentrated feeds. Forages also contribute multiple quality attributes to an animal diet (energy, protein, minerals, functional fiber). This creates challenges for the industry to fix an economic value on a variable but important product. Marketing of hay has been historically based upon subjective factors (e.g. leafiness, leaf attachment, stem coarseness, color, weeds, molds, and odor) and more recently analytical laboratory values (CP, ADF, NDF, NDFD). Various indices or calculated values (RFV, TDN, RFQ, NEL) have been used based upon measured lab values. While corn silage is seldom marketed, the marketing categories of Supreme, Premium, Good, Fair, and Low (or utility) grade of alfalfa and grass hay are recognized by marketers, based upon both visual and lab analyses. Each of these forage types has a role in animal feeding, but prices are typically dictated by the value of high quality forage for high-producing dairy cows – other classes are secondary. The importance of forage quality changes in the market based upon the supply-demand situation in any given year, with quality being most important in ‘low price’ years. Marketing of forages requires a uniform description of quality characteristics (“guidelines”) and an understanding of the value and limitations of laboratory measurements. Reliable, repeatable lab measurements greatly facilitate marketing of hay and silage, with fewer measurements required for marketing than for ration balancing. There is a very low percentage of corn silage that is marketed, but it is anticipated a greater amount may be marketed in the future. A ‘hierarchical’ approach to marketing of forages is suggested, with multiple measurements (particularly NDF, CP, NDFD and ASH, and starch in the case of

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silage) playing important roles, depending upon class of animal, ration approach, and production system.

INTRODUCTION

The marketing of forage crops (hay, silage, or green forage) has a relatively recent history, since traditionally forages have been mostly fed 'on-farm' without a point of sale. However, in recent years, and in some regions, forages have fully entered regional and even world trade. Only a small minority of corn (*Zea mays*) silage or other silage crops are marketed, but a greater amount of alfalfa and grass hays are marketed; thus the evolution of quality-market relationship for forages have concerned primarily hay, and predominately alfalfa (*Medicago sativa*) hay.

In the western United States, it is likely that >80% of the alfalfa and grass hay is marketed either domestically or in world trade. This is less common in eastern United State, where a vast majority of hay is fed on-farm, but marketing is becoming more common in those regions as well. Exports and dairy purchases in particular have expanded greatly in recent years, necessitating quality-defined price discovery. In this paper, I explore the current methods for defining quality in forage crops for markets, and speculate on how this might change in the future.

HISTORY OF HAY MARKETING STANDARDS & GUIDELINES

Hay market standards or guidelines have evolved considerably since the USDA first described hay quality standards (most recently in the

Table 1. The first Hay Quality Standards for alfalfa & alfalfa/mixed hay (USDA,1949) were based mostly upon visual designations. Over the past 60 years, these subjective standards have largely been replaced by lab values combined with observational qualities. Color is now generally believed to not have an effect on animal performance.

US Grade (1949):

US No.1. 40% leafiness, 60% green color, 5% foreign material (must have at least one-fifth of the leaves clinging to the stem, and shall not include hay in which a majority of the alfalfa stalks bear brown and/or black seed pods)

US No.2. 25% leafiness, 35% green color, 10% foreign material

US No.3. 10% leafiness, 10% green color, 15% foreign material

Sample Grade: Hay which contains more than a trace of injurious foreign material, or which has any objectionable odor, or which is undercured, heating, hot, wet, musty, moldy, caked, badly broken, badly weathered, badly frosted, badly overripe, or very dusty, or which is otherwise of distinctly low quality.

Additional designations:

Extra Leafy hay –“The leafiness of the alfalfa is 50% or more with most of the leaves clinging to the stems

Leafy Hay –“The leafiness of the alfalfa is 40% or more with at least one-fifth of the leaves clinging to the stem.

Extra Green Hay—“Hay which has 75% or more green color.

(These designations shall not include hay in which a majority of the alfalfa stalks bear brown and/or black seed pods)

--Handbook of Official Hay and Straw Standards effective July 1, 1949. USDA Production and Marketing Administration, Washington, DC.

1950s-Table 1). These were based primarily upon leafiness, color and foreign material. It is striking that the quality hay that was fed in the 1950s were likely genuinely much lower quality than the alfalfa hay fed today; the highest quality designation (US No. 1 Extra Leafy) would still tolerate up to 49% of the stalks having seed pods!! (Table 1). There is virtually no high quality hays fed today which have seed pods, suggesting that demands for quality have changed considerably since that time.

Over time, laboratory measurements in particular have played a much more important role, as dairy farmers and nutritionists took a more scientific approach to animal feeding. The system for trading hay today is based upon laboratory analysis, particularly fiber and protein, and more recently, fiber digestibility, but subjective evaluation of quality (including the long-recognized attributes of leafiness, leaf attachment and weeds).

CURRENT PRACTICE IN THE UNITED STATES

Currently, USDA recognizes five categories of hay (Table 2), which are tracked for price and volume (USDA-Market News Service, Table 2). It should be emphasized that these are guidelines, not standards, and that industry participants are generally free to modify these designations to a considerable degree. For example some hay may meet the ‘Supreme’ quality designation based upon RFV (greater than 180 RFV), but contain, for example, 20% CP – but a marketer may still designate the hay ‘Supreme’ due to its high TDN, high leafiness, softness, but communicate with the buyer that it is somewhat lower in protein. {See box on the following page for listing of abbreviations}. It’s important to realize the differences between **guidelines** (which are approximations of quality) vs. **standards**, (which require minimum quality values). Guidelines are more appropriate for forages, given their variability.

Table 2. Current USDA Quality Guidelines for reporting economic data of alfalfa hay (not more than 10% grass) adapted in 2002 (2003 USDA Livestock, Hay & Grain Market News, Moses Lake, WA). Guidelines are used along with visual appearance to determine quality. It should be pointed out that these guidelines also differ somewhat within the United States, due to regional differences in available hay and attitudes. All figures are expressed on 100% DM

except as noted.

Physical Descriptions of Hay Quality to be used in combination with lab tests for alfalfa hay quality categories (USDA-Market News):

- **Supreme:** Very early maturity, pre bloom, soft fine stemmed, extra leafy. Factors indicative of very high nutritive content. Hay is excellent color and free of damage.
- **Premium:** Early maturity, i.e., pre-bloom in legumes and pre head in grass hays, extra leafy and fine stemmed-factors indicative of a high nutritive content. Hay is green and free of damage.
- **Good:** Early to average maturity, i.e., early to mid-bloom in legumes and early head in grass hays, leafy, fine to medium stemmed, free of damage other than slight discoloration.
- **Fair:** Late maturity, i.e., mid to late-bloom in legumes, head-in grass hays, moderate or below leaf content, and generally coarse stemmed. Hay may show light damage.
- **Utility:** Hay in very late maturity, such as mature seed pods in legumes or mature head in grass hays, coarse stemmed. This category could include hay discounted due to excessive damage and heavy weed content or mold. Defects will be identified in market reports when using this category.

Chemical Descriptions of Hay Quality Guidelines to be used in combination with Physical Descriptions for alfalfa hay quality categories (USDA-Market News):

Category	ADF	NDF	*RFV	*TDN	*TDN (90% DM)	CP
	-----%-----					
Supreme	<27	<34	>180	>62	>55.9	>22
Premium	27-29	34-36	150-180	60.5-62	54.5-55.9	20-22
Good	29-32	36-40	125-150	58-60	52.5-54.5	18-20
Fair	32-35	40-44	100-125	56-58	50.5-52.5	16-18
Utility	>35	>44	< 100	<56	<50.5	<16

RFV is calculated from ADF and NDF: $RFV = (88.9 - (.779 \times \%ADF)) \times ((120 / \%NDF) / 1.29)$

TDN = $\{82.38 - (0.7515 \times ADF)\}$ according to Bath & Marble, 1989.

TDN (90% DM) = TDN X 0.9.

WHAT IS QUALITY?

What does a nutritionist want in a forage?

Before discussing markets, it's important to ask 'what is quality?'. Forage quality is broadly defined as the potential to produce a desired animal response from a given intake of forage. Forage quality is always a complex mix of nutritional traits. In a discussion with animal nutritionists, several key qualities emerge as important requirements from forages. These are:

Attribute I. High Quantity of Digestible Energy.

In many cases, the primary consideration for forage quality is the total potential digestible energy per dry matter weight unit (lb, kg, or ton) of forage. This is often the most important forage quality factor, since biological energy drives the animal functions of maintenance, growth, and milk production. Unfortunately, the total potential biological energy in feeds cannot be measured directly but is predicted with equations derived from several laboratory analyses.

Digestible energy primarily comes from rapidly-available forms are compounds such as sugars, starches, and pectins, but also from the fiber fraction, fats, and protein. Considerable energy in forages is contained in the cell wall portion (particularly cellulose, hemicellulose and lignin), which may be 35-55% of the plant, but the cell wall may be only 30 to 70% percent digestible.

In general, as NDF goes up, potential energy often goes down. Energy (TDN, NE, NE_L, or ME) has been predicted from a linear relationship to a fiber measurement (ADF or NDF) in alfalfa, or summative equations that use NDF, NDFd, ash, EE, CP, and other factors to predict energy.

NDF and ADF and NDFD are often considered important measurements for energy estimations, but soluble carbohydrates and NFC (non fiber carbohydrates) are a key calculation

Abbreviations:

ADF = Acid Detergent Fiber
NDF = Neutral Detergent Fiber
NDFD = NDF digestibility
NFC=Non Fiber Carbohydrates
CP = Crude Protein
DCAD=Dietary Cation-Anion Difference
EE = Ether Extract (fat)
TDN = Total Digestible Nutrients
IVDDM = In Vitro Digestible Dry Matter
RFV = Relative Feed Value Index
RFQ = Relative Forage Quality Index
RUP = Rumen Undegradable Protein
ME = Metabolizable Energy
NE = Net Energy
NEL = Net Energy for Lactation
NFTA = National Forage Testing Assoc.

(subtracting NDF, ash, protein and EE from the total dry matter. Carbohydrates can be directly measured, but are not often measured in hay. Estimation of digestible energy is typically the most important factor for predicting quality of forages, although intake is often considered a close second.

Attribute II. High Intake Potential

High quality forages have high intake potential—this is an important attribute of high quality. Intake is critical for high producing dairy cows – but unfortunately, once again, it’s difficult to measure and must be predicted. Some forages (such as legumes) are digested very rapidly in the rumen, while other feeds (such as warm season grasses) typically require extended periods for complete digestion. This might be affected by the nature of the forage particle (the physical disintegration of the fiber or ability of the cow to masticate), and by the rate of fiber digestibility by rumen microbes. Additionally, there are factors that cause animals to consume more or less of a forage, often termed “palatability,” that are affected by species, taste, condition of the hay, odor, weed content, stem quality, and plant maturity.

Lower intake levels result in lower energy availability per unit of time, reducing animal performance and lowering the forage quality. Higher-fiber alfalfa or forages with long rumen residence time cause animals to become “filled” and stop eating. This rumen fill limits intake, which ultimately reduces energy intake and animal performance. However, too-rapid rates of degradation result in poor rumen function and negatively affect animal health, causing acidosis and other health problems.

Digestibility of the NDF fiber fraction (NDFd) and other in vitro techniques (IVDDM) are important measurements for predicting intake. Intake potential is one of the most important quality factors for lactating dairy cows.

Attribute III. Good level of Protein, including ‘by-pass’ protein

Since amino acids from proteins are building blocks for muscle, milk, and animal enzymes, they are important nutritional attributes of forages. Additionally rumen microbes require protein for optimum function. Alfalfa typically contains between 17 and 25% CP and

corn silage from 6-8%. Although the concentration of protein (estimated by CP) is important, many nutritionists may also be interested in the amount of protein that passes undegraded from the rumen and is digested in the small intestine. Rumen degraded protein (RDP) provides an estimate of CP availability in the rumen and Rumen Undegraded Protein (RUP) that which does not degrade in the rumen. Acid detergent insoluble CP (ADICP) estimates the undigestible (typically lignified and heat-damaged) CP. While heat damaged protein is a negative factor, excessive degradation of CP in the rumen is also a negative quality factor if the rumen microbes do not fully utilize the ammonia nitrogen (N) for microbial protein. This excess ammonia N is absorbed through the rumen wall, and must be excreted in the urine. High rumen degradable protein can be a problem with very leafy immature alfalfa.

Crude Protein and RUP and ADIP are typical measurements for protein analysis. Since high CP concentrate feeds are generally available, protein in alfalfa forages are often not as strongly valued compared with its energy content in alfalfa hay markets. As the cost of CP supplements rises, the economic value of protein in forages will become greater, particularly “rumen escape protein” (RUP).

Attribute IV. Ruminally-Effective Digestible Fiber

Although high fiber forages reduce energy and intake, we don't want zero or very low fiber in forage crops. The NDF itself has value in rations in its own right. Many nutritionist highly prize alfalfa & corn silage for its NDF content itself, especially when these feeds are fed at a low % of the ration. The provision of ruminally-effective fiber with a high level of digestibility is a major attribute provided by alfalfa hay to ruminant rations.

Unfortunately, one can immediately see a contradiction between attributes #I , vs. #IV. Markets are faced with a quandary on this issue. Since energy and intake are important, we need to reduce NDF to influence digestibility, and growers are paid extra money to lower their NDF or ADF concentrations. But we don't want to get rid of NDF entirely in forages (even if we could). Reduction of forage fiber to very low levels creates problems in rumen function since dietary fiber stimulates rumination, chewing, and saliva production; the latter helps to stabilize rumen pH, preventing acidosis. High-producing ruminants can suffer physical problems with rumen health when “effective fiber” is too low in their diet. Thus, the fiber in alfalfa provides

positive physical and chemical attributes to ruminant rations, and should be valued in and of itself.

It is clear that both a low concentration of the NDF and the high digestibility of the fiber fraction (NDFD) are important attributes of high forage quality, but the NDF fraction itself is a valuable quality attribute, especially when highly digestible (high NDFD). The value of effective-fiber (vs. low-fiber, high-energy) hay varies, depending on the levels in the diet and class of animals.

V. Low Ash and good Mineral Balance

In general, as ash increases, the level of digestible energy declines, since minerals do not contain energy or protein. Therefore, lower ash levels are generally desirable quality characteristics.

Ash is an estimate of total mineral content in a forage, which could originate from normal mineral uptake by the plant, for example, phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), chlorine (Cl), and sodium (Na) from excessive salt accumulation, or contamination with soil (dirt).

Although low ash is generally a good thing, alfalfa provides several essential minerals contained in the ash fraction. The balance (or type) of mineral ions, such as Ca, P, and K in alfalfa may be more important nutritionally than the quantity. For example, high K is a negative attribute for dairy animals just before and just after calving (often termed the “close-up” or “transition” period), since excessive K contributes to an increased incidence of milk fever. In many markets, dairies will pay a premium for ‘low Potassium’ hay for transition animals. Additionally, excessive concentrations of micronutrients (such as selenium [Se] or molybdenum [Mo]) can be toxic when present in high amounts in the diet. Conversely, hays can provide necessary micronutrients that otherwise might be limiting in diets. Nutritionists frequently are most interested in the balance of mineral nutrients in forages (e.g. DCAD).

VI. Other Quality Factors—Weeds, dust, molds, toxic factors, condition, texture and aroma

Animals, like humans, respond to aromas and subtle cues like mouth-feel and dust and molds. Weeds are an obvious negative trait for quality of alfalfa hay, particularly grassy weeds and poisonous weeds, and weeds with spines or thorns. There are other, less-well-defined attributes of quality, such as secondary plant compounds that affect sensory preference by animals, but these may be important primarily as they affect intake (Factor II) and general animal health. Toxic weeds or insects (e.g., blister beetle) can be important anti-nutritional or toxic factors, and important quality factors in hay. Each year, many animals are sickened or die from poisonous weeds, excess nitrate (from weeds), or excess micronutrient concentrations in hay or silage. These are all attributes under the umbrella of “forage quality.” Nitrates must be measured, while weeds and condition must be judged via visual inspection, not lab analysis.

EVALUATION OF HAY QUALITY

Table 3. Quality Attributes of Alfalfa Hay and the methods used to evaluate quality. A combination of visual analysis and lab analysis is always recommended. Note that color is not often recommended as a quality indicator.

Factor	Attributes	Suitability for Prediction:	
		Visual Analysis	Lab Analysis
Estimated Via Visual Analysis			
Weed Content	Providing weed-free alfalfa is one of the most critical components of high quality hay, especially coarse grasses.	Excellent	Fair – lab analysis can pick up grassy weeds (NDF).
Noxious Weeds	Poisonous or noxious weeds can harm animals and are a major anti-quality factor.	Excellent	Poor
High Leaf Percentage	High alfalfa leaf percentage is one of the strongest predictors of high quality.	Good, but must have trained eye	Good – NDF and CP are good predictors
Leaf-Stem Attachment	Poor attachment leads to loss of quality during feeding.	Excellent	Poor
Molds/Dust	Molds and dust can reduce intake and affect animal health	Excellent	Poor
Texture	Very dry hay causes mouth irritation, softer hays can exhibit better animal acceptance.	Excellent	Poor
Odor	Animals respond to hay with attractive odors and can reduce intake with poor smells	Good to Excellent	Poor
Estimated Via Lab Analysis			

Fiber Concentration	Low, but not too low, fiber concentration (NDF) predicts good energy and intake potential	Poor to Fair – can estimate fiber ‘by feel’	Excellent – measure NDF/ADF
Fiber Digestibility	The degradability of the fiber fraction, which makes up 1/3 to ½ the plant, is a critical quality predictor	V. Poor	Excellent- requires invitro methods (NDFd)
Protein Concentration	Supplying sufficient protein is a key quality attribute	Poor to fair- leaf % is OK	Excellent – measure CP
Protein Degradability	‘By-pass’ protein may improve protein efficiency	V. Poor	Good
Ash	Low ash is important for high quality forages, and can indicate dirt contamination	V. Poor	Excellent – Measure ash
Ash/Mineral Balance	Low potassium is important for some animals, not for others. Look for balance of CA, Mg, and K, Na for some animals – consult nutritionist.	V.Poor	Excellent – measure ash and DCAD

Evaluating Forage Quality

As the above discussion reveals, forage quality is not just one thing, and cannot be reduced to just one number. It’s not just the fiber content (the ADF or NDF which determine RFV or TDN) that determines quality, but also the crude protein content, mineral balance, the digestibility of the fiber fraction, functional fiber, and the rumen undegradable protein, but let’s not forget the presences of weeds, molds, and foreign materials, and condition of the hay.

We also shouldn’t forget that quality is dependent upon type of animal and type of ration. Just as a race-car is not the best for hauling hay to feed your animals, different types of hays are best suitable for different types of animals. A low-fiber, high protein pure alfalfa hay with high digestibility is the most appropriate for a high-producing dairy cow, but a medium-fiber hay with moderate forage grass content is appropriate for many non-lactating horses and beef animals. Additionally, higher fiber in hay is not a bad thing when hay becomes a small part of the diet,

because it provides ‘functional fiber’ to the animal for good rumen function in TMR rations. There are different qualities for different purposes

Use both Visual and Lab Methods

It is always recommended to utilize multiple methods (visual inspection and lab analysis—Table 3), as well as several lab analyses (Table 2) to evaluate forages.

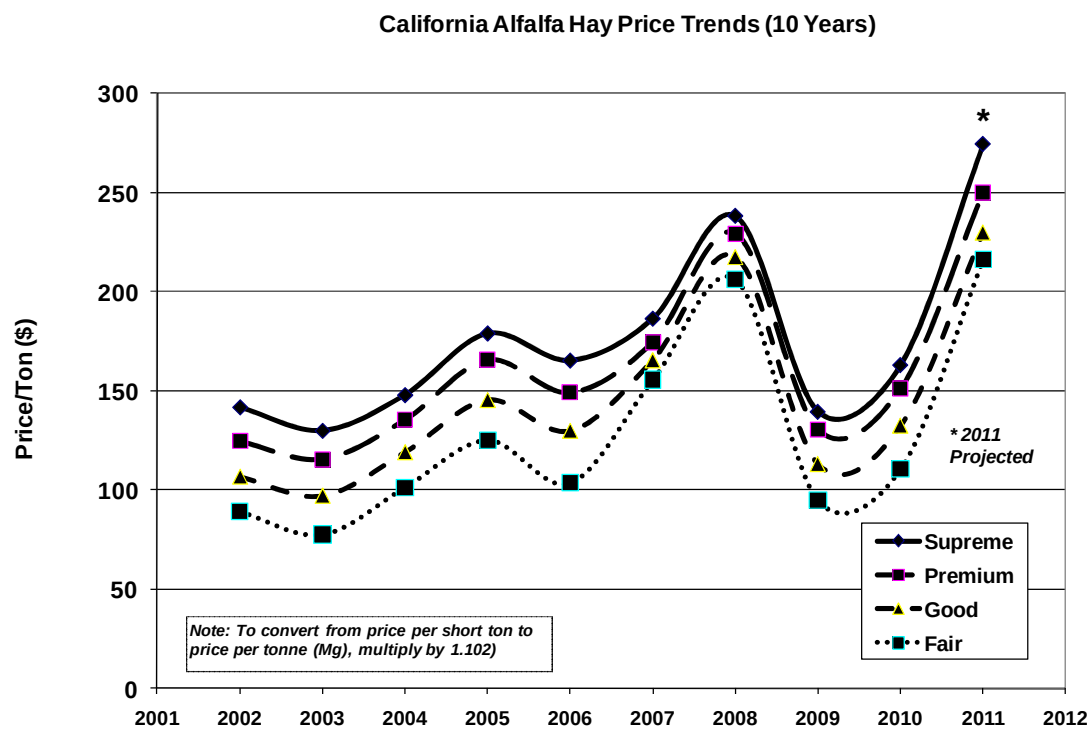


Figure 1. Long term trend of alfalfa hay prices, California, 2002-2011. Source USDA-Haymarket News Service.

CURRENT FIBER-BASED MARKETING SYSTEMS

Nationwide, in the US, alfalfa hay is essentially marketed using ‘fiber-based’ marketing systems. RFV and TDN are the most common languages of the hay trade, and appear to be superficially different. However, these are simply calculated from ADF and/or NDF lab measurements, so they are essentially similar in approach. They are both ‘fiber-based systems’. Higher fiber equals lower price in current markets. TDN is 100% equivalent to ADF alone, and RFV is essentially 99% equivalent to NDF alone in pure alfalfa hays. CP and other factors figure in to some degree, but it’s basically driven by fiber. (See NFTA www.foragetesting.org for calculations of RFV and TDN).

The state of California provides an interesting glimpse into the marketing of forages, since >95% of the hay in this state is marketed (compared with probably <10% in most eastern and Midwestern states), it has a long history of marketing, with large volumes. The marketing system in CA is similar to many of the other western states of OR, WA, ID, UT, NV, AZ, and NM, where 35-40% of the US alfalfa is grown. In CA, forage quality designations probably account for between \$250-300 million/year in crop value of a \$1 billion/year cash crop.

During the past 10-year period, average hay prices in this state varied between about \$70/ton to about \$280/ton, and were influenced by both supply-demand in any given year, and upon quality factors (Figure 1). On a volume averaging 7.4 million tons (6.7 million Mg), averaged across 13 marketing districts, differences in price changed an average of \$6.14 per unit change in % ADF (Figure 2). Differences due to quality were about \$45/ton or an average of 45.8% percent of the lowest price value in any given year. (To convert from \$/ton to \$/Mg, multiply times 1.102).

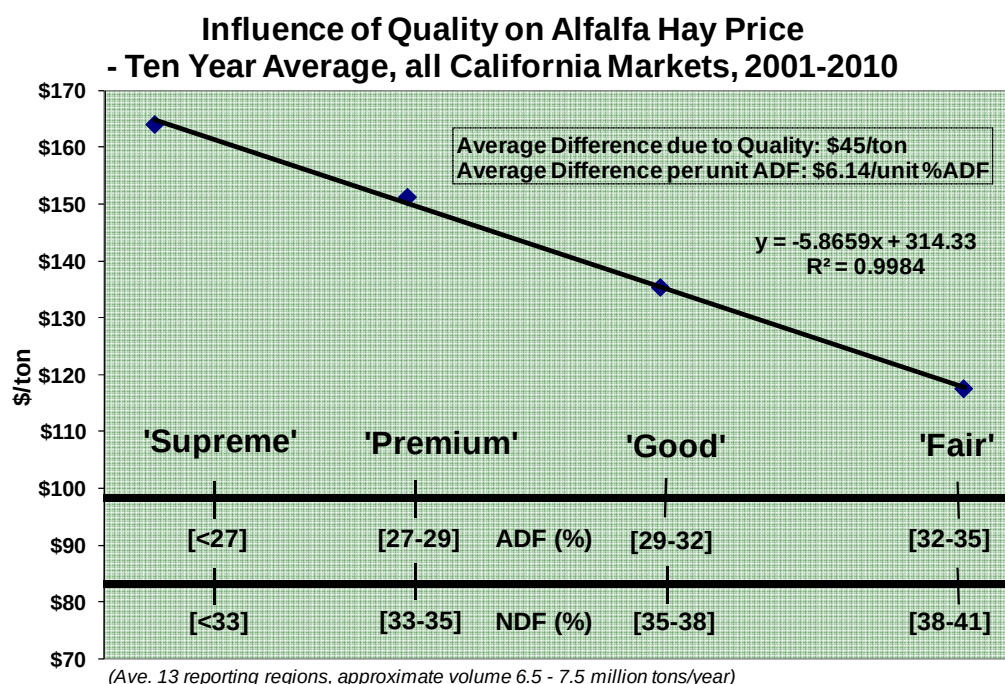


Figure 2. Average Changes in Fiber concentration (ADF & NDF) and its influence on price, 10 years in California markets. This does not account for ‘utility’ hays or hays sold mostly for horses.

Supply-Demand Influence on Quality-Price Relationship

The influence of quality on hay price is highly dependent upon the supply and demand situation in any given year (Figure 1). It’s worth noting that in ‘high price years’, differences due to quality are smaller than in ‘low price years’ (Figure 1), both numerically and as a percentage of price. This relationship can be clearly seen in Figure 3. In the lowest priced years, high quality hays were worth up to 90% more than the low quality hays, but in a high priced year, the quality premium was only 20-30% more. This is because buyers can be less choosy in a high-price year, and must accept hays of lower quality. Additionally, hay growers are less-apt to sacrifice yield for quality in high price years, producing less amounts of high-quality forage since it is in their economic interests. Conversely, in low price years with high hay supply, lower quality hay often just simply doesn’t sell, so growers must produce high quality in those years just to be able to sell hay.

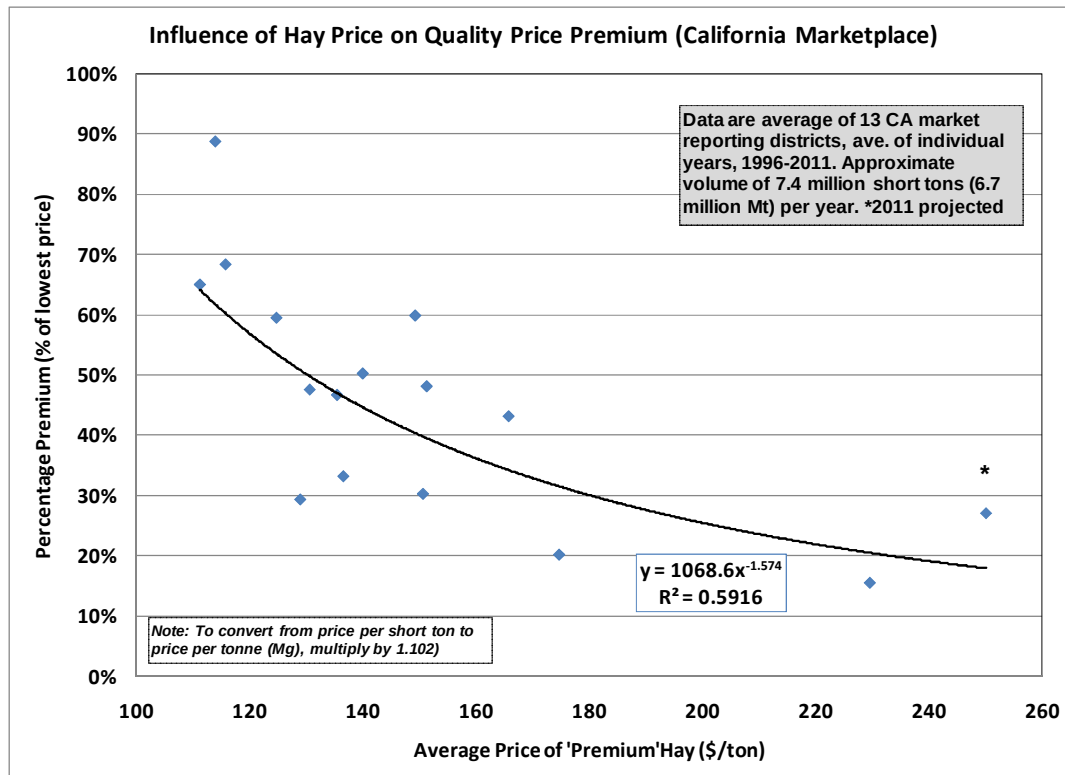
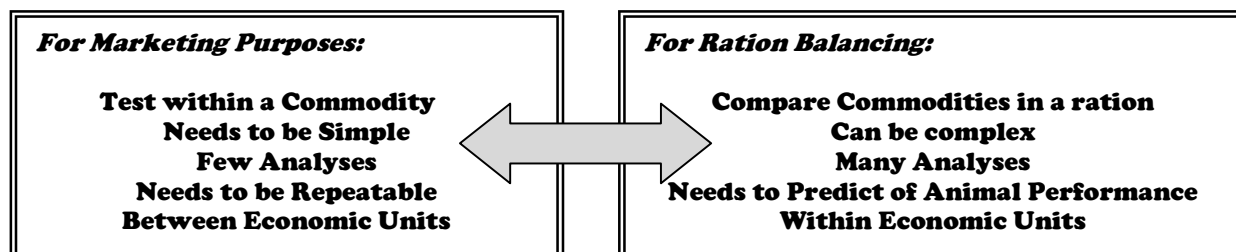


Figure 3. Price premium due to quality as a function of hay price (top vs. bottom), California markets.

LIMITATIONS OF A FIBER-BASED MARKETING SYSTEM

TDN and RFV systems (which are calculated from the fiber analyses of ADF and NDF) have been very useful in enabling hay markets to develop, and have enabled dairy producers to increase animal output substantially. The 'fiber based' market system is not unreasonable, due to the fact that most nutritionists want high energy and protein in their forages, which are generally found in low-fiber hay products. It is important to realize that the analyses required for marketing may be different from the analyses required for ration balancing (Figure 4). While we need to have simple, dependable systems and test WITHIN a commodity for marketing, we need to COMPARE commodities when balancing rations. Marketing measurements need to be simple, whereas lab values used for ration balancing can be more complex (Figure 4).

Figure 4. The important attributes of forage testing changes for marketing purposes vs. ration balancing. Repeatability and simplicity are critical for marketing purposes, while a high degree of predictability and relationship with other feeds are important for ration balancing.



However, the ‘fiber-based’ marketing system creates some warping of the quality-value relationship that must be addressed. There are major yield penalties to the hay grower by trying to produce ever-lower ADF or NDF hays in search of ‘dairy quality’ hay-the farmer suffers yield losses and stand losses as a result. But there are nutritional problems as well.

Valuing forage simply targeting ‘low fiber’ makes no sense from a nutritional viewpoint. The role of forage fiber in dairy rations is changing. With all the concentrates currently fed, digestible effective fiber (NDF) has played a much more important role in rumen function and animal health, as compared with the need to lower fiber itself to increase energy in forages. Marketing based upon fiber values has the advantage of simplicity, but may fail to differentiate important differences in forage quality within a critical range where the change in price due to fiber value is dramatic (Figure 5).

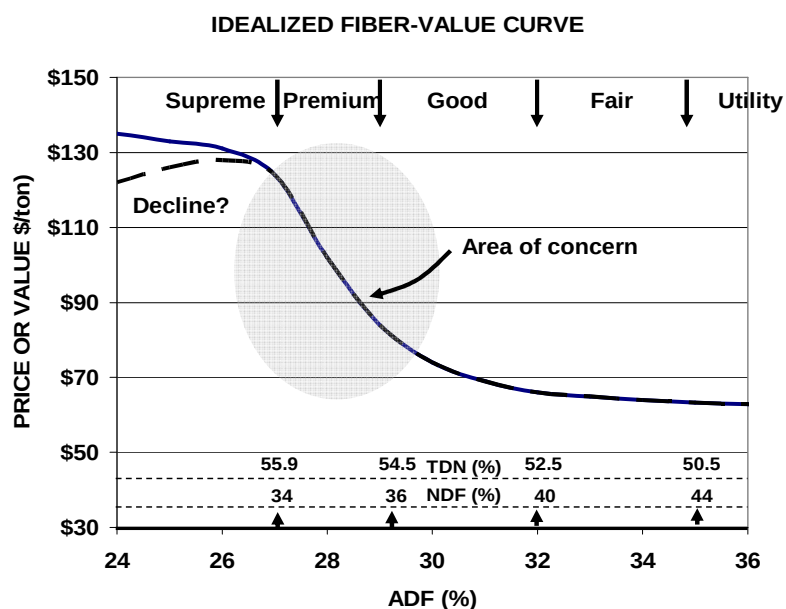


Figure 5. Idealized relationship between fiber value and price. While portions of this curve are linear others are not. A critical area of concern is the ‘cutoff’ between dairy quality and non-dairy hay (linear portion), where small differences in fiber result in large price differences and where there is the most abuse of the fiber-marketing system. This is the region prone to the most misuse, and where additional analyses should assist marketers.

Figure 5 shows an idealized quality-value curve – this is a reflection of the general reality of fiber-vs.-value in most alfalfa hay markets (the numbers are examples only). When %NDF or %ADF are high, markets do not distinguish much due to changes in lab values (these are mostly considered low quality hays fed to dry cows or low producers). But between about 34 and 40% NDF, small changes (e.g. 0.3%) in NDF or ADF result in a large change in price. Additionally, markets do not necessarily reward very low NDF or ADF values at the other extreme.

The influence of such small changes in NDF on price (‘area of concern’, Figure 5) does not make sense, since 1) Lab measurements themselves have a greater degree of variation than would be justified by this practice, and 2) it is unlikely that the true feeding value varies that tightly with small changes in fiber content. A better approach would be to define a range of acceptably low fiber levels (e.g. 33-36 NDF) for high energy forages, and add the different dimensions (such as NDFD, Ash, CP) which would assist in the price identification.

If we follow the ‘low fiber’ price structure to its logical conclusions, we would want zero fiber in forage, with duckweed or algae as our optimum forage! However, nutritionists will rapidly tell you that this is silly – the fiber itself from alfalfa and corn silage forages is important to animal health and are nutritionally valuable.

Greater use of NDF, NDF digestibility, ash, protein and its derivatives, or other measurements may assist buyers and sellers in differentiating hay products and improve prediction of the feeding value, especially if those measurements can be shown to be rapid and repeatable. Buyers can then take a ‘heirarchical approach’ to valuation, emphasizing some analyses more for some classes of animals, and other analyses for other situations. For example, if protein becomes expensive, dairy managers may be much more interested in the protein levels vs. energy or intake.

RECOMMENDATIONS FOR IMPROVEMENTS

The standard forage test consisting of ADF, NDF, CP, and DM has been a standard hay test for several decades. Growers mostly understand them, although some confusion remains vis-à-vis lab-to-lab consistency and sampling standardization (See www.foragetesting.org for standardized hay sampling techniques and lab methods). Calculations of RFV, TDN are

somewhat confusing, but these are simply derived from analyzed lab values. There is a need to seek ways of improving this system, in particular to prioritize what we measure, and what weight is given to each measurement. The predictability of the hay measurement, or series of measurements, must be balanced with the need for the system to be simple and repeatable. This may result in a revised ‘standard hay test’ (Table 4). The highest priority of these include:

- **Use of NDF** as a starting place, a first approximation of quality for markets. NDF represents a more meaningful dietary measurement for most nutritionists. Low, but not extremely low NDF frequently predicts high quality. For alfalfa hays there is no need to measure both ADF and NDF. Some nutritionist value lignin measurements as well.
- **Continued use of CP.** Crude protein continues to be useful and important for ration balancing – often second to NDF in importance.
- **Use of NDFD** into routine analysis for marketing and hay evaluation. The digestibility of the NDF fraction is clearly needed to differentiate hays which are genuinely different in feeding value but have the same fiber value. NDFD provides clearly different information about forages compared with NDF or ADF (Figure 6). However, repeatability of NDFD needs further evaluation and testing.
- **Use of Ash in Marketing.** There remain large differences in ash between different hay types which are currently ignored by markets.
- **Expression of lab values on 100% DM basis** (including ADF, NDF, CP as well as TDN). Confusion arises when other forms of expression are used.
- **Clear separation between analyzed and calculated values** on lab tests to reduce confusion in the marketplace.
- **Continued attention to the importance of hay sampling and lab standardization**, via voluntary effects such as NFTA.

Table 4. Suggested direction for a revised standardized hay test. While a wider range of analyses can be used, this represents a smaller sub-set for ascribing a majority of the value of alfalfa hays. This would replace the

current practice of utilizing DM, ADF, NDF, and CP in standard hay tests.
<i>Analytical Determinations</i> Dry Matter (DM) (as received) Neutral Detergent Fiber (aNDF) (100% DM) NDF Digestibility (NDFD) (100% DM) Crude Protein CP (100% DM) Ash (100% DM) <i>Calculated Values (100% DM) as Needed</i> TDN NEL ME RFV RFQ

COMMENTS ON CORN SILAGE

It is likely that a small minority of the corn silage in the US is marketed between farmers, and even less is marketed based upon quality traits. Thus the experience of the industry of marketing corn silage based upon quality is still in its infancy. The majority of corn silage is grown by dairies and fed on-farm-but this is changing as farm units increase in size; more corn silage is likely to be marketed in the future.

Currently, growers of corn silage who sell to neighbors typically prioritize yield factors over quality, and will often compare grain yields with potential silage yields economically. But from the feeding perspective, there is quite a bit of interest in improved genetics (such as the BMR trait, leafy corn varieties, low lignin, and high oil silage types) and how these will affect animal importance. This begs the question of how to value these traits in exchanges between farmers. This problem is complicated by the fact that ‘quality’ in silages is influenced to a high degree by management factors during ensiling, such as particle size, moisture, inoculation, compaction, spoilage and containment (ability to exclude air). So the question as to whether silage is marketed BEFORE ensiling or AFTER is important. Wisconsin and California (the two

largest dairy states) are also the largest corn silage producing states in the US. In CA, quite a bit of the corn is grown by neighbors, but the ensiling is accomplished by contracted operators or by the dairies themselves. Thus yield and DM content are typically the only considerations setting price in this market—quality is primarily determined within a dairy after ensiling.

There are traditional ‘rules of thumb for marketing corn silage: e.g. multiplying the price of a bushel of corn by 10 times to equal the price of a ton corn silage at 35%DM. The price of corn grain has frequently been used, and is a reasonable approach from a grower’s point of view, since they can always choose to harvest the crop as a grain, with well-understood price structures. Alternatively, some may multiply the going price for top-quality alfalfa hay by 50-66%.

However, methods that link silage price to the price of the grain do not value the forage stover portion of the crop, and the potential differences in feeding value. Jim Linn at University of Minnesota has developed an adjustment method which starts with a base price per ton of DM based upon the planting and harvest costs, and then adds a starch adjustment of a dollar amount per unit corn grain, and an NDF digestibility adjustment based upon a milk price (http://www.ansci.umn.edu/dairy/topics/high_corn_silage_diets.pdf).

It is obvious that improved marketing methods for corn silage will need to be divided into marketing of the ‘Crop in the Field’ vs. marketing ‘Corn Silage Delivered’. The requirements for these are likely to be different. It appears that widely used measurements of DM, NDF, Starch, CP and NDFD are likely to play important roles for both types. For ‘Corn Silage Delivered’, additional factors such as ‘success in fermentation’ – particle size, presence of mycotoxins or spoilage, nitrates are likely to play a more important role.

Moisture content is a critical value, since ‘true tonnage sold’ can only be determined by accurate moisture percentages. This is sometimes done sloppily. The same, unfortunately, is true of the hay markets, but with less impact, since DM doesn’t vary as much. DM should primarily determine tonnage, not quality, although very high or very low moistures are an indicator of the possibility of quality problems, especially with silages, but also with hays.

It is painfully obvious that there is a need for improved incorporation of quality measurements in the marketing of corn (and other) silages to take advantage of the improved feeding value of improved corn silage hybrids and better methods of ensiling. Utilizing corn grain values alone ignores the significant differences in fiber digestibility that are known to exist

between hybrids and growing conditions. DM, NDF, Starch, CP and NDFD are all likely to play an important role, with physical can chemical considerations also coming into play.

In my view, a 'hierarchical' approach here again makes sense, since these different analyses may be more valuable in some rations and markets vs. others.

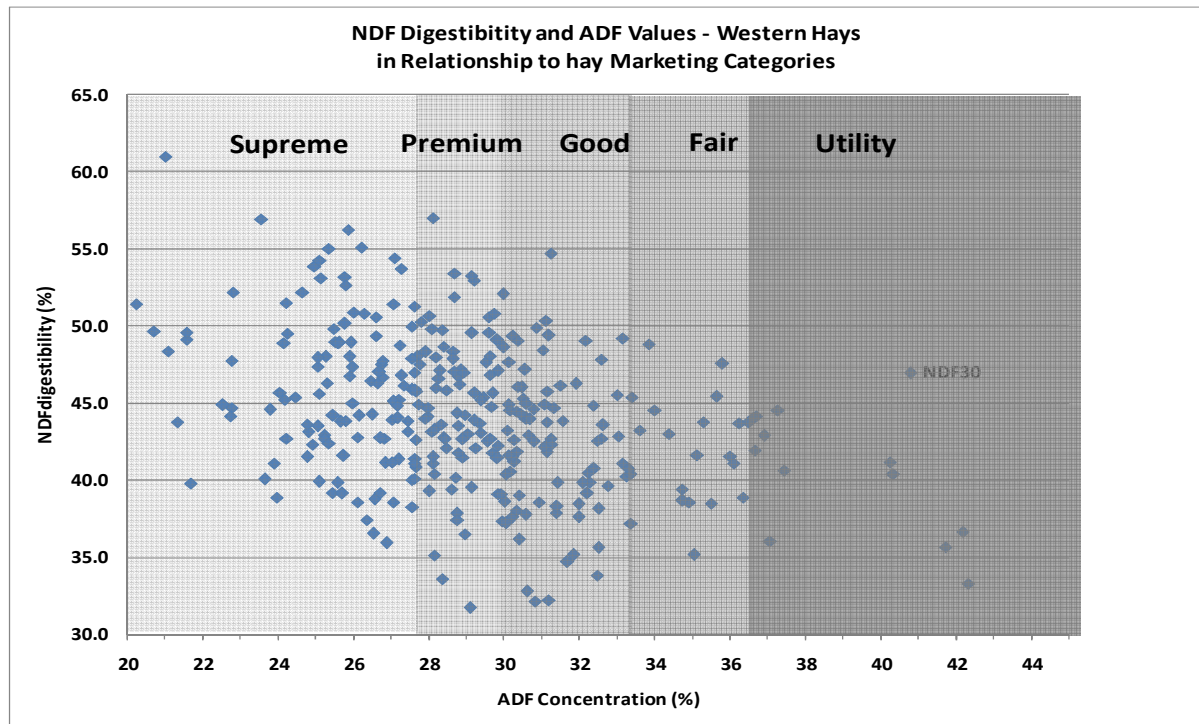


Figure 6. Relationship of NDF digestibility to currently-used hay market categories of Supreme, Premium, Good, Fair, and Utility, as defined by USDA-Market News (using ADF). ADF or NDF concentration, though important, fail to predict digestibility of the fiber fraction, of major importance to nutritionists. (Dataset from grower-submitted western states alfalfa hays, Cumberland Valley Labs, Cumberland MD).

Contribution of silage volatile compounds for the animal nutrition

João Luiz Pratti Daniel¹

Luiz Gustavo Nussio¹

1. Introduction

Silages are produced by fermentation of forage which contains enough moisture. The anaerobiosis, which is the most important condition to the process, allows the microbial growth and formation of organic acids from soluble carbohydrates oxidation. It is the most common way to inhibit undesirable microorganisms and to preserve the ensiled substrate (McDonald et al. 1991; Pahlow et al., 2003).

Under normal condition, lactic acid is the major fermentation end-product in silages. In the homolactic pathway, lactic acid is the single product and the microorganisms obtain 2 ATP per hexose fermented, therefore, dry matter and gross energy are almost entirely preserved. On the other hand, microorganisms which produce volatile fatty acids extract 4 ATP per hexose, however, the substrate is partially converted to gases and emitted into the atmosphere, mainly as CO₂, which usually represents energy loss (McDonald et al., 1991, Van Soest, 1994; Pahlow et al., 2003). From a microbial point of view, more glucose should be broken down by homolactic fermentation in comparison with VFA pathway to obtain the equivalent amount of energy, due to the differences in ATP yield. Nevertheless, lactic acid is stronger (pKa ~ 3.8) than VFA (pKa ~ 4.8), so silage pH drop faster through homofermentative pathway, which decreases the microbial activity and anticipates the stability phase where losses become lower (McDonald et al., 1991).

In addition to the carboxylic acids, other end-products such as alcohols, aldehydes, esters, and ketones have been identified in silages (Morgan and Pereira, 1962; Weiß et al. 2009; Chmelová, 2010) (Tables 1 and 2). In sugarcane silages, alcohols are the major fermentation end-products (Zopollatto et al., 2009) (Table 3). This pathway is characterized by high loss of dry matter, while the gross energy is preserved due to the high heat of combustion of the ethanol.

In general, silage fermentations lead to losses, but the end-products are essential to the preservation. Moreover, these compounds have high energy content and they are significant sources of nutrient to the animal.

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Table 1. Concentration of fermentation end-products in corn silages (Weiß et al., 2009)

Compound	Minimum	Maximum
Lactic acid, g/kg DM	6.9	78.9
Acetic acid, g/kg DM	5.8	79.4
Propionic acid, g/kg DM	<0.1	11.2
Ethanol, g/kg DM	0.9	64.0
Methanol, mg/kg DM	114	1030
Propanol, mg/kg DM	14	19096
Butanol, mg/kg DM	<3	9
iso-Butanol, mg/kg DM	<3	23
2-Butanol, mg/kg DM	12	1314
Allyl alcohol, mg/kg DM	<4	28
2-Methylbutanol, mg/kg DM	<3	10
3-Methylbutanol, mg/kg DM	<3	73
Hexanol, mg/kg DM	<3	21
2-Phenylethanol, mg/kg DM	<4	44
1,2-Propanediol, mg/kg DM	<5	13630
Methyl acetate, mg/kg DM	<3	154
Ethyl acetate, mg/kg DM	12	789
Propyl acetate, mg/kg DM	<5	379
Ethyl lactate, mg/kg DM	16	1263
Propyl lactate, mg/kg DM	9	77

Table 2. Aldehydes in farm-scale silages (Chmelová, 2010)

Aldehyde (mg/kg FM)	Corn silages (n = 8)			Grass silages (n = 13)		
	Mean	S.D.	Range	Mean	S.D.	Range
Ethanal	25.8	16.1	9.4-48.2	29.8	14.2	9.9-49.4
Propanal	70.2	17.8	51.3-99.1	76.6	12.0	54.4-97.6
Butanal	77.9	16.9	52.2-100.0	71.9	17.2	46.1-98.7
2-Methylpropanal	28.9	15.1	10.4-49.0	25.7	15.8	9.2-50.4
Pentanal	150.0	37.8	97.8-200.0	147.0	30.3	105.0-189.0
3-Methylbutanal	78.3	17.4	50.6-97.3	76.0	18.5	46.7-100.0
Hexanal	153.0	38.9	97.1-196.0	150.0	30.3	104.0-190.0
Heptanal	24.4	17.6	7.9-50.3	23.1	11.8	10.3-46.5

Table 3 – Fermentation end-products in sugarcane silage (adapted from Zopollatto et al., 2009)

Compound (% DM)	n	Mean	Minimum	Maximum
Lactic acid	12	3.3	0.8	6.3
Acetic acid	14	4.0	1.6	9.3
Lactic/Acetic	-	0.83	-	-
Propionic acid	9	0.7	0.2	1.9
Butyric acid	6	0.1	0.0	0.1
Ethanol	19	7.8	0.3	21.8†
Total		15.9		

† *L. plantarum* was used as additive (Freitas et al., 2006)

2. Methods to determine dry matter content in silages

In conventional chemical analysis, feed samples are dried in a forced air oven. The weight loss is assumed as water and, in turn, the residue is called dry matter. Thus, this gravimetric method is not accurate to achieve the dry matter content of samples containing volatile compounds other than the water itself. In silages, much of fermentation products are lost by volatilization during heating (Colovos et al. 1957; McDonald and Dewar, 1960; Fox and Fenderson, 1978). It leads to deviations on nutrient and energy contents of silages, dry matter intake, diet digestibility and energy efficiency (Stone et al., 1960). The determination of dry matter by using forced air oven also brings on errors for calculations of dry matter losses during silage storage (Figure 1).

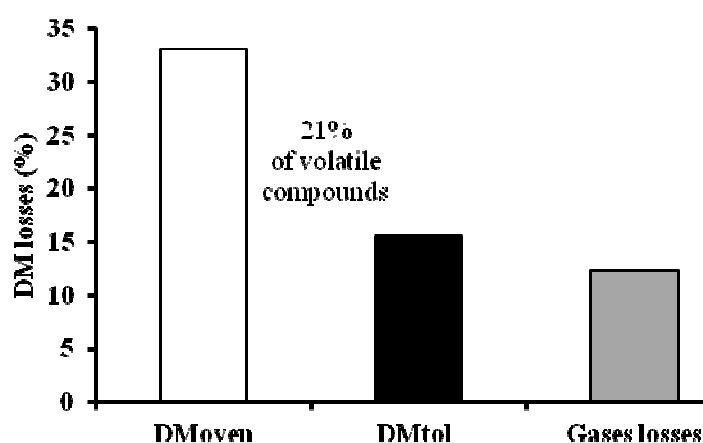


Figure 1. Dry matter and gases losses in sugarcane silage without any additive. Dry matter contents were determined by oven (DMoven) or toluene distillation (DMtol) (Daniel, 2011).

For long time it was known that silage had volatile substances that could be lost during the drying process. Until early 1940s, most studies had ignored this fact. Therefore, Perkins (1943) carried out a trial to compare the traditional method (oven drying at 100°C) with a new method of volumetric determination of water content in fodder samples. The alternative method was based on sample distillation with excess of solvent, the toluene (boiling point 110°C). Although the volumetric methods were less accurate than that gravimetric, there were no deviations relative to volatile substances. Perkins (1943) showed that both methods achieved exactly the same content of water in green fresh plants. On the other hand, the water content was always higher when silage samples were dried at 100°C. Then, the authors suggested that the simultaneous application of both methods to allow quantifying the volatiles fraction of silages.

Ever since, it was suggested that in the toluene's method, part of the volatile fraction could be found in the distilled water and increase its volume. Although measurable, the amount of volatile compounds represented less than the lower scale interval of the device, and therefore it can be negligible (Perkins, 1943). In addition, the volume of volatile components found in the aqueous phase from distillate could numerically compensate the small amount of residual water in the sample (Undersander et al., 1993). Thus, the dry matter determined by toluene distillation with no correction can be considered more appropriate.

In 1960s, McDonald and Dewar (1960) studied the nature of the volatilized fraction from silages at heating. The volatility averaged 87.9% for acetic acid and 89.4% for butyric acid. In all samples, lactic acid also evaporated, with volatility ranging from 1.4 to 16.4%. Nitrogen losses occurred mainly in the silages with higher pH. At the same decade, Wilson et al. (1964) compared the oven drying (DM_{oven}) to the toluene distillation (DM_{tol}). The DM_{oven} was up to 22% lower than the DM_{tol}.

Haigh and Hopkins (1977) determined DM_{oven} and DM_{tol} content of 205 silage samples. The relationship between both methods was described as: $DM_{tol} (\%) = 0.96 * DM_{oven} (\%) + 2.2$ ($r^2 = 0.98$, $P < 0.01$). DM_{tol} averaged 11% higher than the DM_{oven}. In sugarcane silage, Miranda (2006) noted that the DM_{oven} content at 100°C averaged 80% of the value achieved by toluene distillation. Thus, 20% of the dry matter from sugarcane silages was composed of volatile compounds. This value was recently confirmed by Daniel (2011) (Figure 1).

Table 4. Percentages of dry matter lost estimated by various methods, using the toluene distillation as the reference method (Aerts et al., 1974)

Sample	Freeze drying	Microwave	Oven 70°C	Oven 100°C
Corn silage	6.71	-	6.21	7.47
Grass silage	9.73	6.84	8.58	10.72
High moisture corn silage	3.59	3.56	3.42	4.35
Fodder-beet	12.71	-	11.39	13.79
<i>Mean</i>	<i>7.58</i>	<i>5.37</i>	<i>6.89</i>	<i>8.42</i>
<i>(Range)</i>	<i>(1.14 – 28.38)</i>	<i>(1.14 – 20.30)</i>	<i>(1.41 – 22.77)</i>	<i>(0.65 – 23.70)</i>

Freeze drying is an alternative to drying silage samples. Aerts et al. (1974) compared several methods for determining dry matter in comparison to the toluene distillation. They observed 7.58% of dry matter lost during freeze-drying (1.14 to 28.38%) (Table 4). Alomar et al. (1999) determined the chemical composition and gross energy of silage samples after freeze-drying or drying in an oven at 65°C. There was no effect of drying method on the dry

matter and energy content, because both methods led to losses. It was not surprise to the authors, since they could smell the aroma of volatiles compounds during freeze-drying. In fact, it happens due to the lower pressure during freeze drying, which decreases the boiling point of compounds (Atkins, 1994).

Currently, there are other methods to determine the volume of water in feed samples, such as gas chromatography and Karl Fisher titration. In both, water is extracted from the sample with organic solvents, usually alcohols. In the former, the extract is injected into chromatograph coupled to a thermal conductivity detector (Fenton et al., 1981). In the Karl Fisher method, the extracted water is titrated with iodine in the presence of pyridine and methanol (Galletti and Piccaglia 1988). These methods led to results similar to the toluene distillation (Table 6). Chromatography, although a quick and accurate method, requires access to a gas chromatograph coupled to thermal conductivity detector, which has high cost. On the other hand, the reagents used in the Karl Fisher titration are potentially harmful to the health, as well as toluene.

One promising method to correct the dry matter content obtained by dehydration is to determine the concentrations of fermentation end-products in fresh silage and input those values into the equation containing the respective volatility coefficients (Weissbach, 2009). In corn silage, whose pH is usually low, the volatility coefficient of VFA could be assumed as 95%. In grass silage, however, this coefficient was defined as: Volatility (%) = 105 - 5.9 * pH. The volatility of lactic acid averaged 8%. Alcohols with one hydroxyl group evaporated almost completely at oven drying. Other alcohols such as 1,2-propanediol and 2,3-butanediol showed volatility coefficients of 77% and 87%, respectively (Table 5). The following equations were proposed for the dry matter correction (Weissbach, 2009):

Corn silage

$$\text{DM} = \text{DM}_{\text{oven}} + 0.95 \cdot \text{VFA} + 0.08 \cdot \text{lactic acid} + 0.77 \cdot \text{1,2-propanediol} + 1 \cdot \text{other alcohols} \quad (\text{g/kg FM})$$

Grass silage

$$\text{DM} = \text{DM}_{\text{oven}} + (1.05 - 0.059 \cdot \text{pH}) \cdot \text{VFA} + 0.08 \cdot \text{lactic acid} + 0.77 \cdot \text{1,2-propanediol} + 0.87 \cdot \text{2,3-butanediol} + 1 \cdot \text{other alcohols} \quad (\text{g/kg FM})$$

Table 5. Incidence, content and volatility of fermentation end-products in corn silages (Weissbach, 2009)

	Corn silages			Grass silages		
	Incidence (%)	Content (g/kg FM)	Volatility (%)	Incidence (%)	Content (g/kg FM)	Volatility (%)
Acetic acid	100	9.98	95	100	8.27	78
Propionic acid	100	0.28	97	100	0.45	78
iso-Butyric acid	16	0.01	100	63	0.19	84
Butyric acid	62	0.09	100	91	2.06	88
iso-Valeric acid	78	0.06	100	98	0.36	71
Valeric acid	9	0.01	100	55	0.10	93
Caproic acid	21	0.01	100	68	0.19	92
Lactic acid	100	15.20	8	100	14.63	10
Ethanol	100	5.80	100	100	2.50	99
Propanol	50	0.25	100	49	0.20	100
Butanol	1	0	100	14	0.01	100
1,2-Propanediol	92	0.70	77	70	0.60	77
2,3-Butanediol	52	0.08	100	80	0.26	87

3. Losses of volatile compounds from silages

During storage and especially at silage feed-out, part of fermentation end-products can be either emitted into the atmosphere (Mitloehner et al., 2009) or oxidized by aerobic microorganisms (Spoelstra et al. 1988; Driehuis et al., 1999). However, when they are actually consumed by the animals, these compounds have nutritional significance (Daniel et al., 2011a).

The loss of volatile compounds occurs because all liquid substances and some solid evaporate continuously. The rate of evaporation or volatilization is defined by the vapor pressure, which depends on the temperature and type of substance. Materials with higher vapor pressure releases more molecules to the vapor phase and volatilized faster than that under lower vapor pressure (Atkins, 1994).

Temperature affects the kinetic energy of molecules and hence the vapor pressure. As higher is the temperature, more particles may evaporate. Moreover, substances with different polarities have dissimilar intermolecular forces. Polar molecules are strongly attracted to their neighbors because of the positive and negative ends, which increase the resistance to vapor loss. The opposite is applicable for nonpolar molecules (Atkins, 1994). A good example is the comparison between lactic and propionic acids, which differ just by the presence of a

hydroxyl group bonded to the alpha carbon. The hydroxy acid is more polar and therefore less volatile, even at higher temperatures.

In the trial carried out by Weissbach (2009), the volatility of lactic acid was 8% compared to 95% for the propionic acid in corn silage exposed to 105°C. In the same study, the authors confirmed the influence of pH on the volatility index of silage compounds. The pH and pka established the ratio of dissociated and non-dissociated molecules, with the last being volatile component of the equilibrium. Therefore, as lower is the pH, as higher is the rate of acids volatilization and as lower is the alkali volatilization (Atkins, 1994).

Another factor contributing to the volatilization is the silage moisture. Porter and Murray (2001) found higher rates of evaporation of alcohols within drier silages, probably due to the higher gradient concentration between the sample and environment. In practice, mixing silage and concentrates increases the dry matter content, which might favor the volatilization of silage fermentation end-products, although there are no published data on this issue.

When organic compounds volatilize, they play a central role in atmospheric chemistry, because: 1) react with OH radicals, 2) form ozone by interaction with nitrogen oxides in the presence of sunlight, and 3) form secondary organic aerosols (Andreae and Crutzen, 1997). While the ozone layer in the upper atmosphere is beneficial, tropospheric ozone is extremely pollutant and proven risk to the human health. Respiratory problems, nervous damage, liver and kidneys injure, allergies and cancer are some of deleterious effects of pollutants related to the volatile organic compounds (Mendell, 2007, Bernstein et al., 2008).

The difficulty of linking health problems to these pollutants is because the clinical symptoms appear slowly and after chronic exposure (NRC, 2008). Currently, high levels of ozone are found not only in areas with high population density, but also in areas with intense agricultural activity (U.S. Climate Change Science Program, 2008). The control of this pollutant has been difficult since it is not emitted directly into the atmosphere, but formed by photochemical process. In this way, volatile organic compounds play a central role and its control has been advocated as an effective means to reduce levels of ozone in the troposphere (EPA²).

In the north hemisphere there are some studies about emissions of volatile organic compounds (VOC) from silages. Mitloehner et al. (2009) identified 24 VOC from silages and total mixed rations (TMR), being 6 alcohols, 5 VFA, and 13 esters. Alcohols, especially ethanol, predominated in all silages and TMR. The highest emissions of ethanol and propanol

² Environmental Protection Agency – United States of America, www.epa.gov

were detected in corn silages. The second most abundant group of VOC were VFA, being acetic acid the main component due to the high concentration in silages. Montes et al. (2009) measured the emission of VOC from silages under controlled conditions. The emission rates were higher at beginning of the tests, declined faster within 2 hours and then declined slowly over a 24 h period (first-order kinetic). The range of emissions, however, differed across the VOC. Ethanol was the main VOC from corn silage, while the alfalfa silage had the highest emissions from methanol. The VOC concentrations explained the differences between the rates of emissions in different forages, but not among the compounds. Regardless of the higher concentration, acetic acid had lower emission rates than that of ethanol, due to the lower volatility.

Krauter and Blake (2009) conducted a survey of VOC emissions on dairy farms in San Joaquin Valley, California, USA. The TMR based on silages were the main source of VOC (60% of total). In that study the authors showed that the emission rate of VOC was 4.5 times higher in disturbed silage piles compared to the intact piles, due to the higher mass porosity. Stored undisturbed silages represented 7% of total VOC emission.

Despite the exhausting search, no publications were found addressing VOC emissions from tropics silages. The research efforts to characterizing VOC from agricultural operations might be useful for proposing mitigation strategies, reducing environmental pollution and nutrients losses.

4. Usage of organic acids and ethanol on animal nutrition

While some part of fermentation end-products can be lost, the fraction consumed by the animals have nutritional significance (Daniel et al. 2001a), since they have high energy content per unit mass (Table 6). In the reticulo-rumen, silage VFA are incorporated into the rumen pool, removed by absorption and passage (Peters et al. 1990; Resende Júnior et al., 2006), and thereafter used by tissues as metabolizable energy (MacLeod and Orskov, 1984; Bergman, 1990; Orskov, 1995). Across major VFA, only butyrate is significantly metabolized by the rumen epithelium (Kristensen et al., 2000, Kristensen and Harmon, 2004). Such high quality silages have low butyric acid levels, and, in general, silage VFA must contribute significantly to the nutrient pool in the portal blood.

Table 6. Heat of combustion of major fermentation end-products in silages (Clymer, 2004)

Compound	Heat of combustion (kcal/g)	Compound	Heat of combustion (kcal/g)
Lactic acid	3.7	Ethyl lactate	6.5
Acetic acid	3.7	2,3-Butanediol	6.6
Propionic acid	5.1	Ethanol	7.1
Methanol	5.4	Propanol	8.1
Ethyl acetate	5.6	Pentanal	8.5
1,2-Propanediol	5.8	2-Butanol	8.7
Butyric acid	6.1	Hexanal	8.9

Additionally to the VFA, silages are rich in lactic acid. In the rumen, lactate can be absorbed or converted to VFA (Waldo and Schultz, 1956, Hueter et al., 1956). If absorbed, lactate is a substrate for hepatic gluconeogenesis (Reynolds et al., 1988). However, about 80% of the lactate is converted to propionate via acrylate pathway, mostly by *Megasphaera elsdenii* (Counotte et al. 1983; Stewart and Bryant, 1988). In this pathway, lactate is esterified by coenzyme-A, dehydrated and subsequently reduced by a flavoprotein. This path does not include steps of carboxylation or decarboxylation, and ATP synthesis has not been demonstrated (Russell and Wallace, 1988). So, duodenal flow of non-ammonia nitrogen (NAN) might decrease when rumen fermentable carbohydrates are replaced with lactic acid (Jaakkola et al., 2006, Jaakkola and Huhtanen, 1989) (Table 7).

Table 7. Effects of replacement of sucrose with lactic acid (adapted from Jaakkola and Huhtanen, 1989)

	Sucrose	Suc.+Lactic acid	Lactic acid	P linear
<i>Rumen</i>				
pH	5.93	6.15	6.44	<0.05
NH ₃ , mM	9.64	11.21	9.29	NS
Lactic acid, mM	6.05	1.37	0.55	<0.05
Total VFA, mM	111.1	118.3	108.5	NS
Acetic acid, mmol/mol	593	599	609	NS
Propionic acid, mmol/mol	179	225	226	<0.05
Butyric acid, mmol/mol	189	144	134	<0.05
Protozoa, 10 ⁵ /mL	6.8	5.8	4.1	<0.10
<i>Duodenum</i>				
NAN flux, g/d	97.9	96.0	90.9	<0.05
Microbial N / RFOM, g/kg	29.5	30.1	28.9	NS

On the other hand, the rumen environment spend two electrons per mole of lactate converted to propionate, leading to a product more energetic (propionate, 378 kcal/mol) than its substrate (lactate, 336 kcal/mol) and contribute to the rumen pH buffering (Table 7). The lowest lactate concentration in the rumen fluid from animals infused with lactic acid (Jaakkola and Huhtanen, 1989) occurs because of the microbial adaptation and the increased rate of metabolism of this intermediary (Huntington and Britton, 1979).

Rinne et al. (2009) carried out a meta-analysis to review the need of including new elements in feed protein evaluation models relative to the silage fermentation end-products, which provide little energy to the rumen microbes. The application of a discount factor leads to higher prediction error for milk protein production. Authors justified that partial loss of rumen microbial synthesis due to the partial offset by sparing glycogenic amino acids, because of the increasing of propionate flux as the main end-product from ruminal lactic acid fermentation. Thus, including new elements theoretically correct may not improve the precision of protein evaluation systems. In a recent trial (Daniel, 2011), the presence of volatile compounds from sugarcane silage did not depress the rumen microbial synthesis and unaffected the efficiency of protein synthesis (g/kg of TDN).

Ethanol is also an end-product from silages (Morgan and Pereira, 1962; Zopollatto et al., 2009) and had high gross energy content per gram (Table 6). When consumed by animals, ethanol is absorbed or metabolized by the rumen microorganisms (Jean-Blain et al., 1992; Moomaw and Hungate, 1963).

Durix et al. (1991) infusing ^{14}C -ethanol into the semi-continuous fermentor (Rusitec). Ethanol had a negligible effect on the digestibility of solid feedstuffs, although the microbial protein synthesis was decreased. VFA production was enhanced by 40% and acetate represented 80% of VFA formed from ethanol. The largest part of reducing equivalents (electrons) from ethanol oxidation was used to reduce CO_2 to CH_4 .

Although some species of rumen microorganisms are negatively affected by ethanol (Caldwell and Murray, 1986), this alcohol could trigger positive effects in the rumen. In the experiment carried out by Chalupa et al. (1964), the in vitro digestibility of cellulose increased 6% when 2% ethanol was added (DM basis). This improvement was attributed to ethanol serving as a source of readily available energy to microorganisms. Moreover, the additionally supply of electrons by oxidation of ethanol to acetate could decrease the redox potential (Eh), shorten the lag phase and accelerates the microbial growth. Otsuki et al. (1991) studied the effects of ethanol on rumen metabolism of steers fed high-concentrate diets. There

were no effects on pH and Eh, while the concentrations of acetate and ammonia were increased and rumen protozoa counts were markedly reduced by alcohol intake.

In addition to the acetate flow enhanced by 2-carbon compounds, rumen biohydrogenation might be related with the higher milk fat content in ruminants fed ethanol. Gould (2000) observed that ethanol decreased the proportion of biohydrogenation intermediates in the fatty acids flow in the duodenum. The additional electrons supply by the oxidation of ethanol to acetate may have stimulated the rumen biohydrogenation. Randby et al. (1999) verified lower proportion of C18:1 and C18:2 fatty acids and higher fat content in milk from cows fed ethanol. Plascencia et al. (1999) suggested that ruminal biohydrogenation was positive correlated with methane production, which is often observed with ethanol supplementation (Table 8).

Table 8. Effects of ethanol on rumen VFA and methane production (in vitro) (adapted from Yoshii et al., 2005)

	Ethanol supplemented (mmol)	
	0	34
Ethanol metabolized in 24 h (mmol)	-	13.4
Ethanol metabolized (%)	-	39.4
Methane (mmol)	14.4	21.3*
Acetate (mmol)	32.2	45.2*
Increase of acetate (mmol)	-	13.0
Propionate (mmol)	13.6	13.2
Butyrate (mmol)	3.8	4.1
VFA (mmol)	49.6	62.5*

* $P < 0.05$

Kristensen et al. (2007) evaluated the metabolism of silage alcohols in dairy cows. Alcohols were partially metabolized by rumen microorganisms and ruminal epithelium and the remainder reached the portal blood. Most of absorbed ethanol was metabolized in the liver (Table 9). In another study by the same research group (Raun and Kristensen, 2009), ethanol metabolism was evaluated in fresh cows. Dry matter and ethanol intakes increased while the portal recovery of ethanol decreased with the advance of the period of lactation, due to the rumen adaptation (epithelium and microorganisms). Pradham and Hemken (1970) also found higher rates of ethanol metabolism in rumen fluid from adapted cows. Moreover, high concentrate diets leads to higher rates of ethanol oxidation.

In the Danish studies and in the recently work published by Daniel et al. (2011b), ruminal and hepatic metabolisms resulted in low arterial concentrations of ethanol. In both

trials there was no evidence for saturation of hepatic metabolism, since the low arterial concentrations. Considering Michaelis-Menten kinetics (V_{max} and K_m) and the dose of ethanol potentially fed to ruminants by silage intake, Jean-Blain et al. (1992) concluded that the enzymatic systems would not be saturated and the plasma concentration of ethanol would predominantly be low.

Table 9. Ruminal and splanchnic metabolism of ethanol in dairy cows

	Kristensen et al. (2007)	Raun e Kristensen (2009)		
Days in milk	257	4	15	29
Dry matter intake (kg/d)	15.8	14.7	16.5	19.7
Dietary content of ethanol (% MS)	1.42	1.9	1.9	1.9
Ethanol concentration in the rumen (mM)	2.86	3.2	3.3	2.8
Portal recovery (%)	-	39%	28%	24%
Net portal flux (mmol/h)	113	71	97	114
Net hepatic flux (mmol/h)	-131	-	-	-
Net splanchnic flux (mmol/h)	-18	-	-	-
Arterial concentration (mM)	0.165	0.046	0.073	0.074

As the fact of ethanol is converted to acetate, the Table 10 shows theoretical values of metabolizable energy (ME) and net energy for lactation (NEI) of ethanol and acetate mixtures absorbed by ruminants. For simulation, acetic acid was assumed the only product from ethanol oxidation. Considering that the portal recovery of ethanol is lower than 25% (Raun and Kristensen, 2009), it is expected that NEI of ethanol is close to 2.9 Mcal/kg. Considering NEI conservation over silage fermentation process, 1 kg of ethanol is produced in the silo at the expense of about 2 kg of sucrose (McDonald et al., 1991), containing 4.5 Mcal of NEI (2×2.26 Mcal/kg, NRC, 2001). Thus, alcoholic fermentation led to loss of NEI of approximately 35% $[(4.5 - 2.9) / 4.5]$. This proportion may be even greater if the volatilization is taking into account.

Although ethanol is metabolized by rumen microorganisms and at the epithelium, a considerable amount of it reaches the liver. In hepatocytes, three major metabolic pathways are described. In the cytoplasm, ethanol is oxidized to acetaldehyde by alcohol dehydrogenase, and requires the oxidized form of coenzyme nicotinamide adenine dinucleotide (NAD^+) to carry the electrons. In peroxisomes, ethanol is oxidized by catalase and hydrogen peroxide is the electron acceptor. When ethanol reaches high concentrations in the liver, microsomal cytochrome P4502E1 (CYP2E1) plays an important role in metabolism. Acetaldehyde generated in all pathways is metabolized to acetate by

acetaldehyde dehydrogenase in mitochondria. Coenzyme NAD⁺ is also the electron acceptor to this step (Lieber and Abittan, 1999).

Table 10. Theoretical energy content of ethanol and acetic acid mixtures absorbed by ruminants

<i>Ethanol, %</i>	Mixtures				
	<i>100</i>	<i>75</i>	<i>50</i>	<i>25</i>	<i>0</i>
<i>Acetic acid, %</i>	<i>0</i>	<i>25</i>	<i>50</i>	<i>75</i>	<i>100</i>
ME, Mcal/kg	7.10	6.25	5.40	4.55	3.70
NEL ¹ , Mcal/kg	4.54	4.00	3.46	2.91	2.37
NEL / Heat of combustion of ethanol, %	64%	56%	49%	41%	33%

¹ NEL was assumed as 0.64*ME (Moe et al.,1972; NRC, 2001)

As a result, the hepatic oxidation of ethanol generates an excess of reducing equivalents. The large amount of NADH, the production of acetaldehyde and, the activity of microsomal enzymes capable to generating reactive oxygen species (ROS) are involved in the ethanol hepatotoxicity (Lieber and Abittan, 1999). The plasma activity of the enzyme gamma-glutamyl transferase has been used as a marker of liver damage caused by ethanol (Souza et al. 2004; Tennant and Center, 2008).

Moreover, the amount of reducing equivalents could exceed the liver capacity to keep the intracellular redox homeostasis, resulting in metabolic disorders. Most of reducing equivalents are transferred to the mitochondria and decrease the activity of Krebs cycle and lipid oxidation, stimulating the synthesis of fatty acids. As the supply of ethanol also promotes lipolysis in adipocytes, alcohol consumption can lead to liver fat accumulation (Lieber and Abittan, 1999).

The lower plasma glucose concentrations observed by Pradham and Hemken (1970) after rumen infusion of ethanol, may be due to the inhibitory effect of ethanol on the conversion of propionate to glucose, with concomitant intracellular accumulation of malate and depletion of phosphoenolpyruvate (Demigné et al ., 1991). The inhibitory effect of ethanol on gluconeogenesis seems mediated by increasing of NADH:NAD⁺ ratio (Demigné et al., 1991). Thus, the consumption of high doses of ethanol would be extremely undesirable in fresh cows, which naturally are prone to ketosis and fatty liver.

5. Effects of fermentation end-products from silages on voluntary feed intake and animal performance

The nutritional value of silages differs from its fresh or dried forage source. Physical and chemical characteristics of silages may interfere on dry matter intake, microbial and visceral metabolism, and in turn on animal performance (Weiss et al., 2003). High concentrations of organic acids, alcohols, and other volatile organic compounds instead of water soluble carbohydrates is the main nutritional differences between well preserved silages and their original crops.

The performance of ruminants fed silage based diets depends mainly on feed intake and diet nutritive value, usually the digestibility. Across the variation on digestible energy intake, 60% to 90% is related to feed intake and only 10% to 40% to digestibility (Mertens, 1994). Unlike fresh or dried fodders, the relationship between intake and digestibility of silages is poor, probably due to the fermentation end-products (Huhtanen, 2002).

Several meta-analysis were published by Scandinavian researchers in attempt of understanding the relationship involving silage composition, feed intake and animal performance. Huhtanen et al. (2002, 2003 and 2007) and Krizsan and Randby (2007) showed that both silage intake and animal performance were negatively correlated to the silage fermentation extension (ammonia, lactic acid, VFA and total acids concentrations). Hetta et al. (2007) established a negative correlation between silage intake and acetic acid concentration, but found a positive relation between feed intake and ethanol content. The authors discussed the opposite association between ethanol and ammonia concentrations ($r = -0.67$), since sugar-rich ensiled crops usually present high ethanol and low ammonia concentrations (Driehuis and Van Wikselaar, 2000).

It is remarkable that correlations obtained by meta-analysis can leads to misleading conclusions due to the collinearity across variables. The relationship between silage pH and intake is a good example of confounding. Although many studies did not show strong correlation between these variables (Offer et al. 1998; Steen et al. 1998; Huhtanen et al., 2002), pH tends to be negatively correlated to silage consumption, because high pH silages have higher concentrations of ammonia and higher VFA/lactic acid ratio. Furthermore, high concentrations of ammonia do not seem a causative factor per se, but a marker of proteolysis end-products (Rook et al., 1990). For this reason, several studies were carried out in order to isolate effects of silage fermentation end-products on voluntary feed intake.

Hutchinson and Wilkins (1971) carried out a well-known study about the effect of acetic acid on silage intake. Ryegrass silage was treated with increasing doses of acetic acid (2%, 5% and 8.8% of DM) before feeding adult sheep. The pH and moisture content of silages were kept by adding of NaOH and water. Daily dry matter intake was similar across

treatments, but ingestive behavior pattern was altered by acetic acid. Ingestion time was lower during first 4 hours after feeding, but the opposite behavior occurred between 16 to 20 hours after feed offering. The deviation on ingestive pattern with maintenance of feed intake was also found by Daniel (2011) in high producing dairy cows (Figure 1). Feed refusal in initial period after morning feeding may be related to the higher volatilization rate of fermentation products (Figure 2).

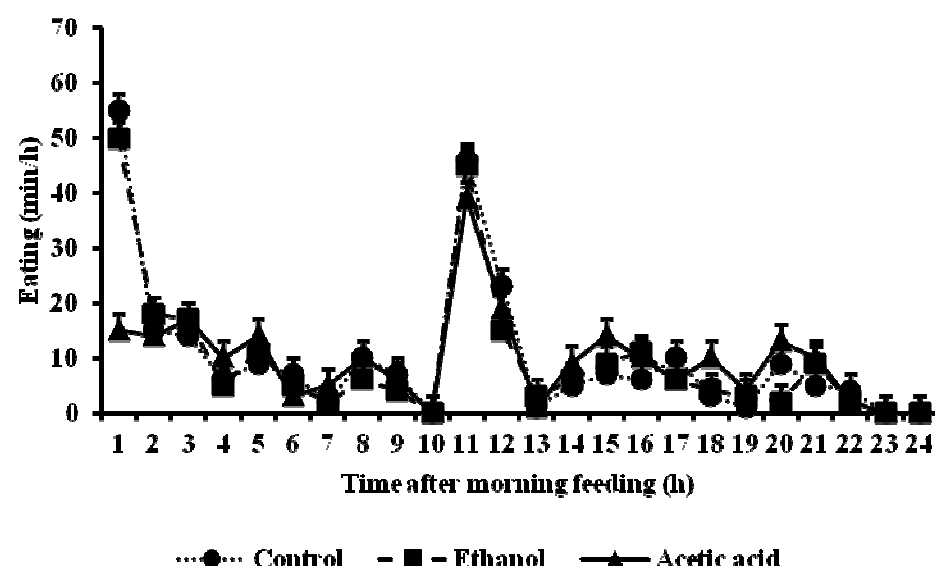


Figure 1. Eating pattern of dairy cows supplemented with ethanol or acetic acid (Daniel, 2011)

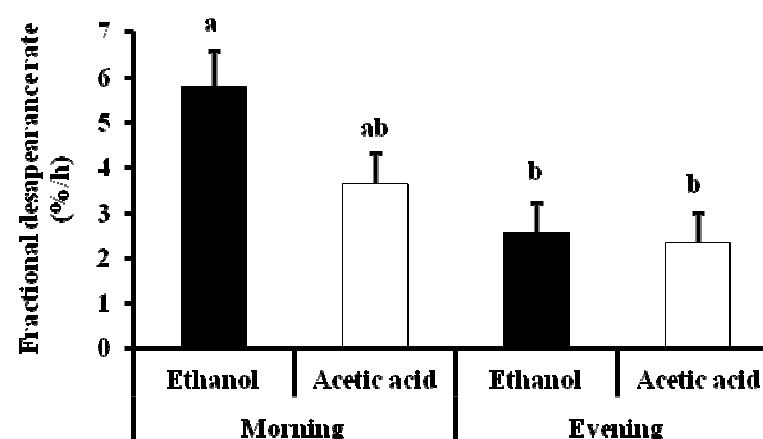


Figure 2. Fractional disappearance rates of ethanol and acetic acid added onto the total mixed rations (Daniel, 2011)

Wilkinson et al. (1976) studied the performance of steers fed corn silage or fresh plants added or not with a mixture of acetic and lactic acids. Sucrose was used to balance the

energy content across treatments. The ensiling process did not affect the dry matter intake, but decreased the daily weight gain. On the other hand, the acid mixture reduced the feed intake without affecting the daily weight gain. The lower value for daily intake may have been caused by the adopted method to determine the dry matter content (oven drying at 70°C).

Krizsan et al. (2006) supplemented steers with acetic acid, caproic acid, tryptamine or a mixture of substances. Acetic acid or its combination reduced the silage intake. Though, when the compounds mass were taken into account, daily intake was similar across treatments. So, it is possible to suggest that the method used to measure the dry matter content was the main determinant of the decrease on silage dry matter intake when it was compared to unfermented crop.

Especially in 1990s, the use of by-products as ruminant feed ingredients was increased around the world. From these sources, wet distillers grains emerged due to the alcohol production from corn through industrial fermentation. At that time, the distillation process was less efficient and the by-product contained as much as 10% of ethanol (Larson et al., 1993). This fact triggered an increase interest among researchers, who investigated the nutritional value of ethanol (Ham et al., 1994). Partial replacement of 5% or 10% of corn with ethanol did not affect the performance of lambs (Table 11). In view of high levels of ethanol in sugarcane silages, Daniel (2011) studied the performance of high producing Holstein cows fed ethylic alcohol. Energy efficiency of cows supplemented with ethanol was similar to the control group (Figure 3). The net energy for lactation (NEl) of ethanol was estimated as 2.6 Mcal/kg.

In both studies the higher theoretical energy value of ethanol compared to carbohydrate was not demonstrated. Rumen conversion of ethanol to acetate and concomitant increase of methane production might be reasonable explanation to the deviation on the predicted energetic value based on heat of combustion. With NEl of ethanol, the authors speculated that approximately 90% of the ethanol was converted to acetate, which is in agreement to the absence of ethanol in the peripheral blood (Daniel et al., 2011b). From forage conservation point of view, it was possible to extend the traditional knowledge regarding energy recovery through this metabolic pathways operating in silages (Table 12).

Table 11. Effects of replacement of ground corn with ethanol on the performance of lambs (Ham et al., 1994)

Ethanol (% MS)	0	5	10	EPM
DM intake (kg/d)	1.92	2.03	2.03	0.14
Weight daily gain (kg/d)	0.34	0.34	0.34	0.04

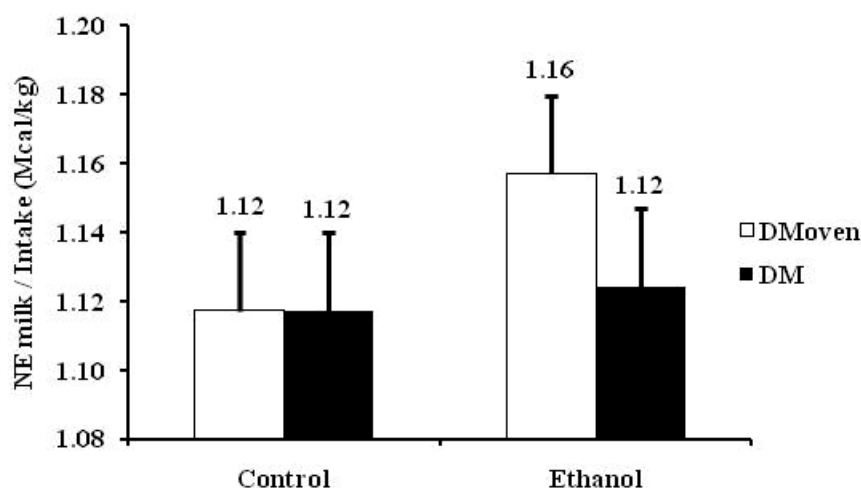


Figure 3. Energy efficiency of dairy cows fed ethanol (Daniel, 2011)

Table 12. Energy recovery by fermentation of glucose into alcohol in silages

		McDonald et al. (1991)	McDonald et al. (1991)	
		DM recovery (%)	GE recovery ¹ (%)	NEI recovery ² (%)
Pathway	Products			
Yeast	Ethanol and CO ₂	51	99	69 [†]

¹ GE = gross energy; ² NEI = net energy for lactation

[†] Heats of combustion considered: glucose 676 kcal/mol, ethanol 329 kcal/mol and, acetic acid 222 kcal/mol. Assume that 90% of ethanol is converted to acetate

Randby et al. (1999) fed 600 g/d of ethanol to Norwegian Red cows, in a crossover design with 9 days-periods. When ethanol was taken into account in their calculations, the dry matter intake and energy correct milk yield were higher than that of control, due to the higher milk content of fat and protein. Organoleptic milk quality was reduced with ethanol intake. Probably, the trial length did not allow the complete adaptation of enzymatic systems related to ethanol metabolism, resulting on milk with lower sensory quality. In the trial of Daniel et al. (2011a), ethanol supplementation induces to higher daily yields of milk and protein. As the milk fat content was slightly decreased by ethanol treatment (not significant), 3.5% fat corrected milk was similar across treatments (Table 13). Unlike the results of Randby et al.

(1999), sensory milk quality was unaffected by diets, probably due to the trial length, and in turn, tissue metabolic adaptation to the ethanol (Daniel et al., 2011c).

Table 13. Performance of dairy cows fed ethanol or acetic acid (Daniel, 2011)

Parameter	Control	Ethanol	Acetic acid	SEM	<i>P</i>
Dry matter intake, kg/d	22.16 ^{ab}	22.72 ^a	21.42 ^b	0.58	<0.01
Milk yield, kg/d	35.50 ^b	37.82 ^a	35.70 ^b	1.38	<0.01
3.5% fat corrected milk, kg/d	35.79	36.93	36.60	1.14	0.39
Fat, %	3.55	3.37	3.69	0.11	0.12
Protein, %	3.32	3.33	3.29	0.03	0.56
Fat, kg	1.25	1.28	1.31	0.05	0.73
Protein, kg	1.19 ^{ab}	1.26 ^a	1.15 ^b	0.03	0.03

^{a, b} Means with different superscripts differ (Tukey; $\alpha = 0.05$)

6. Take home messages

- ✓ Volatile compounds represent up to 20% of silage dry matter;
- ✓ Lactic acid, acetic acid and ethanol are major fermentation end-products, however, several novel compounds has been found;
- ✓ Due to the volatile compounds, the method adopted to determine dry matter of silages can alter the nutrient and energy contents, dry matter intake, digestibility and energy efficiency of diets. Moreover, it may brings on errors for calculations of dry matter losses during silage storage;
- ✓ During storage and especially at silage feed-out, part of the volatile compounds can be either emitted into the atmosphere or oxidized by aerobic microorganisms. However when they are actually consumed by the animals, these compounds have nutritional significance;
- ✓ Under optimal silage management, approximately 80% of silage volatile compounds may be ingested by the animals. This value might differ depending on the silage feed-out and feeding frequencies;
- ✓ Alcoholic fermentation is the major pathway in sugarcane silages which might lead to high recovery of gross energy with low dry matter recovery rate;
- ✓ The net energy for lactation from ethanol estimated based on animal performance is lower than the predicted values traditionally available in the literature;
- ✓ Animal performance data suggest that silages volatile compounds might be responsible for additional 10% over the energy content predicted based on chemical analysis.

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Effects of spoiled silages on animal performance

Mateus C. Santos¹ and Limin Kung, Jr.²

Introduction

Ensiling is the anaerobic process of preserving moist crops by lactic acid fermentation. Compared to other methods of forage preservation, silage retains the largest amount of nutrients from the original material, it can be made from a wide variety of crops and it is less dependent of the weather at harvest (Pahlow et al., 2003). The silage microbiota play a key role in the successful outcome of the conservation process. The biota can basically be divided into two groups, namely the desirable and the undesirable micro-organisms. The desirable microorganisms are lactic acid bacteria (LAB) whereas undesirable organisms include those that can cause poor fermentations (e.g. clostridia and enterobacteria) or aerobic spoilage (e.g. yeasts, bacilli, *Listeria* and moulds). Under optimal ensiling conditions, epiphytic LAB predominantly ferment plant water soluble carbohydrates into lactic acid that acidifies the crop resulting in preservation of nutrients (McDonald et al., 1991). The preservation of these crops as silage can be improved by the utilization of microbial inoculants. Lactic acid bacteria are the most common microorganisms used and they are usually applied at harvesting. Homolactic bacteria improve fermentation efficiency by increasing lactic acid production whereas heterolactic bacteria are used with the objective of preserving forage quality during storage and after silo opening by increasing the production of anti-fungal compounds.

During storage and feed out silage is exposed to oxygen. If populations of lactate-assimilating yeasts are high in silage, exposure to air stimulates their growth and initiates aerobic spoilage of nutrients. The type of yeasts and their metabolism in silage has not been well studied. In addition, cows are often fed silages that have gone through various degrees of aerobic spoilage and can have high numbers of yeasts. To date, the effects of these organisms on normal ruminal metabolism and animal performance are still unknown.

Thus, this review will consider the role of yeasts in silage preservation and the impacts of feeding spoilage silages on animal performance.

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Yeasts and silage fermentation

All forage crops at ensiling contain aerobic and anaerobic microorganisms and a range of both bacteria and fungi that affect silage quality (Muck, 2010). The surface of plants accommodates a varied microflora and yeasts play an important part in this microflora. The majority of epiphytic yeasts on forage crops belong to genera *Sporobolomyces*, *Cryptococcus*, *Rhodotorula* and *Torulopsis*, which can range in population density from less than 10 to 10^6 or 10^7 colony forming units (cfu)/g of fresh material (Lindgren et al., 1985; Middelhoven and van Baalen, 1988). Middelhoven and van Baalen (1988) reported that these species vanished after two days of ensiling and none of them were able to tolerate acetic acid or assimilated this acid at pH 4.0. Most of the yeasts that are associated with the fresh plant in the field are able to decompose the plant's cuticle, thus promoting leakage of nutrients from which the microflora benefits (Ruinen, 1965). Moreover, yeasts of the genera *Rhodotorula* spp., *Lipomyces* and *Cryptococcus* are classified as obligate aerobes, with exclusively oxidative metabolism (Rosenfeld and Beauvoit, 2003) and may explain why they are vanished after a few days of silage preservation.

After anaerobiosis has been established in the ensiled forage, aerobic fungi are succeeded by a fermentative flora of yeasts (Di Menna et al., 1981; Middelhoven and van Baalen, 1988). These yeasts tend to grow best aerobically but can ferment sugars anaerobically and produce ethanol and CO_2 when trace amounts of oxygen are present (McDonald et al., 1991). During silage preservation, anaerobic as well as aerobic yeast activity is considered undesirable. Ruxton and McDonald (1974) reported that yeasts do not contribute to the preservation of an ensiled forage and actually compete with lactic acid bacteria for available water soluble carbohydrates. The ethanol fermentation in silage not only decreases the amount of sugar for lactic acid production, but it can also decrease the dry matter recovery because of excessive production of CO_2 . Bolsen (1997) reported DM losses in the top layer (0.5 m) of bunker silos ranging between 43 to 61% and 24 to 27% in unsealed and sealed silos, respectively.

The two primary factors required in making good silage are the rapid exclusion of air from the forage mass at the start of ensiling and the continued exclusion of air from the silage mass during storage. Excessive exposure to air at the start of fermentation prolongs the metabolism of unwanted microbes that thrive in air and delays the growth of beneficial bacteria that produce lactic acid. This can lead to undesirable fermentations and a loss in nutritive value. The amount of air ingress during silage storage can affect the yeast flora composition. Jonsson and Pahlow (1984) found that if anaerobic conditions were achieved

and maintained in the silage, fermentative, but non-lactate assimilating *Saccharomyces cerevisiae* prevailed, whereas if air penetrated into the silage during storage, lactate assimilating yeasts of the genera *Candida* and *Hansenula* (= *Pichia*) predominated.

The low pH achieved by the lactic acid fermentation, together with anaerobiosis usually provides conditions adverse to microorganisms. The degree of anaerobiosis during storage period and the concentration of organic acids are important factors that affect the survival of yeasts. Jonsson and Pahlow (1984) stated that the presence of oxygen enhances survival and growth of yeasts, whereas a high level of acetic acid reduces their viability during the storage period.

Yeasts and aerobic deterioration of silages

When silage is exposed to air, during feed-out in the silo or in the feed bunk, aerobic microorganisms can become active, causing heating and spoilage. It is generally acknowledged that the population of yeasts present upon exposure to air governs the vulnerability of silage to aerobic deterioration. In a summary of 2009 corn silage analysis, Cumberland Valley Analytical (Maugansville, MD) reported that more than 50% of samples analyzed (n = 121) had a population of yeasts with $> 1 \times 10^7$ cfu/g (Figure 1).

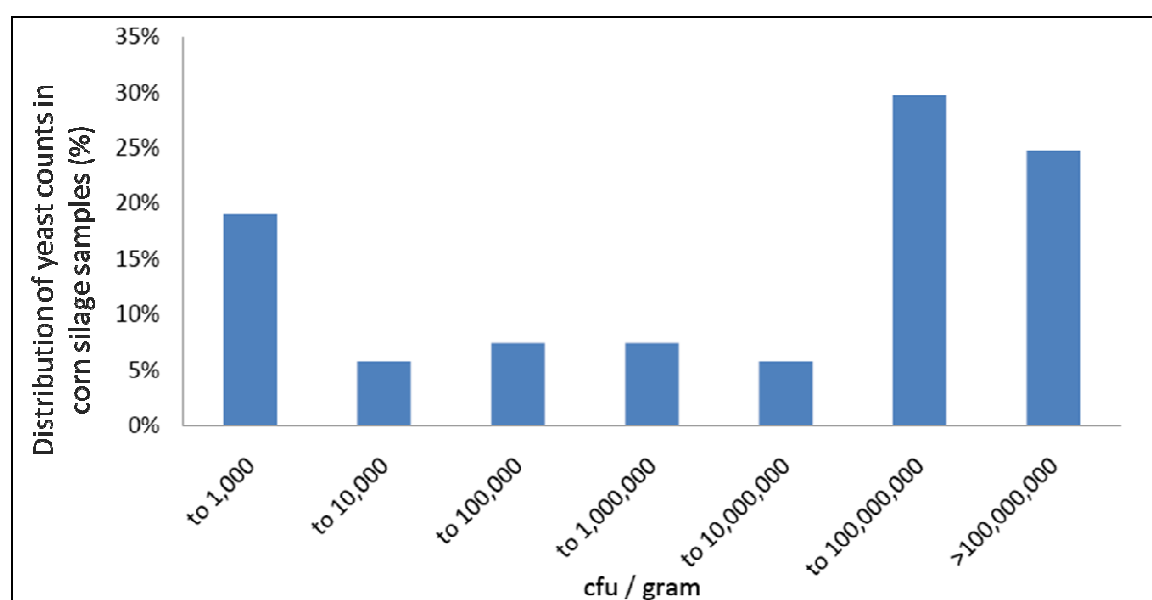


Figure 1. Distribution of yeast counts in 121 samples of corn silage analyzed by Cumberland Valley Analytical Services (Cumberland Valley Analytical Services, 2009).

Daniel et al. (1970) reported that silages having a population in excess of 10^5 cfu/g DM are particularly prone to aerobic deterioration. Middelhoven (1998) also reported that

silage with a large yeast population, e.g. 10^5 cfu/g, will be spoiled by these organisms upon exposure to air. Kung et al. (1998) reported that aerobic stability was negatively correlated to the number of yeasts present at the time the silo was opened (Figure 2). Corn silage with low yeast populations (10^3 cfu/g) remained cool for up to 3 times longer than silages with high initial yeast populations (10^6 cfu/g). Preventing silage from spoiling when it is exposed to air can improve the efficiency of a farm by preserving forage as high quality silage that is palatable to cows.

In silages, the population of yeasts associated with aerobic spoilage can be divided into two physiological groups. First, there are the yeasts with a high fermentative ability for sugars but a variable ability to assimilate lactic acid. These organisms include *Saccharomyces cerevisiae* and species of *Torulopsis*. The second group is composed of yeasts that have a high respiratory affinity for lactic acid. These organisms include species of *Candida*, *Issatchenkia*, *Pichia*, *Hansenula*, and *Endomycopsis* (Moon and Ely, 1979; Jonsson and Pahlow, 1984; McDonald et al., 1991).

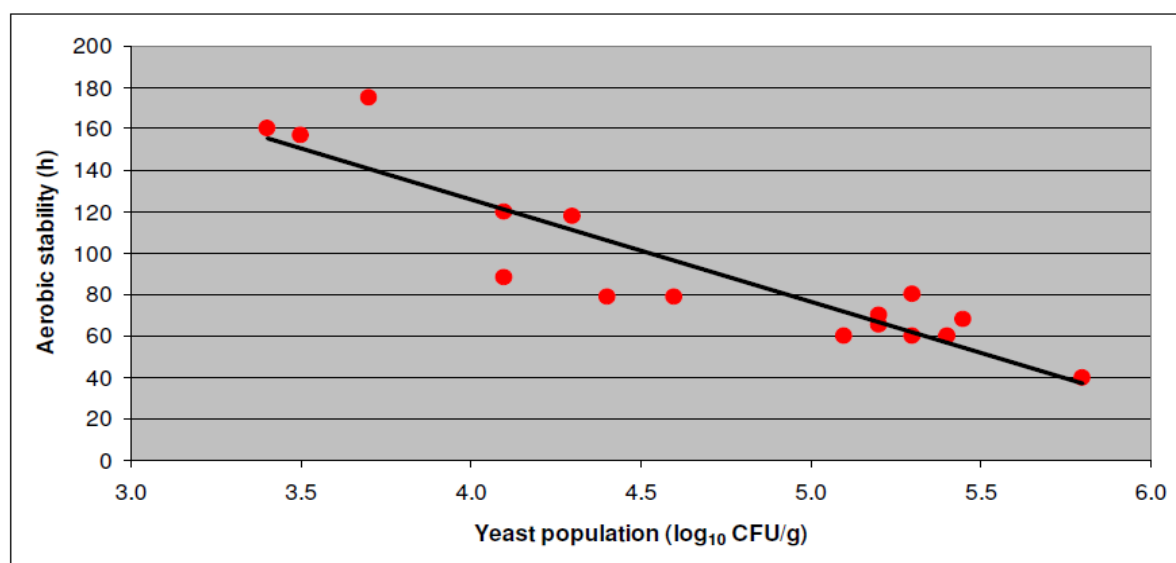


Figure 2. The effect of yeast population on the aerobic stability of corn silage (Kung et al., 1998).

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. *Candida valida* (including its teleomorph *Pichia membranifaciens*) shows notable resistance towards acidic pH and high concentrations of preservatives (Deak et al., 1992). Moon (1983) used a growth medium containing 2% of glucose to study the effects of acetate, lactate and propionate on the growth of acid tolerant

yeasts isolated from deteriorated silages and/or spoiled lactic acid fermented foods. The author observed that all of the yeasts (*Saccharomyces uvarum*, *Geotrichum candidum*, *Endomycopsis burtonii* and *Hansenula canadensis*) grew well with glucose or 1% lactate but not 1% propionate as an energy source. All yeasts except *S. uvarum* could grow in the presence of 1% acetate after a two week incubation period. When acids were present in the growth medium, an increase in turbidity was considerably delayed when compared with the control medium containing glucose. After two weeks incubation, final turbidities were similar between all tubes suggesting that at these concentrations of the acids delayed growth rather than caused cell death.

Under aerobic conditions, the metabolism of sugars and lactic acid by yeasts results in the production CO₂, heat and H₂O. This inevitable oxidation of sugars usually results in high loss of dry matter (Woolford, 1990) and the loss of important nutritional components (Kung et al., 1998). In addition, the degradation of lactic acid causes a rise in silage pH, which in turn triggers the growth of many other spoilage organisms (McDonald et al., 1991). Kung (2005) reported that exposure to air initiates a chain of reactions that ultimately results in spoiled feed (Figure 3). Specifically, yeasts that degrade lactic acid in the presence of air are the primary microbes that cause spoilage in silages. Degradation of lactic acid also increases the pH of the silage to a level that allows opportunistic bacteria (e.g. Bacilli) and molds (e.g. *Aspergillus*, *Fusarium*, and *Pencillium*) to grow and further reduce silage quality (McDonald et al., 1991). In some cases bacteria from the genus *Acetobacter* may initiate aerobic spoilage in corn silages but their role is less understood (Muck and Pitt, 1994). The authors conducted six trials to evaluate the microbial development at 5, 20, 35, and 50 cm from the exposed face of corn silages and observed that most microbial activity occurred at the 5-cm level in all six trials. Acetic acid bacteria initiated heating in all trials although yeasts were significant in two trials. Because silages are often incorporated into total mixed rations (TMR), the stability of the TMR may also be an issue on farms. In a small survey of TMR sampled in DE, PA and MD over two years, more than 50% of 30 TMR that were sampled within 1 hour of being made spoiled in less than 12 h when incubated at a controlled laboratory temperature of about 23°C (Kung, Mulrooney and Morges, unpublished data Univ. of Delaware).

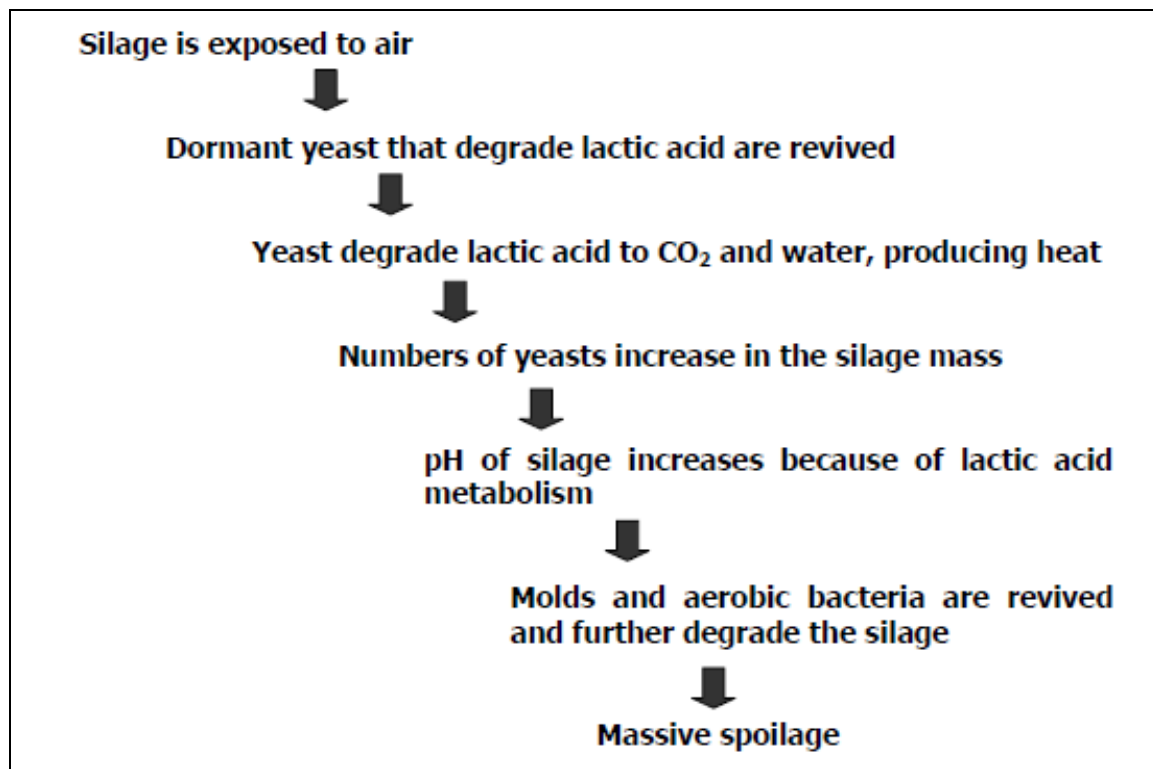


Figure 3. The “domino effect” of air causing aerobic spoilage in silages (Kung, 2005).

Impacts of feeding spoilage silages on animal performance

When animals consume spoiled silages, the exact causes of reduced intake and/or performance are not fully understood. Detrimental yeasts may affect rumen microorganisms or produce end products that might alter rumen fermentation, the direct consumption of spoiled nutrients may reduce performance, and the production of undesirable end products (e.g. mycotoxins) from further spoilage by molds and other organisms may also be problem.

Yeasts occur naturally in the microbial community of the rumen (Lund, 1974). Up to 1.3×10^5 yeasts/ml grew when dilutions of bovine rumen fluid were incubated at 25°C, but only 3.5×10^3 /ml grew at 39°C, suggesting that the yeasts present normally are essentially transient members of the community, entering with the fodder (Lund, 1974). Kung et al. (1997) added a direct fed microbial strain of *S. cerevisiae* to sterile ruminal fluid and reported that this organism was able to maintain viable counts through 24 h of incubation but some cell death apparently took place by 48 h. Thus yeasts, and particularly *S. cerevisiae*, are not normally significant members of the rumen microbial community (Wallace and Newbold, 1995). The temperature and chemical composition of rumen fluid tended to be inhibitory to growth of these microorganisms.

It is well known that yeasts have the potential to alter the fermentation process in the rumen (Wiedmeier et al., 1987; Harrison et al., 1988), and yeast products based on *Saccharomyces cerevisiae* are increasingly used in ruminant diets to improve animal performance (Desnoyers et al., 2009; Robinson and Erasmus, 2009). However, improvements in animal performance are not always achieved, and in fact publications have reported that these effects are strain specific. Chung et al. (2011) investigated whether the risk of subacute ruminal acidosis (SARA) in dairy cows would be decreased by feeding diets with selected yeast strains based on *S. cerevisiae*. The authors compared a commercial product widely used in dairy production (strain 1) with a novel strain that is not used commercially (strain 2) and with a control diet. They observed that the concentration of lactate and total VFA in the rumen fluid were similar among treatments, however yeast strain 2 decreased ($P = 0.04$) the proportion of acetate in rumen fluid compared with yeast strain 1 and increased ($P < 0.01$) the proportion of propionate in ruminal fluid compared with the control or yeast strain 1. Cows that received yeast strain 2 experienced lower ($P \leq 0.03$) average daily minimum, mean, and maximum pH compared with cows that received no yeast or yeast strain 1. The authors concluded that yeast strain 2 increased the risk of acidosis when compared with the commercial product containing a different strain of *S. cerevisiae*.

When spoiled silages are fed to animals, depression in intake and performance has been observed in dairy cattle (Hoffman and Ocker, 1997) and in beef cattle (Whitlock et al., 2000). In these cases, the high number of spoilage yeasts that is ingested by the animal may also affects ruminal fermentation parameters, resulting in poor animal performance. In the study conducted by Hoffman and Ocker (1997), 18 mid-lactation cows were fed a total mixed ration containing fresh or aerobically deteriorated high-moisture corn silage. The fresh material was removed from the silo each day before being fed to the herd and the aerobically deteriorated silage was removed from the silo and piled on a concrete floor at the beginning of each experimental period (14 d). The researchers found significant differences in milk yield ($P < 0.01$). Cows that were fed a total mixed ration containing the aerobically deteriorated high-moisture corn produced 3.2 kg less milk per cow/day when compared to cows fed aerobically stable silages (Figure 4).

water-soluble carbohydrate to organic acids, resulting in silages with good nutritive value and with a beneficial population of microorganisms.

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The future of forage conservation

Friedrich Weissbach

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Introduction

The Organizing Committee of this Conference has invited me to elaborate on this demanding and challenging topic. Already to have accepted the invitation appears to be presumptuous because nobody has the power to look into the future.

However, we all are living in an era with many uncertainties and without reliable orientation for the future. Numerous of the old certainties are not valid any longer. Questions like: How do we feed the growing world population? How do we produce sufficient energy? How do we limit global warming to an acceptable level? – are asked with increasing pressure and discussed internationally, but satisfying solutions are not in sight, which are accepted by the international policy, and realizable.

Simple answers are not there anyway due to the massive impact of these problems and their resolution. But what is already clear today is: we all must be prepared for bigger changes than the generations before us and for new challenges to research and development. This also applies to such a special research field which is forage conservation.

To acquire orientation, it is crucial to summarize and urgently re-evaluate our knowledge. What portion of it is fit for the future and what we need in terms of gain of knowledge can only be determined out by a critical and simultaneously constructive discussion process. The attempt to contribute thereto can be the only aim of my presentation, not more.

In the following I would like to explain my view as to why we need to preserve more feed by silage making than in the past and how we can improve the current preservation methods and technologies.

Forage conservation and the problem of methane emission

It is commonly accepted that one of the pre-requirements for the high productivity in cattle farming in the developed regions of the temperate climate zone was the introduction and utilization of efficient methods of forage conservation. The proportion of conserved forages significantly increased in relation to the total yearly feed production, and feed quality has markedly improved during the last 50 years.

In Central and Northern Europe as well as in some parts of North America, this was achieved by replacing hay by silage and by using improved technologies of ensiling grasses and legumes. The spread of growing silage maize in cooler regions then before brought also a further big progress. Today, there is even competition between different silage sources and grassed pasture, which, formerly, had been considered superior in terms of quality and costs. Dairy herds with an average milk yield of 8,000 kg per cow and year, and even more, are kept indoors throughout the year and fed on silage as sole roughage source because this seems to be the only way to meet their very high quality requirements.

From a historical perspective, forage conservation has served one fundamental function – to ensure adequate nutrition of animals also in seasons without or with limited plant growth. There are hardly any regions on the globe in which fresh pasture feed is available throughout the year at constant volume and quality. The alternation is more typical between seasons with and – more or less – without vegetation, between summer and winter, or between wet and dry seasons. Animal production, on the contrary, is a continuous process that requires a constant supply of feed in quantity and quality. Animals do have requirements for maintenance, and only feed intake exceeding this will lead in performance. Each day, on which the genetically and physiologically determined performance potential is not fully used due to insufficient nutrition, will ultimately result in losses in productivity.

Thus, forage conservation resolves the discrepancy between continuity in feed demand and discontinuity in vegetation. It therefore ensures the supply of feed based on demand throughout the year. Moreover, only the conservation of forages enables the fixation of optimal quality which would otherwise change during the course of vegetation. This makes it possible to fully exploit the performance potential of animals throughout the year. Therefore, we must conclude that forage conservation and storage is an essential issue.

Nevertheless, public funding for research in forage conservation has dramatically declined during the last decades in many European countries although there is still a lot to do. Institutes of a high international reputation were closed, and in others was the experienced staff drastically reduced. Existing knowledge could not be passed on to the next generation so that know-how for future use was lost.

However, there are still research needs in forage conservation even in developed countries. In this regard it may be reminded of the frequently occurring, serious problems with food hygiene in the globalized world, which are often caused by hygienic problem of fed silages (Fenlon, 1988; Roberts, 1988; Pahlow et al. 2003; Driehuis and te Giffel, 2009). Even more important is there the need for improvements in emerging nations and developing countries. In these countries is the low level in animal performance often caused by shortages of suitable technologies for forage conservation and therefore a too low extent of storage of feed.

On the other hand, currently there has been enormous financial support for research projects on methane emissions from ruminants in numerous institutes across the globe. Beside carbon dioxide, methane is known to be the most relevant trace gas regarding climate change and contributes to the greenhouse effect. Its emissions should possibly be limited in the interest of limiting global warming.

But methane emissions by ruminants and its potential consequences have been widely known for long. The public, and thereby politics seem to have been become aware of it during the last few years only. Beside astonished realization of the facts, questions have arisen as to whether how much methane is produced by ruminants, if this emission can be reduced, and if not, if we can still afford to keep cattle, sheep and goats? These questions shall be answered by results from ongoing research activities obviously.

However, on the basis of numerous experimental data, which have been available for decades, it is already well possible to take a scientifically sound standpoint on this topic. The formation of methane is an undesired but unavoidable special characteristic of the ruminant's digestive system. Methane production results in energy loss. On average, 7-9% of ingested gross energy is lost (Schiemann *et al.*, 1971).

Trials have frequently been conducted which aimed at reducing ruminal methanogenesis by special feed additives or by diet formulation. As far as feed additives are concerned (e.g. ionophores like monensin), it has been shown that their effects are limited to the first days of administration and that thereafter methane emission reached pre-trial level soon (Kirchgeßner *et al.* 1995). New ideas, approaches and concepts for the control of rumen fermentation by chemical additives (Takahashi, 2010) or bacterial additives (Davis, 2010) still remains largely a matter of speculation. As of yet, no results from animal trials supporting these hypotheses have been published that showed a sustainable reduction in methane emission by additives of any type without affecting animal health or performance. Thus, a solution of the problem by using feed additives is not to be expected in the near future.

The possibilities to influence methane emissions by diet formulation can be evaluated by using regression equations describing the relationship between nutrients and methane formation. The evaluation of the most comprehensive data collection (337 metabolic trials in cattle using 3 to 12 animals per diet, 5 days balance period and about 1,500 data sets) resulted in the following multiple regression equation (Jentsch *et al.*, 2009):

$$m = 1.32 x_1 - 0.56 x_2 + 1.68 x_3 + 2.78 x_4 \quad r^2 = 0.858$$

where m is the methane energy [J] and x_1 to x_4 are the apparent digestible nutrient fractions [g]: x_1 crude protein, x_2 crude fat, x_3 starch + sugar ($\approx$ NFC) and x_4 N-free organic residue ($\approx$ NDF). It is obvious that the content of cell wall substances (NDF), which are typical for diets for ruminants, has the biggest impact on methane formation. Rations containing high non-fibre carbohydrates (NFC) concentration require high inclusion rates of grain and are therefore not a viable option. The same data sets were used to describe the relationship between dry matter intake by cattle and their methane production (Piatkowsky *et al.*, 2010):

$$M = 32.76 - 0.384 x \quad r^2 = 0.224$$

where M is the methane weight [g/kg DM] and x the feed intake [DM g/kg live weight]. Taking into consideration typical feed intake figures, methane emissions by different cattle categories can be calculated as well as methane emission as a function of milk yield (Table 1).

Table 1: Methane emission from cattle

(Piatkowski et al., 2010)

Performance	Methane	
	g/kg DM intake	g/kg Milk
Dairy cows		
Maintenance	28.3	
4000 kg milk/year	24.8	29.5
6000 kg milk/year	23.0	22.0
8000 kg milk/year	21.8	17.4
10000 kg milk/year	20.7	14.6
Heifers		
200 - 300 kg	25.7	
300 - 400 kg	24.7	
Beef cattle		
250 - 350 kg	25.7	
350 - 450 kg	25.0	
450 - 550 kg	25.9	

It can be concluded therefrom that – regardless of how and where on our globe ruminants are kept and fed – at least 2.1 to 2.6% of the ingested DM is converted into methane, and emitted. Increasing performance leads to reduced emission per kg ingested DM and per kg produced milk and beef. Cattle which do not perform due to insufficient feed supply take in low amounts of feed and consequently emit low amounts of methane per animal and day. But these methane emissions are not only unproductive, they are also extremely high (2.8%), if related to ingested DM.

This is the range which covers the magnitude of methane emissions from a given number of cattle and their consumption of plant biomass. Simultaneously, this suggests the limits within which emission can be influenced. More possibilities are not available, and will not become available in the near future. Therefore, one is tempted to conclude that the number of cattle – as well as of sheep and goat for which the same relationships and emission rates per kg ingested DM are applicable – should be drastically reduced. To consume less food of animal origin, for the sake of the earth's climate, is currently a real request announced in public. Particularly for food products from ruminants is this request entirely unrealistic.

Apart from the fact that the population in emerging or developing countries cannot be denied higher consumption of animal products as a consequence of increasing living standard, the vast portion of agriculturally exploitable land in the world is grassland. Data presented in Table 2 (taken from the Statistical Yearbook of the FAO) show that two thirds of this are pastures. In Brazil, even about 75% of the total land which is utilizable for agricultural purposes is grassland.

Table 2: Grassland as proportion of the total agricultural area

(FAO Statistical Yearbook 2010)

	World		Brazil	
	Area Million ha	Proportion %	Area Million ha	Proportion %
Arable land	1381	28	61	23
Permanent crop	146	3	8	3
Pastures	3357	69	196	74
Total agricultural area	4884	100	265	100

Naturally, the vast acreage of grassland is less productive than is arable land. However, its yield cannot be abandoned, neither today, nor in the future in prospect of further increasing world population. Grass can only contribute to feeding mankind by its utilization by ruminants. Therefore, maintaining a similarly numerous cattle stock as we have today can hardly be avoided in the future.

Data from statistical publications of the FAO are summarized in Table 3 and show cattle and buffalo numbers in the world and present countries in which at least 50 million animal of this category are kept already today.

Table 3 : Number of big ruminants

(FAO Statistical Yearbook 2010)

Countries	Cattle and buffaloes (Million heads)		
	1999-2001	2007	2009
India	286	280	279
Brazil	171	201	206
China	125	105	116
USA	98	97	95
Pakistan	45	59	63
Argentina	49	51	51
Ethiopia	35	45	51
World	1479	1540	1571
	100	104	106

Also during the last decade a further increase in cattle stock could be observed. The least that should be achieved is to stop this trend. The increasing demand for food of animal origin which is caused by the steady growth of the world's population can and must be met by improving animal performance. Concurrently, this is the only realistic way of reducing methane

emission per kg product. An even higher aim would be to increase performance to such an extent that the number of ruminants and the amount of methane emitted by them could be reduced.

The relationship between methane emission and milk yield has been described earlier (Kirchgeßner *et al.*, 1995). It can be concluded from figure 1, and also from data presented in Table 1, that the biggest effect can be achieved by increasing the animal's yield at nowadays low performance levels whereas the contribution to further reducing emissions can be neglected at already high milk yields.

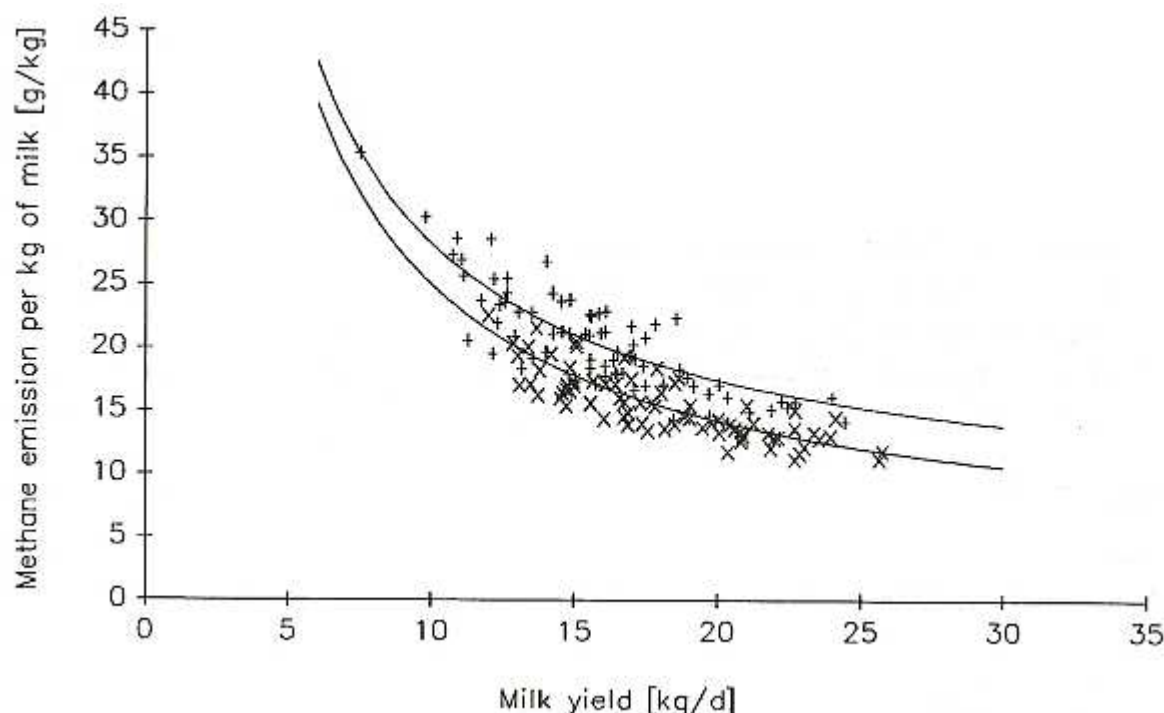


Figure 1: Methane emission per kg of produced milk as affected by performance level (adapted from Kirchgeßner *et al.*, 1995)

Among countries having very big ruminant populations, and especially in among developing countries, there are many with extremely low performance level in animal production and, thus, high potential for performance increases, which would ultimately lead to significant reductions in methane emissions. One of the most effective measures that can be taken for improvements is the implementation of an efficient feed store management. Consequently, the development and implementation of improved technologies for feed conservation, which are adapted to country-specific climatic and socio-economic conditions, becomes an important task of general climate policy.

Further challenges to forage conservation have arisen during the last few years by the increasing use of agriculturally produced plant biomass for energy production. Both, the production of bioethanol and of biogas are continuous biological processes, which, in analogy to animal production, require storage of plant biomass.

As far as moist grains are concerned, the energy saving preservation technology of anaerobic storage needs to be taken into consideration. If whole-plant maize, maize stover, whole-plant cereals, biomass from grasses and legumes as well as sugar beet and possibly also sugarcane in future are addressed for production of biogas, conservation by making silage is unavoidable.

Only as silage can forages be used as substratum for biogas production. In addition to the necessity to make these materials storable, plant biomass also for this utilization needs to be produced at a defined and constant quality. Fermentation in the silo can be considered, at least to a certain degree, the first phase of the whole process of biogas production which subsequently continues in the fermenter until the fermentable organic matter is fully degraded to methane and carbon dioxide. Quality requirements upon silages are similar to those for animal feeding, but not identical in all quality traits.

Production of electricity, heat and fuels from biomass is desired in the future to significantly contribute to the overall production of renewable energies in order to replace fossil sources, thereby relieving the atmosphere of carbon dioxide formed from them. Forage conservation must, and can, bear its crucial share to climate-neutral energy production by providing suitably prepared plant biomass on demand. Also for this purpose must forage conservation be carried out at a larger scale in the future, and the best technologies for that must be developed.

Hay-crop silages and the problem of fermentation quality

The aim of all technologies and procedures in forage conservation is to maintain the highest possible feeding value of the grown forage in terms of quantity and quality at reasonable economic input. Wherever climatic conditions permit the regular production of “field-dried hay“ without any problems in a short period of time, this preservation technology represents the least cost method. If made properly, nutrient loss does not necessarily need to be higher and feed quality lower than that of ensiled biomass.

As this is not applicable to other regions of the world hay making was replaced by producing „hay-crop silages“. This change has led to marked improvements, and its very likely further expansion in the future will be of additional benefit. Furthermore, the utilization of whole-crop maize, whole-crop cereals, and possibly also of sugar cane, for feeding purposes and energy production is linked to the preservation technology of ensiling. The extended use of such crops in the form of silage will, in any case, lead to extension of silage making. Therefore it seems rewarding to have a closer look at the potential of this method of forage conservation, and the still future demand for research and development which is worth to reflect on.

Wilkinson, Bolsen and Lin (2003) carefully analyzed and evaluated the history of silage and silage making. In their summary, they distinguished between three categories of regions and countries, respectively, which differ in current situation and developmental potential in silage production:

- „Europe and North America, where silage making is well established“...and with...“a need for new technologies and inputs to constrain costs of silage production”...and probably...“some replacement of perennial grass silages with silages made from corn, whole-crop cereals and forage legumes.”
- “Some temperate and tropical areas, where silage currently supplies a small proportion of nutrients (e.g. Australia, New Zealand and Latin America), there will probably be increase in silage production to capitalize on the advantages of a silage system in producing a more even seasonal supply of nutrients... compared with grazing or hay systems.”
- “Tropical and subtropical livestock production...where...the potential of silage ... is, as yet, largely unrealized”... and where ...“will be a continued requirement to harvest

material of reasonable nutritional value and to maintain that feeding value and reduce losses during the storage and feeding periods.”

For each of these situations ensiling technologies and strategies are to be searched for, which are suitable for the specific conditions in the respective countries. In this regard, experience from developed countries can be made use of, but it is unlikely that all of it is directly applicable in tropical and subtropical regions.

In the following, technologies and strategies are described that have been developed in Europe and North America. Thereafter, it will be discussed as to whether those might be suitable for other regions. Finally, conclusions are drawn on required research and development activities in particular for tropical and subtropical areas.

The main problem of ensiling grass and legumes in temperate regions is to ensure good fermentation quality of the silage despite changing weather conditions. Good fermentation quality is needed to ensure low fermentation losses, high feed intake and good hygienic status of the silage.

It is well established that the behaviour of a crop, when subjected to silage fermentation, depends on the substratum supply for lactic acid bacteria. The required amount of water soluble and thus fermentable carbohydrates (WSC) is related to buffering capacity (BC) of the herbage. Therefore, in order to characterize the ensilability of a given crop, the ratio between WSC and BC is calculated. The WSC/BC ratio has been suggested to express the acidification potential of the herbage.

Buffering capacity is characterised here – on the contrary to Playne and McDonald (see McDonald *et al.*, 1991) – by the amount of lactic acid which is required to acidify the crop to pH of 4.0 (Weissbach, 1967). The practical advantage of this procedure is that the parameters WSC and BC have the same dimensions (e.g. g/kg DM). If so, the ratio between the two reflects how many times of the standardized lactic acid demand does the herbage contain in fermentable carbohydrates.

Legumes very often have a low content of WSC but, simultaneously, contain higher concentrations of buffering substances than found in grasses. As opposed to what is frequently assumed BC is not primarily affected by the protein content of the herbage but mainly by the alkalinity of its mineral components. The evaluation of data from 52 plant species of different taxonomic families resulted in the following equation (Weissbach, 1998):

$$BC = 0.092 x_1 + 0.442 x_2 - 19.5 (5.88 - x_3) r^2 = 0.842$$

where BC is the buffering capacity as meq/100 g DM, x_1 is the nitrogen content [meq/100 g DM], x_2 is the ash alkalinity [meq/100 g DM] and x_3 is the pH of the herbage.

This equation also takes into consideration the effect of high concentrations of free organic acids, which may occur at high levels in some tropical species (McDonald *et al.*, 1991).

The susceptibility of clostridia to acid increases with decreasing water activity in their environment (Table 4). Thus, bad fermentation can be avoided despite of a low WSC/BC ratio by pre-wilting the herbage. The lower WSC/BC the more the DM must be increased.

Table 4: Critical pH values of silages
(Weissbach, 1968)

DM content %	Water activity a_w	pH required for stability of silage
15	0.985	4.10
20	0.980	4.20
25	0.975	4.35
30	0.971	4.45
35	0.966	4.60
40	0.961	4.75
45	0.956	4.85
50	0.952	5.00

Figure 2 demonstrates different frequency ranges of bad fermentation to be expected at given WSC/BC ratio and DM content.

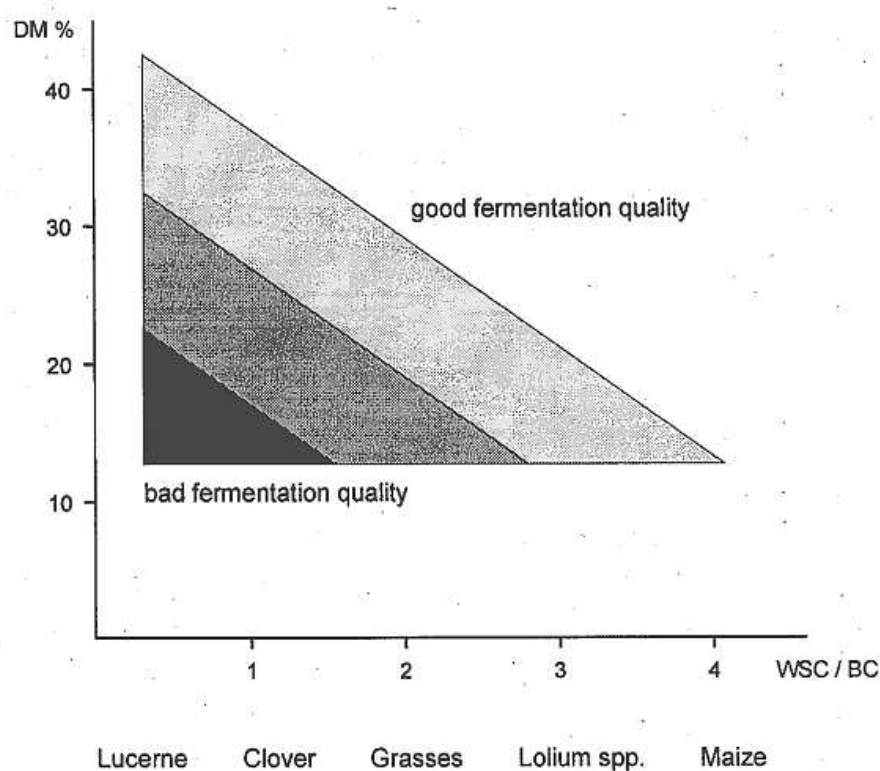


Figure 2: Frequency of bad fermentation (butyric acid containing silages) as affected by WSC/BC ratio and DM

The minimum DM content (DM_{min}), which is required to compensate for substratum deficiency (upper edge of the triangle), increases with decreasing WSC/BC ratio and can be calculated by the following equation (Weissbach *et al.*, 1974):

$$DM_{min} [\%] = 45 - 8 \text{ WSC/BC}$$

The fermentability of a given crop refers to its ensilability as far as the two parameters WSC/BC ratio and DM are concerned. In order to characterize fermentability, the two parameters DM and WSC/BC according to a proposal of L. Schmidt (see Weissbach and Honig, 1996) can be also combined to one parameter, which is named the “fermentability coefficient” (FC):

$$FC = DM [\%] + 8 \text{ WSC/BC}$$

Herbages with $FC < 35$ are considered difficult-to-ensile, whereas those with $FC > 45$ are referred to as easy-to-ensile.

It has been shown that ensuring the minimal DM content ($DM_{\min}$), as described by the equation above, is not always sufficient to consistently inhibit butyric acid fermentation. The crop to be ensiled additionally needs to contain a certain concentration of nitrate (Hein and Weissbach, 1977; Spoelstra, 1985; Kaiser and Weiß, 1997; Weissbach *et al.* 1993; Weissbach, 1996). Nitrate is converted into nitrite during the early stages of fermentation, thereby inhibiting clostridial development until pH has reached the critical level. The minimal required nitrate concentration has been discussed controversially (Kaiser *et al.* 2002; Kaiser *et al.* 2005; Kaiser *et al.*, 2009; Pahlow, 2002; Kaiser *et al.* 2009; Weissbach and Honig, 1996; Weissbach, 1998). However, based on experimental data from numerous ensiling trials with herbage from very different plant species, a minimal content of nitrate of 1 g/kg DM has proven to be sufficient under most conditions. Alternatively, an epiphytic lactic acid bacteria (LAB) count of at least 10^5 cfu/g fresh forage can compensate for a lack of nitrate and support a good fermentation quality.

In contrast, it has been shown that butyric acid free silage can be produced frequently even if the crops had DM concentrations lower than $DM_{\min}$ and did not contain significant amounts of nitrate and high LAB counts. For some plant species it was proven that the causal agents for this observation were secondary plant metabolites (Weissbach, 1998). Apparently is the susceptibility of clostridia to low pH values also affected by the simultaneous presence of bacterial inhibitors. These inhibitors prevent the degradation of lactate at later stages of fermentation.

Based on the two protective effects, namely

- the presence of nitrate (and nitrite, respectively) or high populations of efficient epiphytic LAB at the beginning of the fermentation process and
- the presence of specific inhibitors which protect silage against clostridial activity during the later stages of fermentation and the further storage

strategies can be derived concerning the control of the fermentation process by the use of silage additives.

Table 5 summarizes data on ensilability of the most important silage crops in Europe. Due to higher WSC/BC ratios, rye grasses (*Lolium* species) are easier to ensile than all other grass species, which are easier to ensile than legumes. Whole-crop cereals and maize (*Zea mays*) are generally easy to ensile. In the most unfavourable conditions, a $DM_{\min}$ of about 30 % is needed for rye grasses, 35% for red clover (*Trifolium pratense*) and for all other grasses, and 40 % for Lucerne (*Medicago sativa*).

Table 5: Ensilability parameters of silage crops as expected under normal conditions

Crop resp. Sward type	Cut No. resp. Maturity stage	N supply level	DM %	g/kg DM		WSC/BC	DM _{min} g/kg	FC	
				WSC	BC			unwilted (DM as cut)	wilted (DM 30 %)
Grasses									
Lolium dominated swards									
	Primary growth	low	18	220	55	4.0	< 20	50	62
		moderate	18	180	55	3.3	< 20	44	56
		high	18	160	55	2.9	22	41	53
	Re-growthes	low	22	140	55	2.5	25	42	50
		moderate	22	120	55	2.2	28	39	47
		high	22	100	55	1.8	30	37	45
Other grass swards									
	Primary growth	low	18	120	50	2.4	26	37	49
		moderate	18	100	50	2.0	29	34	46
		high	18	80	50	1.6	32	31	43
	Re-growthes	low	22	100	50	2.0	29	38	46
		moderate	22	90	50	1.8	31	36	44
		high	22	70	50	1.4	34	33	41
Legumes									
Red clover	all cuts		20	100	70	1.4	34	31	41
Lucerne	all cuts		20	60	80	0.8	39	26	36
Whole-crop cereals									
Barley	Milk stage		30	140	40	3.5	< 20	58	
	Dough stage		40	70	35	2.0	29	56	
Wheat	Milk stage		30	120	40	3.0	21	54	
	Dough stage		40	60	35	1.7	31	54	
Maize	Milk stage		25	190	35	5.4	< 20	68	
	Dough stage, early		30	130	32	4.1	13	63	
	Dough stage, full		35	80	30	2.7	24	56	

WSC = water soluble carbohydrates; BC = buffering capacity

DM_{min} = DM minimum; FC = fermentability coefficient

In practice, the DM content varies in a rather wide range during the harvest of a pre-wilted crop and during filling the same silo. The higher the intended average wilting degree, the higher this variation range will be. The recommended strategy is to maintain the DM within a certain range. The lower limit of this range (DM_{min}) is determined by WSC/BC, the upper limit depends on the ensiling technology and the quality of sealing the silo. For bunker and pit silos, a DM_{max} of 45% should not be substantially exceeded. Thus, specific crops require different ranges of DM variation, wherein the wilting degree should be fluctuating ideally. For bales, DM_{max} should be set 60 %, whereas for tower silos, DM_{max} should set based on stacking height (lower section: 60%, middle section: 45 %, upper section: 30%).

The primary aim of the use of silage additives to ensure good fermentation quality is the alleviation of a too low wilting degree and, if required, of the lack of nitrate. Proven chemical silages additives (organic acids and salts thereof, as well as neutral reacting preservatives, including sodium nitrite and hexamine) should possess the strength which equals that of an

increase in crop DM by at least 10%. Inoculants, mainly of the homofermentative type, should be as efficient so that the DM at ensiling can be 5% lower than the DM_{min} . Considering these facts, the resulting crop specific DM ranges at ensiling, which should be adhered to, can be derived. Table 6 shows the recommended ranges for ensiling in bunker and pit silos.

Table 6: Strategy of making wilted hay-crop silage

Silage additive	DM content [%] to be aimed at	
	Available range	Width of the
	DM_{min} ... DM_{max}	available range
<i>Lolium</i> dominated grass swards		
without	30...45	15
homolactic inoculant	25...45	20
chemical additive	20...45	25
Other grass swards and red clover		
without	35...45	10
homolactic inoculant	30...45	15
chemical additive	25...45	20
Lucerne		
without	40...45	5
homolactic inoculant	35...45	10
chemical additive	30...45	15

By strategically using silage additives, it is possible to significantly extend the technologically advisable DM range, and to vastly suppress undesired fermentation pathways. However, the costs for silages additives, especially for chemical products, are rather high as, for consistency of effect, the required application rate depends on the moistest batch of crop which is brought into the silo.

Recent developments in harvesting technology have enabled the use of „precision farming“ tools also in silage production (Savoie and Shinnars, 2009). This has opened up possibilities for the control of silage additive application strictly based on real demand. Choppers are nowadays equipped with sensors which measure throughput and DM content on a real-time basis as well as with on-line controlled silage additive applicators. For each of the chemical silage additive types can crop-specific mathematical functions be created, which permit additive application based on DM. This in turn leads to a significant decrease in additive costs since, on average of the whole silo, only the dosage is used which is required for a given DM content.

Exemplarily, Table 7 shows the expected effects for the ensiling of grass with a liquid chemical additive, composed of sodium nitrite and hexamethylene tetramine. This additive is normally applied at 3 l/t and its effect is equal to increasing DM level by 14% (Weissbach *et al.*, 1989). The minimal dosage of this silage additive, which is constantly applied by using the on-line controlled equipment, supplies sufficient sodium nitrite to inhibit clostrial activity during the early fermentation phase also in nitrate-free forage.

Table 7: Mean requirement of a chemical silage additive with DM controlled application

Degree of wilting	DM content of the grass that enters the silo [%]			Mean application of silage additive for the whole silo, litres/t FM
	Mean content within the whole Silo	Variation during filling the silo		
		Range	Width of range	
Without	18	16 ... 20	4	3.0
Very slingtly	25	20 ... 30	10	2.5
Slightly	30	22 ... 35	13	1.8
Moderately	35	25 ... 45	20	1.2
Rather heavy	40	28 ... 58	30	1.0

What recommendations can be derived from this body of knowledge and state-of-the-art technology for other regions, e.g. Brazil?

There have been published comprehensive reviews (Nussio, 2005; Adesogan, 2009) as well as numerous individual articles by different research groups on the problems, challenges and experiences of silages production in tropical and subtropical regions (Cezario *et al.* 2009; Martens *et al.* 2009; Parvin *et al.*, 2009a; Parvin *et al.*, 2009b). Warm-season grasses are the backbone of the tropical forages. As reported by Ribeiro *et al.* (2009), grass silage production in Brazil is mainly based on the genera *Brachiaria* and *Panikum*, whereby *Brachiaria* species alone represent 85% of the cultivated grassland.

It is well known that these warm-season-grasses, in comparison to temperate grasses, are much lower in WSC and protein, much higher in ADF and NDF and, consequently, substantially lower in digestibility (Adesogan, 2009). However, systematic research seems to be still lacking on chemical composition, digestibility and fermentability of these grasses as affected by plant species, season, level of fertilization, stage of vegetation (maturity). But such information is absolutely necessary to be able to derive optimal cultivation and utilization regimes and strategies for conservation as well.

Experiments, in which individual plant species were cultivated on experimental plots (see e.g. Ribeiro *et al.*, 2009), are a good start, but it is not sufficient to chemically analyze the crop – or even only the silage, as it was done in the cited publication. In fact, such experiments must also aim at different stages and maturity, and should preferably be combined with digestibility studies in sheep. This information should be used in the prediction of the effects of stage of maturity and cutting frequency on nutritive value.

The basic principle of ensiling strategies for grasses in the tropics and subtropics can, as in Europe, only be the combination of pre-wilting and silage additive use. The necessity to wilt already originates from the demand to prevent effluent production, and the loss of nutrients caused by it. In addition, wilting will be necessary to ensure Silages of good fermentation quality. How much the crops need to be wilted to inhibit butyric acid fermentation is likely to be derivable from the same relationships that are known for European crop species. The required DM_{min} depends on the WSC/BC ratio. This approach is certainly applicable also to warm season grasses. On the contrary, not applicable will be the European threshold values for DM_{min}, or even

the recommendations on minimal sugar content as percentage of fresh matter, as has been done in the past.

Regarding the usability of silage additives to compensate for low fermentability, it is strongly recommended in all ensiling trials to analyze the fresh crop for DM, WSC, BC, nitrate and, preferably, also for epiphytic lactic acid bacteria count. This is the precondition to decide whether the additive in question had the chance to show positive effects. Moreover, single laboratory ensiling experiments are not sufficient to evaluate the efficacy of a given silage additive. The factors affecting fermentation quality are too complex, and many factors are not controllable so that the validity of the results of only a few studies is too weak. It should be enforced that a minimum of 5 independent trials using the same species and the same additive be conducted in order to be able to draw sound conclusions.

Adesogan (2009) forcefully pointed at the need to distinguish between low and high input farming systems in the tropics. Both, the aim and the justifiable input differ significantly. Against this background, the use of the cheaper commercial inoculants will be more advisable than chemical silage additives for low-input systems in many cases.

Under such conditions even the use of simple and cheap methods like the inoculation with „previously fermented juice“(PFJ) can be a reasonable option (Oshima *et al.*, 1997a; Oshima *et al.*, 1997b; Nishino and Uchida, 1999; Yu *et al.*, 2009). This method is characterized by the cultivation of epiphytic microorganisms in a plant extract prepared from the crop to be later on ensiled. The diluted plant extract is supplemented by molasses or sucrose and incubated for 2-3 days at 30°C. This suspension containing vital microorganisms (probably mainly LAB) is subsequently added to the crop and used as a starter of silage fermentation. However, it might be even more successful to use samples from good silages (which were made with successful inoculation) for the preparation of PFJ as “self made inoculants”. Research also in this field could be worthwhile.

Maize silages and the problem of aerobic instability

The increasing cultivation of forage maize and its use for silage production offer chances to increase silage production and feed inventories in many warm countries. Recommendations from Europe and North America regarding this crop and specific ensiling technologies can be directly applied. Wherever necessary can those be also adapted very well to low-input farming systems.

Forage maize is generally easy to ensile. Its fermentability coefficient is always higher than 45, and many mistakes must be simultaneously made to produce a maize with silage bad fermentation quality. Maize always contains much more WSC in relation to its BC, and also the epiphytic LAB counts are very high in most cases.

However, the surplus of WSC creates a completely different quality problem. WSC which are not utilized for lactic acid production serve as substrate for yeasts. During anaerobic storage will yeasts thrive on utilizing sugar to produce ethanol. Upon subsequent exposure to air, yeasts switch over to respiratory metabolism and excessive cell multiplication linked with head generation. Subsequently, lactic acid is degraded so that pH increases, thereby creating environmental conditions which allow other undesirable microorganisms to develop. Silages made from forage maize, but also those produced from sorghum and whole-crop cereals, tend to be susceptible to aerobic spoilage, which is associated with dramatic losses in nutrients as well as with the deterioration of hygienic quality. During the last years, it was shown that even spores of the obligate anaerobic clostridia were found at very high numbers in the upper layers of silages which underwent aerobic deterioration (Driehuis and te Giffel, 2005; Tabacco and Borreani, 2009).

These processes are well studied and documented (Pahlow and Muck, 2009). The surplus of fermentable carbohydrates creates the predisposition of silage from maize and sorghum to aerobic deterioration. Mainly yeasts create the aerobic spoiling and air ingress during storage and after silo opening initiate this undesirable process. To avoid the impact of oxygen, or at least to reduce it, is the pre-requirement for any efficient ensiling technology. Technical measures, which lead to improvements regarding this aspect, are also highly economical as a rule (Muck and Homes, 2005; Bernardes *et al.*, 2009; Holmes and Bolsen, 2009; Muck and Homes 2009).

Even though, the particularly high risk of aerobic instability in these crops remains, and measures need to be taken to minimize it. Unfortunately, there is no reliable prediction of the aerobic stability of a given silage possible as of yet. However, what is well known is the inhibiting effect of high concentrations of undissociated acetic acid on yeasts and its beneficial effect on aerobic stability. Figure 3 presents the result of an evaluation of numerous experimental data.

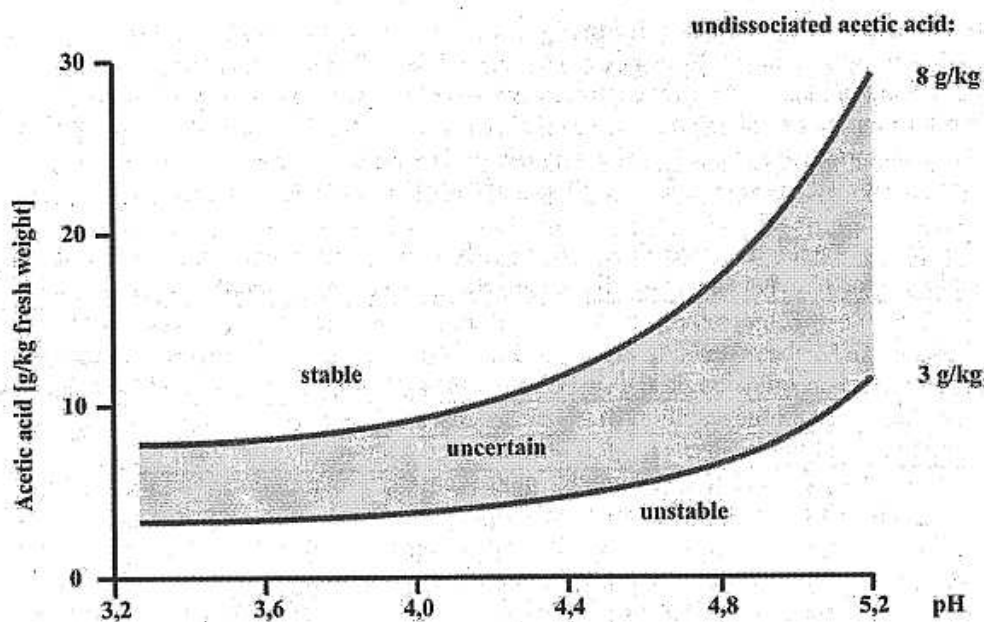


Figure 3: Risk of aerobic instability as influenced by acetic acid and pH (Wolthusen *et al.*, 1989)

Data clearly demonstrate that silages containing less than 3 g/kg FM are mostly unstable, whereas those having more than 8 g/kg FM of undissociated acetic acid are almost always stable. As known, the proportion of undissociated acid (α) depends on pH according to the following equation:

$$\alpha = \frac{[H^+]}{[H^+] + D}$$

where $[H^+]$ is the concentration of hydrogen ions [mol/L] and D the dissociation constant, which is 1.76×10^{-5} for acetic acid. Consequently, to secure aerobic stability, the two criteria must be met, namely sufficiently high acetic acid content and sufficiently low pH.

It can be concluded from Figure 3 that there is still a wide range of undissociated acetic acid concentration (3 - 8 g/kg FM) in which the behavior of the silage upon exposure to air cannot be predicted. Thus, there must be additional factors which affect aerobic stability. Further research into this phenomenon is crucial. With the exception of maize and sorghum, there should be considered secondary plant metabolites and their derivatives which may exert an inhibitory effect on yeasts. This effect is known for Lucerne, but the chemical nature of these compounds still remains to be elucidated. Systematic studies on European wild plants revealed that not only silage from lucerne is resistant to aerobic spoilage, but also such from 5 additional legumes and numerous other dicotyledonous species did not deteriorate at air contact (Weissbach, 1999). It should therefore focus be placed on the experimental question as to whether these effects can be found in tropical and subtropical plant species and to make use of that.

As far as the suppression of yeasts by increased acetic acid concentration is concerned, there was a major breakthrough achieved in the end of the 1990ies. It could be demonstrated that inoculation of crops with specifically selected strains of the heterofermentative species *Lactobacillus buchneri* resulted in elevated acetic acid concentrations and higher aerobic stability of silages (Driehuis *et al.*, 1996; Driehuis *et al.* 1999). Lactate is subjected to secondary fermentation and converted into acetate. Somewhat later the metabolic pathway was elucidated regarding the formation of acetate. The formed hydrogen is utilized to produce 1,2-propanediol (Oude Elferink *et al.*, 1999; Oude Elferink *et al.*, 2001) (Figure 4).

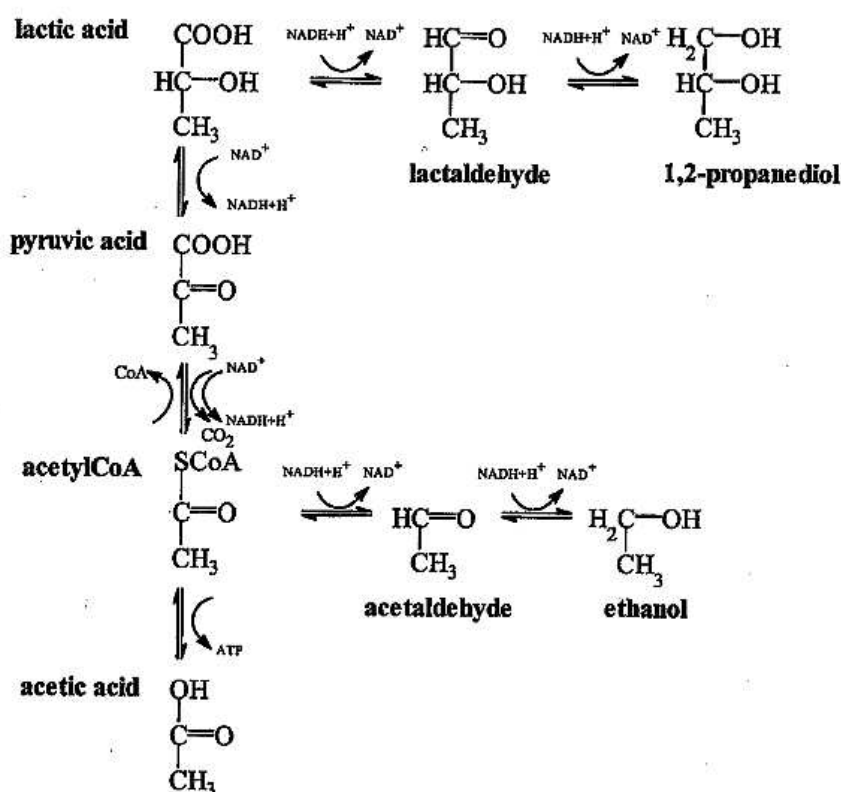


Figure 4: Proposed pathway for anaerobic degradation of lactic acid by *Lactobacillus buchneri* into equimolar amounts of 1,2-propanediol and acetic acid and trace amounts of ethanol

Further on, it was shown that 1,2-propanediol is metabolized by the naturally occurring species *Lactobacillus diolivorans* to give 1-propanol and propionic acid at later stages of silage fermentation (Krooneman *et al.*, 2002). It is well documented that propionic acid is a more potent fungal inhibitor than acetic acid. The formation of this fermentation acid also contributes

to stabilizing the silage. In addition to this, it has to be noted that the described biochemical reactions result in the accumulation of two compounds which have higher energy contents per g than the acids lactic and acetic acids.

During the last few years, different researchers have tested different *L. buchneri* strains, which were shown to be very efficient in improving aerobic stability (Bannemann *et al.*, 2009; Brüsemeister *et al.*, 2009a; Brüsemeister *et al.*, 2009b). In the meantime, evidence has been provided that inoculation with this type of bacteria can lead to acetic acid concentrations which may be higher than tolerated by small and large ruminants. Excessive amounts of acetic acid are suspected to reduce feed intake (Eisner *et al.*, 2006). Even though such potentially negative effects are unlikely to be of practical relevance in temperate regions, it should be studied as to whether these effects and the potential risk associated with it may occur in the tropics and subtropics. Under the prevailing storage temperatures there, which can be higher than those in Europe and North America, the process of lactate degradation by *L. buchneri* may lead to higher contents of acetic acid as desired.

On the contrary to hay-crop silages is the use of homofermentative inoculants in maize and sorghum neither necessary nor advisable. Repeatedly postulated positive effects have not been consistent, or its magnitude has been marginal. Quite the contrary was observed. Stimulation of homolactic fermentation resulted in significant decreases in aerobic stability of these types of silages.

The opinions about the best concept of inoculation of silages expressed by silage specialists have been controversial although the body of evidence is unequivocal (Pahlow *et al.*, 2003; Kung, Jr. *et al.* 2003; Kung Jr., 2009). Davies (2010) opposed to the use of heterofermentative LAB and stated that the production of acetic acid leads to the formation of CO₂, and that energy is lost which cannot be used by the ruminant. Moreover, the higher acetic acid concentration he assumed to detrimentally affect methane production in the rumen if more acetic acid instead of lactic acid enters this part of the digestive tract.

However, these postulates are not substantive. The released CO₂, which is produced as a co-product of acetic acid forming in the silo, originates from photosynthesis and therefore is not loading the atmosphere. Considering this loss of biomass is even indirectly advantageous since for each kg of DM that is lost during fermentation, as much as at least 3 kg of DM can be saved during feed-out upon exposure to air, which may otherwise be degraded during the process of aerobic deterioration (Tabacco *et al.*, 2011). Moreover, the metabolic end products of the heterolactic pathway are not only composed of acetate, but also of the energetically more valuable compounds 1,2-propanediol, 1-Propanol and propionic acid. Potentially, the energy value per g of the silage DM is even increased. Unfortunately, the volatile compounds which are lost during oven drying are sometimes not or incomplete determined. This in turn results in an incorrect calculated, too low nutritional value of silages.

Another problem, which just very recently became obvious, is also associated with volatile fermentation end-products. There have been reports from commercial farms on odd-smelling maize silages which are not taken in well by dairy cows, or silages are even refused. Analyses of samples taken on farms as well as numerous studies at laboratory scale could shed light on the causal agents. The odd smell was associated with the spontaneous formation of esters of fermentation products (Weiß *et al.*, 2009 a; Weiß *et al.*, 2009 b).

By using GC-MS and a special GC analysis a number of highly volatile substances could be identified. The highest concentrations were consistently found for ethyl lactate and ethyl acetate. The content of these esters was mainly influenced by the concentration of ethanol and only to a smaller extent by the concentrations of the organic acids. The higher ethanol level, the more ethyl esters of the respective acids were found. As a consequence, if ester accumulation is to be reduced, then ethanol forming must be lowered.

Mitloehner et al. (2009) also reported on studies into volatile organic compounds (VOC) including esters emitted from open-face silage piles. The purpose of these investigations was to check as to whether and which VOC from dairy farms might be released into the atmosphere and could be a source of ground level ozone formation. But no qualitative and quantitative data and no relations between ester formation and silage properties were given in this article.

Table 8 below summarizes the results of a lab-scale ensiling trial on sorghum (Weiß and Auerbach, 2011), in which the effect of different additive types was studied on ester accumulation. Although *L. buchneri* could reduce the levels of ethanol and forming of ethyl lactate if compared with untreated silages and those treated with a homofermentative *L. plantarum* additive, only the combination of sodium benzoate and potassium sorbate in the tested chemical additive dramatically restricted ethanol fermentation as well as the formation of ethyl esters of lactate and acetate. These findings are supported by Kleinschmidt *et al.* (2005), who also reported on the inhibition of yeasts and ethanol formation in silages caused by chemical additives.

Table 8: Fermentation pattern of sorghum silage made with different additives
(Weiß and Auerbach, 2011)

Treatment (DM = 26 %)	pH	g/kg DMc				mg/ kg DMc	
		Lactic acid	Acetic acid	Ethanol	1.2-Propane-diol	Ethyl acetate	Ethyl lactate
Control	3.8	4.0	2.7	3.4	1.3	120	467
<i>L. plantarum</i>	3.8	3.8	2.2	2.9	0.3	123	463
<i>L. buchneri</i>	3.8	2.5	4.6	1.8	3.6	128	237
Benzate/Sorbate*	3.7	2.4	2.8	0.8	1.2	44	33

* Liquid preparation containing sodium benzoate and potassium sorbate
DMc = DM corrected for volatile organic compounds

Sugarcane silages and the problem of volatile compounds

Sugarcane belongs to the highest yielding crops and is used already today for feeding to some extent (Nussio, 2005). Not only the whole plant is used, but also sugarcane tops, which accrue as by-product during harvest of the crop for sugar production. Obviously, this crop possess the potential of being used for feeding to a higher extend in the future, thereby enabling a more efficient and seasonal even supply of feed for ruminants throughout the year.

An increased use of sugarcane would be easier if efficient silage making technology would be available. Therefore, several studies on ensiling sugarcane were carried out (Kung, Jr. and Stanley, 1982; Pedroso *et al.*, 2002; Pedroso *et al.*, 2005; Avila *et al.*, 2009; Muraro *et al.*, 2009; Nussio *et al.*, 2009a; Nussio *et al.*, 2009b; Pinto *et al.*, 2009). But the results were contradictory. Although positive results were reported from feeding of sugarcane silages to heifers (Nussio, 2005) and also to dairy cows (Queiroz *et al.*, 2005) the experiences with ensiling losses and the measured nutritive value of silages were in contrast to that. Kung, Jr. and Stanley (1982) even concluded that „sugarcane did not lend itself to ensiling because of large dry matter losses ...“

Obviously, the high sugar surplus is the problem of silage making here. Sugarcane contains much more WSC than can be converted into lactic acid. Thus, the vast proportion is subjected to utilization by yeasts. Very high gaseous fermentation losses and an even higher total DM loss have been frequently reported (Pedroso *et al.*, 2005; Nussio *et al.*, 2009a; Nussio *et al.*, 2009b). However, the total DM loss was not associated with effluent seepage, as only low amounts were produced. The reason for the remarkable difference between total DM loss and gaseous loss remained unclear.

What was considered even more important was the dramatic decline in DM content and nutritive value with progressing fermentation. Concentrations of the fiber fractions like ADF and NDF increased during the course of fermentation, and the *in vitro* as well as *in vivo* digestibility measured with sheep was reduced. The more mature sugarcane and the higher its sucrose content, the higher was the loss in nutritive value of the silages.

All these negative observations on ensiled sugarcane, however, are likely to be caused only by volatile organic compounds (VOC), which are contained in the silage but were not or not sufficiently accounted for. As ethanol content can be extremely high in sugarcane silage, the impact of this methodological error is particularly prominent and leads to biased and unrealistic results.

The fact that silages contain VOC is well known (McDonald and Dewar, 1960) and the methods to account for them in DM determination have a long history in silage research (Cherny and Cherny, 2003). Over decades, DM determination by employing the „corrected toluene distillation method” according to Dewar and McDonald (1961) was considered the standard method. The correction was at first only directed on acids which were transferred into the distillate, later on also alcohols were also taken into consideration. Extensive studies were then carried out to obtain the “true CM content” by the use of chemical methods for determination of the water content like Karl Fischer titration or gas chromatography (Porter and Barton, 1997; Porter and Murray, 2001). It has been quite surprising to European silage specialists to notice that the VOC are not considered in the procedure of determination of the nutritive value of silages by AOAC methods which are followed also in some other countries (e.g. Pedroso *et al.*, 2005).

It is accepted that routine toluene distillation is not a viable option any more due to health protection issues and costs. Moreover, there are additional principal reasons which render this method inadequate. All results obtained by this method have never been compatible with oven-drying, to which all other laboratory analyses have been related to (e.g. to express the nutrient contents given on a total DM basis). This applies even if all VOC which were transferred into the distillate would have been determined and considered. The reasoning behind this is that silage is not composed of only solid and liquid substances, but it also contains absorbed CO₂ which is totally only released if the sample is subjected to high temperatures during oven drying or toluene distillation. From the results of an extended study with different kinds of silage carried out by Berg, 1971 (re-evaluated and cited in: Weissbach, 2005) it could be proven that this absorbed CO₂ achieves a noticeable amount and is closely related to DM content. Consequently, the equation:

$$(\text{Silage fresh weight}) - (\text{Water}) = \text{DM}$$

is false. The calculated difference between silage fresh matter and weight of water is, thus, always slightly higher than the real DM. Therefore, toluene distillation cannot serve as reference for simplified analytical methods, as was proposed by e. g. Haigh (1995) and cited in: Cherny and Cherny (2003). This also applies to all methods based on chemical determination of the water content and proposals for DM correction based on them.

The only feasible method, which is in full agreement with the current state of knowledge, must include the determination of all VOC from silages and the addition of the volatile proportion of them to the DM measured by oven-drying. In doing this the specific volatility of each individual VOC must be taken into account and subsequently their volatile proportions calculated. These volatility coefficients were determined by employing a standardized procedure of oven drying (pre-drying of the sample at 60-70°C, followed by final drying at 105 °C for 3 hours) (Weissbach and Strubelt, 2008a-c; Weissbach, 2009a). The following equations have been proposed to correct the DM for the loss of volatiles during oven-drying for these drying conditions.

Maize silages

$$DM_c = DM_n + 0.95 FA + 0.08 LA + 0.77 PD + 1.00 AL$$

Hay crop silages

$$DM_c = DM_n + (1.05 - 0.059 pH) FA + 0.08 LA + 0.77 PD + 0.87 BD + 1.00 AL$$

Sugar beet silages

$$DM_c = DM_n + 0.95 FA + 0.08 LA + 1.00 AL$$

where DM_c is the corrected DM, DM_n is the uncorrected DM, FA is the sum of volatile fatty acids ($C_2 \dots C_6$), LA is lactic acid, PD is 1,2-propanediol, BD is 2,3-butanediol and AL is the sum of all other alcohols ($C_1 \dots C_4$). All these parameters are to be used in the equations with the dimension g per kg fresh matter. The equation on silages from forage maize can be also used for such from sorghum and whole-crop cereals, and that on sugar beet should be also suitable for sugarcane.

As a consequence of correcting the DM content, all other analytical parameters, which are expressed as part of the DM, need to be corrected as well. Those of them which are directly measured in the pre-dried sample and are usually expressed as percent of DM_n (e. g. ash, ADF or NDF) must be multiplied by the quotient DM_n/DM_c . Difference fractions (e. g. OM or NDS = neutral detergent solubles) have to be calculated once more by using the figures expressed as percent of DM_c . The same applies to the calculation of the *in vitro* or *in situ* digestibility of such fractions to which the VOC belong (e.g. digestibility of DM or digestibility of OM). As an example, the dried residue of the IVTDM test, given as part of DM_n , has to be multiplied by DM_n/DM_c . The difference between 100 and this number is then the real IVTDM.

The article of Kung and Stanley (1982) contains experimental results on sugarcane silages, which were harvested at different stages of maturity (6, 9, 12, 15 und 24 months of growth), as well as on a maize silage. Silages made from sugarcane after 6, 12 and 24 months of growth and the maize silage were tested in digestibility trials with sheep. Fortunately, this article provides all of the needed data to enable subsequent re-calculation based on corrected DM. Data summarized in Tables 9-11 compare the results based on uncorrected and corrected DM.

Table 9: Composition of sugarcane silage with and without VOC correction calculated from experimental data published by Kung, Jr. and Stanley (1982)

Herbage and stage of maturity	DM content, %			Ethanol		ADF	
	Fresh herbage	Silage not corrected	Silage corrected	Silage not corrected	Silage corrected	Silage not corrected	Silage corrected
	DM	DMn	DMc	g/kg DMn	g/kg DMc	g/kg DMn	g/kg DMc
Corn	33.4	33.1	34.0	8	7	308	299
Sugarcane							
6 mo	22.3	18.1	20.5	75	68	434	395
12 mo	29.0	20.4	24.0	155	131	441	375
24 mo	31.5	22.4	26.6	175	147	443	372

DMn = not corrected for VOC; DMc = corrected for VOC

With increasing age of the sugarcane, DM increases due to accumulation of sucrose. The DM of the silages decreases mainly as a result of ethanolic fermentation, what can be explained by dramatic mass losses. Whenever ethanol is formed, 49% of the mass disappears (based on monosaccharides) (McDonald *et al.*, 1991). Fermentation leads to decreased amount of DM, and the content of all components in the dry matter, which are not affected by fermentation, e.g. ADF, increases. The magnitude of this increase is lower if the content is calculated based on DM_c (Table 9).

In digestibility trials, DM intake increases due to correction of silage DM, while DM excretion remains unchanged, leading to increased DM digestibility. In the sugarcane silage of 24 months of age reaches the difference between corrected and non-corrected digestibility of DM about 10 percent units (Table 10).

Table 10: Digestibility of sugarcane silage with and without VOC correction calculated from experimental data published by Kung, Jr. and Stanley (1982)

Herbage and stage of maturity	Digestibility of DM %		Digestibility of energy %		TDN % of DM	
	not corrected	corrected	not corrected	corrected	not corrected	corrected*
Corn	61.4	62.5	67.6	68.4	62.3	63.9
Sugarcane						
6 mo	49.5	54.0	57.6	62.3	51.6	60.8
12 mo	47.3	55.2	58.5	66.7	48.1	65.2
24 mo	41.4	50.8	49.5	60.6	41.5	61.3

* TDN corrected with regard to DM and energy

In the calculation of nutritive value it still needs to be considered that during ethanolic fermentation about 97% of the gross energy of glucose or fructose is maintained in the metabolic end-product, the ethanol. Ethanol, which is included in the corrected DM, is not only fully digestible, but also contains much more energy per g than do other components of digestible OM.

It was attempted in this data evaluation to incorporate the high energy value of ethanol. The following assumptions were made: gross energy of ethanol is 7.12 kcal/g, gross energy of volatilized fatty and lactic acids 3,50 kcal/g and the average gross energy of the total of other components of digestible organic matter is 4.17 kcal/g (Schiemann *et al.*, 1971; Jentsch *et al.*, 1969). Accordingly, 7.12 kcal/g ethanol and 3,50 kcal/g acids were added to gross energy (GE) and digestible energy (DE) (Table 10).

A rather unconventional approach was chosen in order to take account of the high energy content of ethanol in the calculation of TDN. In analogy to digestible fat, whose concentration is multiplied by 2.25 when calculating TDN, the ethanol as part of the digestible carbohydrates was multiplied by 1.71 g per g ($7.12/4.17 = 1.71$). In this way, one can get to a TDN figure which is not only DM-corrected but also energy-corrected. The TDN figures given in Table 10 are obtained by this calculation. The difference between corrected and non-corrected TDN achieves 20 percent units, where 10 are coming from higher digestibility of DM_c and about 10 units from the higher energy content of the digestible DM_c.

This evaluation supposes that the energy of ethanol is utilized like the energy of other nutrients. Based on our current physiological knowledge there is not any doubt that the ethanol will remain unchanged when passing through the rumen and the rumen wall, and that its energy will be then utilized in intermediary metabolism of the animal like that of other absorbed nutrients. Jentsch *et al.* (1969) found an energy utilization rate of 72% for ethanol in pigs, which is quite normal in comparison to other nutrients like, e.g., glucose or lactic acid, while the energy of acetic acid was less effectively used (about 60%).

The calculations performed here led to substantial higher values for the energetic feeding value of sugarcane silages than was suggested before. Although the tested maize silage was not of best possible quality, the sugarcane silages - on average - achieved the same level of energetic feeding value as did the maize silage. This may explain why with sugarcane silages in the mentioned feeding experiments much better results were obtained than from the data on its apparent nutritive value could have been expected (Nussio, 2005; Queiroz *et al.*, 2005).

This conclusion is supported by the energy contents of the silages (Table 11). If the correction were done the digestible energy of sugarcane silages reaches the same level as the tested maize silage did.

Table 11: Energy content of sugarcane silage with and without VOC correction calculated from experimental data published by Kung, Jr. and Stanley (1982)

Herbage and stage of maturity	Gross energy kcal/g DM		Digestible energy kcal/g DM		Methane forming potential litres**/kg DM	
	not corrected	corrected*	not corrected	corrected*	not corrected	corrected
Corn	4.74	4.73	3.20	3.24	279	271
Sugarcane						
6 mo	4.92	5.04	2.83	3.14	276	251
12 mo	4.78	5.07	2.80	3.38	324	276
24 mo	4.62	4.97	2.29	3.01	321	270

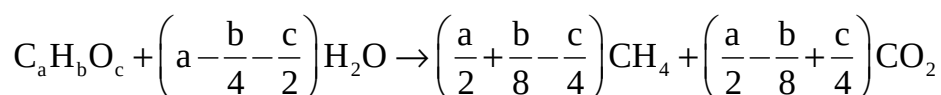
* corrected for DM and energy of VOC

** volume at standard temperature and pressure

In Table 11 also the values of the methane forming potential of these silages are given for the case that sugarcane silage is to be used as substratum for biogas production. These numbers were calculated by using a method which has been established during the last years and described (Weissbach, 2008; 2009a; 2009b; 2009c; 2009d; 2009e; 2009f; 2009e).

The method is based on the assumption that the content of true digestible OM, which was measured in sheep fed at maintenance level, normally can be used as a measure of the biogas forming potential of a crop. The true digestible OM is regarded here as the “fermentable organic matter” (FOM). From the measured contents of apparent digestible OM, 60 g per kg DM_c as metabolic faecal fraction were subtracted in order to get to the content of true digestible OM.

The contributions of the individual chemical compounds to biogas formation were calculated by using stoichiometric equations, like that of Buswell and Mueller (1952) for the nitrogen-free-compounds:



An analogous equation for N-containing compounds was formulated by Boyle (1976). For instance, the stoichiometric methane forming potential is 393 L per kg sucrose and 730 L per kg ethanol according to this equation. As the result of extensive calculations on the basis of the chemical composition of the respective crop, the following equations have been deduced for predicting the methane forming potential:

Maize silage:	MFP = 0.420 FOM
Sugarcane silage:	MFP = 0.400 FOM + 0.34 AL
Sugar beet silage:	MFP = 0.375 FOM + 0.32 AL

where MFP is the “methane forming potential” [litres/kg DM_c], FOM is the fermentable organic matter [g/kg DM_c] and AL is the sum of all alcohols [g/kg DM_c]. The MFP is given as gas volume at standard temperature and pressure.

As can be seen in the last column of Table 11, MFP is similar between all 4 tested silages (approximately 270 Liter methane per kg DM_c). It can be concluded from this finding that fermented sugarcane produces as much methane as maize silage, and that this does not depend on the extent of conversion of sugar into ethanol.

However, if MFP was related to the non-corrected DM (see second last column in Table 11), then the results were markedly biased and indicated an seeming increase in MFP by 19% due to increasing sugar transformation into ethanol. This seeming increase has been observed in many laboratory batch trials on biogas production in which correction of DM for the loss of VOC was not carried out. It is easy to recognize that DM correction is also a crucial pre-requirement in the evaluation of biogas yield and the possibilities to influence it.

A recently published microbiological study has attracted significant attention. This article described the capability of certain *Lactobacillus buchneri* strains of producing ferulate esterase (Nserenko *et al.*, 2007), thereby generating hope to be able to increase cell wall digestibility of maize and grass silages by the use of these bacteria as inoculant. Other scientists also focused on this topic (Berzahi, 2009; Brüsemeister *et al.*, 2009a; Brüsemeister *et al.*, 2009b; Kung, 2009; Nussio *et al.*, 2009; Santos *et al.*, 2009; Spielbauer *et al.*, 2009). Summarizing the results it must be stated that the expected effect on digestibility of NDF was inconsistent and its extent marginal. Also a colonizing within the digestive tract when inoculated silage was fed could not be found (Harman *et al.*, 2009). However, the already well known positive effects of *L. buchneri* on aerobic stability have been confirmed also for these strains.

Another study aimed at improving biogas yield of grass and maize silage by using a favored *L. buchneri* strain (Ruser *et al.*, 2009). As could be seen in table 11, the effects on gas yield primarily depend on if and how comprehensive DM was corrected for the loss of VOC during drying. In order to be able to undoubtedly relate the higher biogas yield to improved cell wall digestibility, it should be excluded that the observed differences were caused by differences in fermentation pattern of the tested silages. This, in turn, renders the full analysis of the silages for all relevant VOC and the subsequent correction of DM. Pronounced improvements in biogas yield by inoculation of silages with *L. buchneri* strains can already be explained entirely by increasing aerobic stability, which reduces aerobic biomass losses. This has been demonstrated in numerous trials (Banemann *et al.*, 2009).

As shown above, it is well possible to produce valuable silages from sugarcane with low energy losses, to be used in the feeding of ruminants and as substratum for biogas production. However, the question of how to design the ensiling technology and procedures for each specific use has not been answered yet, and remains to be addressed in further research activities. Also in this regard, high expectations and hopes were expressed about the potential and commercial use of *L. buchneri*-type inoculants (Nussio *et al.*, 2009a and 2009b; Muraro *et al.*, 2009) to inhibit the growth of yeasts and ethanolic fermentation. Novel bacteria strains have been searched and tested for, which would eventually exert a specific suppressing effect on yeasts (Avila *et al.*, 2009; Pinto *et al.*, 2009) or whose antifungal effect could be attained especially in certain sugarcane varieties. All these attempts have not demonstrated promising results, and even more importantly, the basic problem of excessive WSC surplus in this crop could not be solved, which was likely to be expected.

It appears meaningful, if at all possible, to use the existing body of research data and re-evaluate them after DM correction for the loss of VOC during drying. Furthermore, additional studies are to be carried out which include the determination of all relevant silage parameters. Based on these experiences, novel concepts regarding technological developments can be proposed, thoroughly tested and, finally, implemented. Many questions still remain open, and the answers to them can only be given by specifically-designed trials. These questions are:

- How does untreated sugarcane silage behave under practical conditions if it is produced by employing technologically best ensiling measures (chop length and quality, compaction, air exclusion) as is done with maize?
- How is the aerobic stability of such sugarcane silage after sufficiently long fermentation and storage length (e.g. 6 months)?
- How is the acceptance and feed intake of such sugarcane silage by animals despite high ethanol concentrations?
- Does the use of *L. buchneri*-type inoculants really lead to benefits, how much acetic acid is produced during extended periods of storage and how does this affect feed intake?
- Are there any advantages of using antimycotic chemical additives that preserve the sugar in sugarcane silage and concurrently ensure high aerobic stability, and do positive effects pay for the cost of this type of additive?

It also needs to be asked as to whether energy production from whole-crop sugarcane does have an economically chance in the future. In comparison with the use of sugarcane for bioethanol production not only the sucrose yield would be utilized but the total plant biomass as

far as fermentable. Which requirements would then need to be met by the sugarcane silage, and which ensiling technology offers the most profitable solution?

Not very long ago, the use of sugar beet as substratum for biogas production was initiated in Germany (Wagner *et al.*, 2009). Also this crop cannot be stored without special technologies and must be ensiled if it is intended to use it continuously throughout the year. The best technology has not been determined yet, and different options are currently in discussion and testing. Mainly two concepts are followed.

The first preservation option is to crush the sugar beets and let them ferment spontaneously and uncontrolled. We do not see any constraints to the unavoidably occurring conversion of sucrose into ethanol by yeasts during extended fermentation periods since the MFP of the sucrose is almost fully retained in the fermentation end-product ethanol. However, there is still the challenge of providing cost-efficient inventory capacity for anaerobic storage. The disadvantage of sugar beets, if compared with sugarcane, is the production of excessive volumes of effluent, which further increases the costs for storage capacity. One solution to this problem may be the storage of whole beets in plastic tubes. Whole beets release significantly less effluent than do crushed beets. The needed processing of the whole beet for biogas production will be done just prior to the feeding into the biogas fermenter. The second option is that of chemical preservation of whole or crushed beets. The use of inoculants we do not consider for a viable approach in the preservation of such sugar-rich plant biomass.

Exemplarily, Table 12 and 13 shows the results of a study on the effects of a chemical additive on fermentation pattern, fermentation losses and biogas production parameters (Thaysen and Auerbach, 2011).

Table 12: Effect of a chemical additive on silage fermentation in sugar beet

(Thaysen and Auerbach, 2010)

	DMc %	g/kg DMc				FOM g/kg DMc	MFP L/kg DMc
		Sugar	Lactic acid	Acetic acid	Ethanol		
Fresh beet	25.0	732			0		352
Silage from whole beet							
Control	20.7	289	77	28	162	920	403
Benzoate/Sorbate*	24.0	641	24	21	12	927	352
Silage from crushed beet							
Control	21.2	271	78	31	171	912	402
Benzoate/Sorbate*	24.2	455	66	40	9	924	350

* Liquid preparation containing sodium benzoate and potassium sorbate

DMc = DM corrected for volatile organic compounds

MFP = methane forming potential

Untreated sugar beet markedly lost DM due to the degradation of sugar. However, the recovered DM had a substantially higher MFP since ethanol levels were elevated. In the contrary, in the treated sugar beet silage ethanolic fermentation was almost completely suppressed, thereby maintaining most of the sugars.

Table 13: Effect of a chemical additive on fermentation losses in ensiling sugar beet (Thaysen and Auerbach, 2010)

Treatment	Losses during fermentation, %			
	DMc		MFP	
	Mean	SD	Mean	SD
Ensiling of whole beet				
Control	21.0	8.1	9.0	7.5
Benzoate/Sorbate*	7.6	4.3	7.1	4.8
Ensiling of crushed beet				
Control	20.3	3.4	8.3	2.9
Benzoate/Sorbate*	6.4	1.3	5.7	0.9

* Liquid preparation containing sodium benzoate and potassium sorbate

DMc = DM corrected for volatile organic compounds

MFP = methane forming potential

Fermentation losses were determined by balancing input and output of corrected DM and by balancing the MFP of fresh beet and beet silage. MFP was calculated from the contents of FOM in fresh beet and in beet silage considering the ethanol content of the silages and using the equation shown above.

The DM losses which were mainly caused by CO₂ release during ethanolic fermentation amounted to more than 20 % in untreated silages and was substantially reduced by the chemical additive. Because of high energy content of the ethanol the losses of MFP were much lower and only little decreased by additive treatment. From this results, it can be stated that regarding recovery of energy both options of ensiling crops with high sugar contents can be accepted.

Conclusions

Forage conservation in general and silage production in particular presents an extremely important topic of high actual priority. Sufficient feeding of the world's growing human population in all regions requires the best possible productive use of agricultural land resources, the reduction of losses of grown biomass and its highly efficient utilization.

This holds true for all regions of the world, but in emerging countries contributing significantly to the global agricultural production, there are huge possibilities to increasing productivity, which still have not been turned into reality. In many developing countries, efficient subsistence farming structures must be set up which are adapted to the socio-economic conditions.

All this is not possible without keeping ruminants. How many animals are needed and how much of climate-damaging methane is emitted depends on the performance level of the animals, which, in turn, is affected by the quality of feed inventory management.

A novel challenge to the agricultural industry is posed by the increasing use of plant biomass as renewable energy source and there demand for substratum supply. Also for this purpose, low-loss conservation and subsequent storage of biomass is crucial.

Consequently, forage production and conservation must be increased and improved by employing suitable technologies. Further research is to be carried out on silage production. This applies particularly to tropical and subtropical regions, in which silage production has not been used so far to the possible and needed extent, respectively.

An intensified international exchange of opinions and experiences in forage conservation is fruitful and useful, but the direct transfer of technologies from temperate climates seems limited. Solutions leading to their broad use under practical conditions must be developed for specific conditions, and probably for specific countries. This ultimately creates the demand for systematic research and extension programs on forage conservation and their financial support.

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Genotypic differences in maize phenology, growth and biomass “hybrid” vs. “local cultivars” applied with variable rates of different n-fertilizer sources

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Introduction Maize (*Zea mays* L.) is the second most important crop after wheat in the North West Frontier Province (Khyber Pakhtunkhwa) of Pakistan but its yield per unit area is very low (Amanullah et al., 2009). Nitrogen is one of the most important factors affecting maize growth, phenology and grain yield (Amanullah et al., 2009), leaf area and biomass, grain quality (Amanullah and Shah, 2011) and net returns (Amanullah et al., 2010) in Northwest Pakistan.

Materials and Methods The objective of this experiment was to investigate the response of different maize genotypes [local high yielding cultivars (Azam and Jalal) vs. hybrid (Pioneer-3025)] to variable rates (50, 100, 150 and 200 kg N ha⁻¹) and sources [urea, calcium ammonium nitrate (CAN) and ammonium sulphate (AS)] of N in comparison to control (N not applied) on calcareous soils having wheat-maize cropping system for long time. A field study was conducted at the Agriculture Research Farm of Agricultural University, Peshawar, during summer 2008-2010.

Results and Discussion Nitrogen applied plots delayed physiological maturity by three and half days, produced 19 cm taller plants, 3.29 more leaves plant⁻¹, 81.17 cm² (21 %) higher leaf area, and 3,533 kg ha⁻¹ (35 %) higher biomass than the plots where N was not applied (control). Days to physiological maturity was delayed significantly with each increment in N rate, and enhanced with application of AS compared to urea and CAN. Application of CAN produced taller plants, more leaves plant⁻¹, leaf area and so higher biomass than urea and AS. Although, there was no significant difference in the biomass of CAN (10,352 kg ha⁻¹) and AS (10,062 kg ha⁻¹) applied plots, but was significantly higher than the biomass of the plots applied with urea (9,472 kg ha⁻¹). Plant height ranked first with 150 kg N ha⁻¹. Number of leaves plant⁻¹, leaf area and biomass were statistically among the two higher N rates (150 and 200 kg N ha⁻¹) and were significantly higher than the two lower rates of N (50 and 100 kg N ha⁻¹). The Pioneer-3025 hybrid was 13 days late maturing, produced 36.25 cm (20 %) taller plants, 3.2 (21 %) more leaves plant⁻¹, 88 cm² (20 %) higher leaf area, and 1,939 kg ha⁻¹ (17 %) higher biomass than the average of the two local cultivars.

Conclusion It is concluded from the study that growing maize hybrid Pioneer-3025 applied with 150 or 200 kg N ha⁻¹ either as CAN or urea could result in higher maize productivity and profitability in the studied area.

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Table 1. Preplanned comparison in physiological maturity (PM), plant height (PH), number of leaves plant⁻¹ (LPP), mean single leaf area (MSLA) and biomass of maize as influenced by control (N not applied) vs. rest (N applied)

Control vs. Rest	PM (days)	PH (cm)	LPP	MSLA (cm ²)	Biomass (kg ha ⁻¹)
Control	87.67	146.11	10.11	298.89	6,429
Rest	91.14	164.97	13.40	380.06	9,962
Difference	3.47	18.86	3.29	81.17	3,533

Table 2. Days to physiological maturity (PM), plant height (PH), number of leaves plant⁻¹ (LPP), mean single leaf area (MSLA) and biomass of maize as influenced by different sources of nitrogen

N Sources	PM (days)	PH (cm)	LPP	MSLA (cm ²)	Biomass (kg ha ⁻¹)
Urea	91.61	162.28	13.11	375.33	9,472
CAN	91.78	167.89	13.89	400.56	10,352
AS	90.03	164.75	13.19	364.28	10,062
LSD _{0.05}	0.44	2.60	0.37	20.34	395

Table 3. Days to physiological maturity (PM), plant height (PH), number of leaves plant⁻¹ (LPP), mean single leaf area (MSLA) and biomass of maize as influenced by variable rates of nitrogen

N rates (kg ha ⁻¹)	PM (days)	PH (cm)	LPP	MSLA (cm ²)	Biomass (kg ha ⁻¹)
50	88.37	158.81	11.78	353.15	8,575
100	90.37	162.33	13.00	335.11	9,883
150	92.07	171.96	14.48	418.37	10,543
200	93.74	166.78	14.33	413.59	10,846
LSD _{0.05}	0.51	3.00	0.43	23.48	456

Table 4. Differences in days to physiological maturity (PM), plant height (PH), number of leaves plant⁻¹ (LPP), mean single leaf area (MSLA) and biomass of maize genotypes

Genotypes	PM (days)	PH (cm)	LPP	MSLA (cm ²)	Biomass (kg ha ⁻¹)
Azam	85.86	153.56	12.31	349.03	8,980
Jalal	87.83	152.22	12.36	352.42	9,651
Pioneer-3025	99.72	189.14	15.53	438.72	11,254
LSD _{0.05}	0.51	3.59	0.38	24.18	237

Soybean silage degradability – effects of harvesting stage, inoculant and varieties

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Introduction The soybean silage has more than two times the protein content than others silages species what makes it an interesting alternative to reduce the use of protein concentrate on ration and, in this way, reduce total diet costs (Pereira et al., 2009). The variety and harvesting stage of the forage and inoculant used can affect fermentation in the silo, modifying the final composition and nutrient availability of silage. Diets systems formulations for ruminants are based on knowledge of the degradability and degradation rates of protein fraction. The objective of this work was to determine the dry matter degradability and protein on soybean silage with varieties harvested in different stages of growth.

Materials and Methods The trial was carried out in Ilha Solteira, SP, Brazil (22°22'S, 51°22'W) and the treatments consisted of three factors: varieties (A7002 and conquista); harvesting stages (R6 and R7) and bacterial additives (with or without) with *Pediococcus acidilactici* and *Lactobacillus plantarum* (Kera-Sil) in base of 4g/ton forage. The forage was chopped to a particle size between 3 and 6 mm. The silos used were plastic buckets (15 kg). After 60 days storage silos were sampled, dried and ground (2 mm). Samples of 5g were placed in nylon bags (6.5x13cm) and incubated in the rumen of five steers for 0, 2, 4, 8, 16, 24, 48, 72 and 96 h. The residues were washed, dried and analyzed for dry matter (DM) and crude protein (CP). In addition, analysis for ruminal kinetics parameters (a, b, c, and effective degradability (ED)) were made. These data were used in the Orskov & McDonald (1979) models potential degradation ($PD = a + b(1 - e^{-ct})$) and $ED = a + (b \cdot c / c + k)$.

Results and Discussion It was observed effect of harvesting stage, variety and interaction between stage x variety and variety x inoculant in most of evaluated parameters (Table 1). In Table 2, the R7 stage had higher values of the fractions a, b, c and ED. There was no effect of inoculant for all measured parameters. For varieties, the A7002 had the highest value for a fraction and ED, while the fractions b and c had no difference between varieties. For CP there were detected differences in fraction b and ED, with R7 having the highest value (Table 2). There was no effect of inoculant on CP. The variety A7002 provided the greatest value in fraction a and c, and the lowest in b for CP (Table 2). For the ED of CP, the variety A7002 showed the highest value, in accordance with Bello-Pérez et al. (2008). Favorable results at R7 stage can be due to the higher proportion of grains in the more mature plant. In general the R7 stage and variety A7002 provided better results in all analyzed parameters.

Conclusion Soybeans silage harvested at R7 stage provided more effective degradability of DM and CP. The A7002 variety is more indicated to ensiling. The addition of inoculants does not seem to improve the degradability.

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Table 1. General mean, treatments effects and interactions, in the parameters a, b (%), c (% /h) and ED (%) of the dry matter (DM) and crude protein (CP) of the silages

Item (%)	Mean	Effects			Interactions			
		Stage	Inoculant	Variety	ExI	ExV	IxV	ExIxV
DM a	24.04	**	NS	*	NS	**	*	NS
b	37.67	**	NS	NS	NS	**	NS	NS
c	7.02	**	NS	*	NS	NS	NS	NS
ED	45.85	**	NS	**	*	**	**	NS
CP a	62.17	NS	NS	**	NS	NS	NS	NS
b	24.26	**	NS	*	NS	**	NS	NS
c	10.15	NS	NS	NS	NS	NS	NS	NS
ED	77.08	**	NS	**	NS	NS	NS	NS

NS P > 0.05; *P < 0.05; **P < 0.01.

Table 2. Degradation parameters (a, b, c and ED) of dry matter (DM) and crude protein (CP) of soybean silage due to the harvesting stage, inoculant and variety

Item (%)	Stage		Inoculant		Varieties	
	R6	R7	Without	With	A7002	Conquista
DM a	22.90b	25.10a	24.06a	24.10a	25.50a	22.60b
b	36.40b	38.94a	37.61a	37.73a	37.98a	37.37a
c	6.42b	7.63a	6.95a	7.09a	7.37a	6.67a
ED	43.30b	48.40a	45.80a	45.90a	47.90a	43.80b
CP a	62.35a	61.99a	62.89a	61.47a	66.30a	58.04b
b	20.74b	27.78a	23.01a	25.51a	22.36b	26.16a
c	8.39a	11.92a	9.16a	11.15a	10.42a	9.89a
ED	73.42b	80.74a	77.04a	77.12a	79.85a	74.31b

Bacterial community of direct-cut and wilted guinea grass silage stored with and without molasses

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Introduction Tropical grass ensiling may result in large amounts of acetic acid rather than lactic acid. Even if lactic acid is prevalent in the beginning, the level of lactic acid may decrease and that of acetic acid will increase when ensiling is prolonged. Information is still insufficient on the bacterial community of tropical grass ensiling, and microorganisms associated with acetic acid fermentation have not been identified. In this study, guinea grass was ensiled immediately after harvest or after sun light wilting to prepare high-moisture tropical grass silage. Molasses was added to obtain various fermentation patterns, and changes in the bacterial community were assessed by denaturing gradient gel electrophoresis.

Materials and Methods The first growth of guinea grass was harvested in the late heading stage, on August 4th, 2009. The grass was chopped using a forage cutter at a theoretical length of 13 mm immediately after harvest or after field wilting for about 6 h. The pre-ensiled materials were added with and without molasses (1.0% fresh basis), and stored in a plastic pouch at ambient temperature for 14, 28, and 56 days. Microbial counts, fermentation products, and bacterial community were determined.

Results and Discussion Intensive acetic acid fermentation occurred in untreated direct-cut silage; the lactic acid content was only 0.39 %DM and the acetic acid content was as high as 5.41 %DM at day 14 (Table 1). The acetic acid level remained high during ensiling, and the silage pH remained above 6.0 even at day 56. Marked increases were seen in the butyric acid, ethanol and NH₃-N content due to prolonged ensiling. Addition of molasses increased the lactic acid content and decreased the propionic acid, ethanol and NH₃-N content. Butyric acid was not detectable in direct-cut silage to which molasses had been added, whereas about 5.0 %DM of acetic acid was detected from day 14 onwards.

Wilting enhanced the lactic acid production even without adding molasses (Table 2). Lactic acid was a major product in untreated wilted silage. The lactic acid content decreased and the acetic acid content increased when ensiling was prolonged, and the levels of these acids were comparable at day 56. In addition, the ethanol content decreased and the NH₃-N content increased due to prolonged ensiling. Both the lactic and acetic acid contents were high in wilted silage to which molasses had been added; hence, the lactic to acetic acid ratio was unaffected by molasses addition. Similar to untreated wilted silage, prolonged ensiling decreased the lactic acid content, ethanol content and lactic to acetic acid ratio, and increased the NH₃-N content.

Although bands for various enterobacteria, such as *Pantoea agglomerans*, *Acinetobacter* sp., and *Pantoea ananatis*, were found in direct-cut and wilted materials, bands for these species were not detectable after ensiling (Figure 1). Distinctive bands indicative of *Morganella morganii*, *Lactobacillus plantarum*, *Enterococcus* sp., *Pantoea* sp., *Lactococcus lactis*, and *Enterococcus faecium* were found at day 14 in untreated direct-cut silage. At days 28 and 56, prominent bands for *Morganella morganii* and *Clostridium botulinum* were also observed. In direct-cut silage to which molasses had been added, bands indicative of *Pediococcus pentosaceus* were clearly seen, whereas those indicative of *E. faecium* were not observed. The DGGE patterns for untreated wilted silage were similar to those for direct-cut silage to which molasses had been added;

however, bands indicative of *M. morganii* and *Pantoea* sp. were faint in untreated wilted silage. A distinctive band for *Lactococcus garvieae* was seen in wilted silage to which molasses had been added.

Table 1 Microbial counts and fermentation products of direct-cut guinea grass silage

	Direct-cut + untreated			SE	Direct-cut + molasses			SE	ANOVA		
	14 d	28 d	56 d		14 d	28 d	56 d		S	M	SxM
Dry matter (%)	13.9	13.2	13.2	0.60	15.4	15.3	15.3	0.36	NS	**	NS
pH	6.28	6.67	6.06	0.13	5.06	4.89	4.75	0.06	**	**	*
Lactic acid (%DM)	0.39	0.12	0.01	0.07	3.24	3.72	3.09	0.23	NS	**	NS
Acetic acid (%DM)	5.41	5.14	5.64	0.50	4.86	5.37	5.32	0.41	NS	NS	NS
Propionic acid (%DM)	0.13	0.29	0.34	0.06	0.00	0.14	0.13	0.01	**	**	NS
Butyric acid (%DM)	0.13	1.20	1.65	0.39	0.00	0.00	0.00	0.00	*	**	*
Ethanol (%DM)	1.01	2.90	4.19	0.46	0.64	0.83	0.90	0.16	**	**	**
NH ₃ -N (%DM)	0.34	0.47	0.55	0.05	0.27	0.30	0.25	0.03	*	**	*

S, M, and SxM indicate effect of storage period, molasses addition, and their interaction, respectively.

Table 2 Microbial counts and fermentation products of wilted guinea grass silage

	Wilted + untreated			SE	Wilted + molasses			SE	ANOVA		
	14 d	28 d	56 d		14 d	28 d	56 d		S	M	SxM
Dry matter (%)	23.5	22.5	25.0	1.27	20.5	21.1	21.4	0.61	NS	**	NS
pH	4.90	4.99	4.87	0.05	4.44	4.61	4.67	0.07	NS	**	NS
Lactic acid (%DM)	3.62	3.13	2.84	0.25	5.30	4.75	4.11	0.17	**	**	NS
Acetic acid (%DM)	1.19	2.67	3.08	0.20	2.10	3.19	3.91	0.28	**	**	NS
Propionic acid (%DM)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	---	---	---
Butyric acid (%DM)	0.00	0.00	0.06	0.02	0.00	0.00	0.00	0.00	NS	NS	NS
Ethanol (%DM)	0.58	0.40	0.29	0.07	0.64	0.33	0.33	0.07	**	NS	NS
NH ₃ -N (%DM)	0.10	0.16	0.17	0.02	0.12	0.16	0.21	0.03	**	NS	NS

S, M, and SxM indicate effect of storage period, molasses addition, and their interaction, respectively.

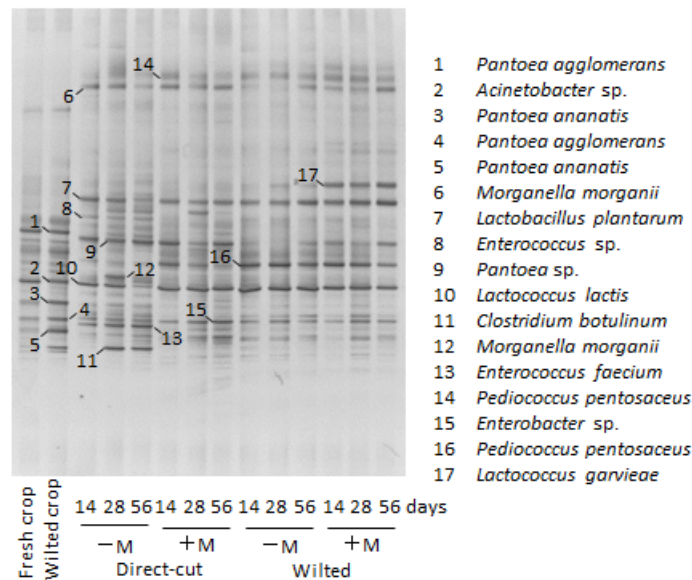


Figure 1 Bacterial community of direct-cut and wilted guinea grass silage stored with and without molasses (M) for 14, 28, and 56 days.

Effect of inoculation of *Acetobacter pasteurianus* at ensiling and after silo opening on fermentation products, aerobic stability, and bacterial community of whole crop corn silage

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Introduction Acetic acid bacteria are obligate aerobes and obtain energy by oxidizing ethanol to acetic acid or oxidizing lactic acid to acetic acid and carbon dioxide. In a survey of bacterial community of bunker-made corn silage, we isolated *Acetobacter pasteurianus* as dominant bacteria grown in Man, Rogosa, Sharpe agar plates. The silage was not spoiled at the time of sampling, although acetic acid bacteria are known to initiate deterioration in the presence of air. To examine how *A. pasteurianus* was involved in fermentation and aerobic spoilage, inoculation experiments were made using the isolate.

Materials and Methods Whole crop corn was harvested at the half milk-line stage on 17 September 2010. Finely chopped crop (300 g) was packed in plastic pouches, and stored at ambient temperature with and without *A. pasteurianus* (10^5 cfu/g) inoculation. Triplicate silages were opened at 2 months after ensiling, and fermentation products and bacterial community were determined at silo opening and after 7-day aerobic deterioration test. Untreated silage was also opened after storage for 6 months, and then added with and without *A. pasteurianus* (10^6 cfu/g) prior to the 7-day aerobic deterioration test. Bacterial community was determined by denaturing gradient gel electrophoresis, and species identification was made based on the partial 16S rRNA gene sequence (DGGE). Presence of *A. pasteurianus* was also evaluated by amplifying PQQ-dependent alcohol dehydrogenase gene.

Results and Discussion Inoculation of *A. pasteurianus* at ensiling increased silage pH, while not affecting lactic acid and acetic acid contents (Table 1). Aerobic stability was improved by *A. pasteurianus* addition; heating was delayed for 2 days and lower pH than untreated control was observed after aerobic deterioration test. Bacterial community was similar between control and *A. pasteurianus*-inoculated silage, and *Lactobacillus plantarum* and *Lactococcus lactis* were found as 2 representative bacteria (Figure 1a). Improved aerobic stability was reflected in bacterial community; although *Bacillus pumilus* appeared upon spoilage in control silage, marked changes were not seen before and after aerobic deterioration test in *A. pasteurianus*-inoculated silage. The band indicative of *A. pasteurianus*, however, was not detectable in any silage, suggesting that *A. pasteurianus* did not survive the ensiling process and was not directly involved in improved aerobic stability.

When *A. pasteurianus* was inoculated to untreated silage after silo opening, spoilage occurred without heating and the lactic acid and acetic acid contents decreased. Spoilage was not accelerated by addition of *A. pasteurianus*, although the band indicative of *A. pasteurianus* became distinctive during the process of aerobic deterioration (Figure 1b). Presence of *A. pasteurianus* in DGGE gel agreed well with detection of PQQ-dependent alcohol dehydrogenase gene in the silage (Figure 2).

Conclusion These findings suggest that *A. pasteurianus* can be a follow-up or an opportunistic bacterium after silo opening, and its contribution to initiating aerobic deterioration may be small compared with that previously reported.

Table 1 Chemical and microbial composition at silo opening and after 7-day aerobic deterioration test of whole crop corn silage inoculated with and without *Acetobacter pasteurianus* (AP) at ensiling

Item	At silo opening			After aerobic stability test		
	Control	AP	SE	Control	AP	SE
Dry matter (%)	41.8	43.3	0.24	45.9	51.1	0.23
pH	3.68 b	3.74 a	0.01	5.98 x	4.94 y	0.09
Lactic acid (%DM)	3.96	4.77	0.66	0.61	1.03	0.12
Acetic acid (%DM)	0.72	0.76	0.08	0.09 x	0.01 y	0.01
Ethanol (%DM)	1.54	1.49	0.18	0.00 y	0.03 x	0.01
Lactic acid bacteria (log cfu/g)	6.10	6.20	0.07	8.89	8.71	0.12
Yeasts (log cfu/g)	5.90	6.30	0.13	9.05	9.08	0.07

Values within the same row with unlike following letter are different ($P < 0.05$).

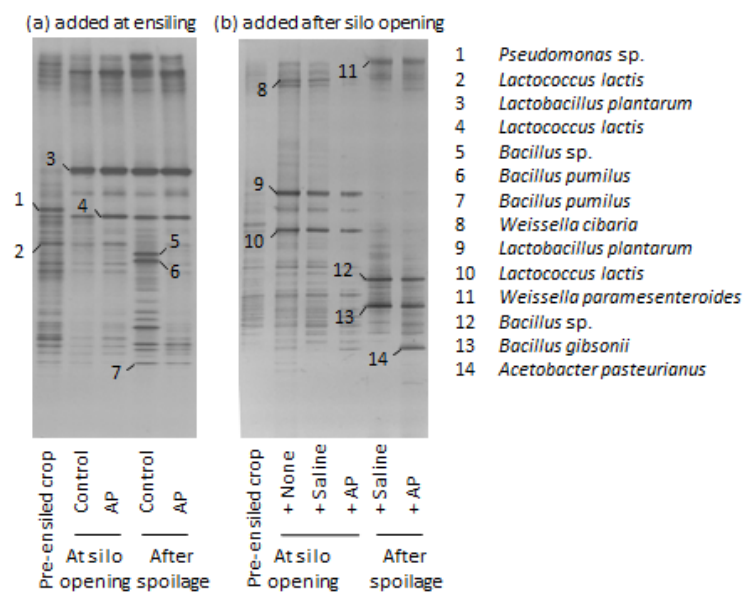


Figure 1 Bacterial community at silo opening and after 7-day aerobic deterioration test of whole crop corn silage inoculated with and without *Acetobacter pasteurianus*. Inoculation was made at ensiling (a) or after silo opening of untreated silage (b).

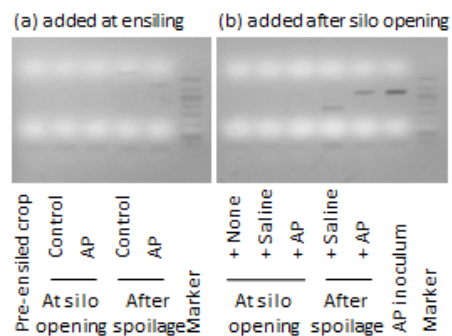


Figure 2 Determination of PQQ-dependent alcohol dehydrogenase gene at silo opening and after 7-day aerobic deterioration test of whole crop corn silage inoculated with and without *Acetobacter pasteurianus*. Inoculation was made at ensiling (a) or after silo opening of untreated silage (b).

Effects of bacterial and enzyme inoculation on the fermentation quality of ensiled whole crop sweet sorghum

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Introduction Although sweet sorghum (*Sorghum bicolor* L. Moench) is usually grown for the production of fuel in many developed countries, it can be used as forage for livestock production. For improving the feed quality of whole crop sweet sorghum (WCSS), ensiling is one of important methods. This method includes the use of bacterial inoculants to improve the fermentation dynamics of ensiled forages by accelerating the decline in pH and improving dry matter (DM) and nutrient retention (Muck, 2010). However, the effects of bacterial inoculants on fibre degradation is not consistent and lactic acid bacteria (LAB) cannot use fibre as an energy source to make lactic acid, and enzymes are added to silage for this reason (Xing et al., 2009). In addition, their effects on aerobic stability of silage are inconsistent due to low levels of acetic acids. As a result, *Lactobacillus buchneri* (LB) has been used to improve the aerobic stability of silage. The present study aimed to evaluate the effect of LB in combination with an enzyme on the fermentation and aerobic stability of ensiled WCSS.

Materials and Methods The WCSS (345g DM/kg, 5.6 pH, 255g WSC/kg DM, 38g CP/kg DM, 439 g NDF/kg DM and 282g ADF/kg DM) was treated with: no inoculant (control), *Lactobacillus buchneri* (LB), *Lactobacillus plantarum* (LP), and a combination of LB and cellulase enzyme (LB+E). Inoculants were applied to obtain 3×10^5 CFU/g of fresh forage. Treatments were ensiled in 48 x 1 liter jars (12 jars/treatment), kept at room temperature of 24-28°C. Three jars per treatment were opened on d 0, 3, 4, 15 and 25 of ensiling, and samples were analyzed for nutrient composition and fermentation characteristics. Data was analyzed by ANOVA using the mixed procedure of SAS software (SAS Institute, Inc., 1991) and significance was declared at a 5 % probability level.

Results and discussions Inoculation reduced the pH and ammonia-N of WCSS silage compared to the control (Table 1), consistent with Fellner et al., (2001), but contrasted Xing et al (2009). High ($P<0.05$) lactic acid concentration was obtained with inoculation, but was highest with LP inoculation. The concentration of acetic acid was increased ($P<0.05$) with the LB and the LB+E treatments, suggesting that the aerobic stability of the silage may be improved with LB even with the presence of an enzyme. The fibre fractions (aNDF and ADF) of the silage were reduced ($P<0.05$) with inoculation compared to the control. However, the LB+E and LP treatments were more effective than the other treatments, consistent to Nadeau et al., (2000) but contrasted Meeske et al., (1993). Inoculation with LB and LP increased ($P<0.05$) the CP content of WCSS silage compared to the other treatments.

Conclusions It was concluded that inoculation improved the nutritive value and fermentation of ensiled WCSS. Further, the addition of enzyme reduced the fibre content of silage and did not restrict the production of acetic acid in the presence of LB.

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Table 1. Effects of microbial inoculation on the nutrient composition and fermentation characteristics of sweet sorghum after 25 days of ensiling (n=9)

	Treatments				SEM	Trt	Significance (<i>P</i>)	
	Control	LB	LB+E	LP			Day	Trt X Day
Nutrient composition (% of DM unless stated otherwise)								
DM	32	30	30	30	84.5	3.01	1.22	3.31
Ash	43 ^b	46 ^a	44 ^b	42 ^b	3.3	0.001	0.001	0.019
CP	4.0 ^b	4.1 ^a	3.9 ^b	4.2 ^a	0.9	0.001	0.001	0.001
EE	1.4 ^c	1.5 ^b	1.6 ^a	1.5 ^b	0.03	0.006	0.009	0.001
aNDF	49 ^a	47 ^b	44 ^c	44 ^c	6.11	0.001	0.001	0.001
ADF	33 ^a	31 ^b	30 ^c	30 ^c	3.01	0.001	0.001	0.001
Fermentation characteristics (% of DM unless stated otherwise)								
pH	3.9 ^a	3.6 ^b	3.6 ^b	3.6 ^b	0.02	0.001	0.001	0.073
WSC	36 ^b	55 ^a	74 ^a	22 ^b	14.3	0.001	0.002	0.432
LA	48 ^c	84 ^b	84 ^b	99 ^a	26.1	0.001	0.001	0.053
AA	24 ^b	39 ^a	40 ^a	6 ^c	17.1	0.001	0.001	0.001
PA	0.04	0.05	0.06	0.33	0.1	0.185	0.510	0.525
BA	0.5 ^b	0.7 ^a	0.9 ^a	0.5 ^b	0.9	0.014	0.927	0.959
NH ₃ -N	15.1 ^a	11.2 ^b	9.6 ^b	10.2 ^b	1.38	0.001	0.016	0.233
(g/kg TN)								

^{a-c} Means with different letters in a row differ significantly ($P < 0.05$)

DM, dry matter; CP, crude protein; EE, ether extract; aNDF, amylase treated neutral detergent fibre; ADF, acid detergent fibre; WSC, water-soluble carbohydrate; LA, lactic acid, AA, acetic acid; PA, propionic acid; BA, butyric acid; NH₃-N, ammonia nitrogen

Effect of an inoculant and enzymes on fermentation quality and nutritive value of erect milkvetch (*Astragalus adsurgens* Pall.) silages

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Introduction Erect milkvetch (*Astragalus adsurgens* Pall.) is widely cultivated in diverse environments in arid and semiarid areas of northern China and it is an alternative feed source for animals. Ensiling may be an appropriate method to preserve its nutritive value. However, the small amount of WSC and high buffering capacity make it difficult to be ensiled. The objective of this study was to assess the effect of inclusion of inoculant, enzymes and their mixture during ensiling on the fermentation quality and *in vitro* degradation of erect milkvetch silage.

Materials and Methods Erect milkvetch (*Astragalus adsurgens* Pall.) for ensiling was harvested at a bud stage from experimental plots in Beijing in August. The forage was chopped into 1 to 2 cm then divided into four equal portions to subject the following treatments: (1) distilled water (Control); (2) addition of *Lactobacillus plantarum* at a rate of 0.01g/kg of fresh forage (I); (3) addition of fibrolytic enzymes at a rate of 0.033 g/kg of fresh forage (E); and (4) combination of I and E (I+E). Each treatment was mixed well and packed into plastic film bags with 200g per bag, which were sealed with a vacuum sealer and stored at ambient temperature (20 to 25°C). 15 bag silos were prepared for each treatment and three bag silos per treatment were randomly opened on days 1, 3, 5, 15, and 45 of ensiling and the content was processed for quality assessment and laboratory analysis. The 45-d erect milkvetch silage samples were used for *in vitro* digestibility determination.

Results and Discussion As shown in Figure 1, for all treated silages, the pH decreased rapidly to below 5.0 during the first 5 days of fermentation except for treatment E. However, it was found that the control silage kept a relative high pH of exceeding 5.3 during the entire periods. Furthermore, all the treated silages had a sustained lower pH than control throughout the ensiling period. In the present study, treatments of both I and I + E exhibited positive effects on silage fermentation as indicated by lower pH and higher lactic acid concentration, than control silage. This may attribute to that the LAB used can improve silage quality by proliferation of LAB and consequently growth inhibition of clostridia and aerobic bacteria. It is also expected that other than the addition of LAB, cellulase increased WSC production which could be then used by LAB for primary lactic acid fermentation. In addition, treatments of enzymes (E, I + E) significantly improved IVDMD and IVNDFD. This is consistent with results reported by other researchers (Eun and Beauchemin, 2008). Silages treated with I significantly increased IVDMD and IVCPCD (Table 1). However, Filya et al. (2007) reported that the addition of I did not show significant effects on IVDMD of alfalfa silage. From more than 60% of trials published between 1990 and 1995, Muck and Kung (1997) summarized that fermentation was improved (i.e. reduced pH, increased lactate to acetate ratio, or both) by inoculants, whereas DM digestibility (*in vitro* or *in vivo*) was increased in only 30% of the trials. Weinberg and Muck (1996), in their review, also reported instances in which fermentation was affected by a LAB inoculant, whereas digestibility was not. Treatments with enzymes (E, I + E) significantly

improved silage IVDMD and IVNDFD. In addition, treatments of I and I + E increased IVCPD ($P<0.05$) (Table 1).

Conclusion All treated silages (I, E and I + E) were well preserved as compared with control silages ($P<0.05$). Results also indicated that additives tested in our study can improve the erect milkvetch silage fermentation quality and *in vitro* digestibility to some extents.

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Table 1. Effects of additive treatments on nutrient compositions of 45-d erect milkvetch silages

Items ¹	Treatments ²				SEM ³	P
	Control	E	I	I + E		
Dry matter, g/kg	259	257	259	260	0.1	0.119
CP, g/kg DM	142 ^d	149 ^c	155 ^b	161 ^a	0.7	<0.001
aNDF, g/kg DM	467 ^a	448 ^b	451 ^b	407 ^c	2.1	<0.001
ADF, g/kg DM	315 ^a	297 ^b	303 ^b	272 ^c	1.5	<0.001
Lignin (sa) (g/kg DM)	86 ^a	76 ^b	77 ^b	74 ^b	0.5	0.006
WSC, g/kg DM	7.1 ^{ab}	7.6 ^{ab}	6.8 ^b	8.4 ^a	0.07	0.034
IVDMD, g/kg	530 ^b	588 ^a	594 ^a	602 ^a	9.3	<0.001
IVNDFD, g/kg	369 ^b	442 ^a	393 ^b	449 ^a	10.2	<0.001
IVCPD, g/kg	719 ^b	701 ^b	844 ^a	837 ^a	20.0	<0.001

¹ aNDF - neutral detergent fibre assayed with a heat stable amylase and expressed inclusive of residual ash; ADF - acid detergent fibre expressed exclusive of residual ash; Lignin (sa) - sulphuric acid lignin; CP - crude protein; WSC - water soluble carbohydrates; IVDMD - *in vitro* dry matter digestibility; IVNDFD - *in vitro* neutral detergent fibre digestibility; IVCPD - *in vitro* crude protein digestibility. ² E - enzymes; I - inoculant; I + E - inoculant + enzymes. ³ SEM - standard error of the mean, $n=3$. ^{a-d} Means in the same row with different superscripts differ ($P<0.05$).

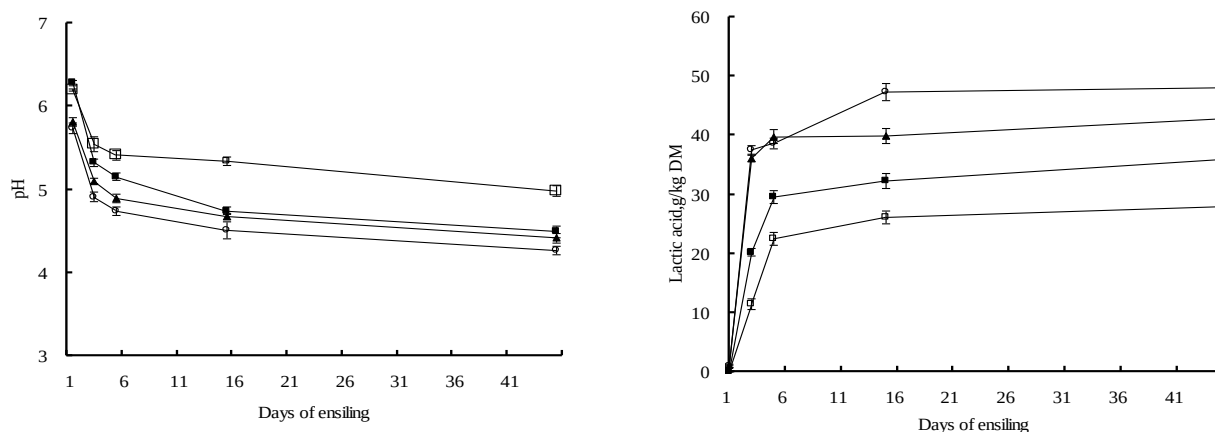


Figure 1. Changes in pH and lactic acid during ensiling of erect milkvetch treated with distilled water ($\square$), inoculant ($\blacktriangle$), enzymes ($\blacksquare$) or inoculant + enzymes ($\circ$)

Characterization and identification of lactic acid bacteria isolated from alfalfa silages

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Introduction Alfalfa (*Medicago sativa*) are now widely used for silage preparation in China. Usually, Lactic acid bacteria (LAB) present on forage crops and grass play an important role during silage fermentation and silage contains many kinds of LAB, including *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Lactococcus*, *Streptococcus* and *Weissella* (Cai et al., 1998). In this study, LAB isolated from alfalfa silages was characterized.

Materials and Methods Six alfalfa (*Medicago sativa*) cultivars were obtained from Linxi, Inner Mongolia in July 2009. Alfalfa were cut into about 2-3cm after mowing and appropriate drying, and put it into polyethylene bags about 150 g per bag, then exhaust air in the bag. LAB were separated and identified after ensiling 1, 3, 45 days. Gram stain of LAB and morphological characteristics were determined after 24h of incubation on MRS agar. Catalase activity and gas production from glucose were determined. Growth at different temperatures was detected in MRS broth after incubation at 10°C and 15°C for 14 d, and at 40°C and 45°C for 7d. Growth at pH 3.0, 3.5, 4.0, 4.5, 5.0, 7.5, 8.0 was observed in MRS broth after incubation at 37°C for 7d. Salt tolerance of LAB was tested in MRS broth containing 3.0% and 6.5% NaCl. Carbohydrate assimilation and fermentation of 49 different compounds with one control were identified on API strips (Ennahar et al., 2003).

Results and Discussion 39 strains of LAB were isolated from alfalfa silage and they were all gram-positive, catalase negative and homofermentative LAB. These strains growth well at 10°C, pH 5.0 and 7.5, and in MRS broth containing 3.0% and 6.5% NaCl. All strains grew weakly or could not grow at 5°C, 10°C, 40°C, 45°C and pH 3.0~8.0 in addition that strain GI44 grew weakly at 40°C and pH 3.5, 4.5, and could not grow at 45°C, pH 3.0, 4.0, and 8.0. These LAB isolates were divided into seven groups (A-G) according to their morphological and biochemical characters. Strains of group A and B belong to the cluster of genus *Pediococcus*. Strains of group C、D and E were identified as *Enterococcus*. Group F and G strains were placed in *Lactobacillus*.

Conclusions The results confirmed that silage contains abundant kinds of LAB, and they all have different characteristics.

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Table 1. Alfalfa cultivars and LAB strains isolated from alfalfa silage

Samples	Cultivars	Kinds	Strain Number
1	Rangelander	<i>Medicago varia</i> Martin	GI14、15、16、17
2	Algonquin	<i>Medicago sativa</i> L.	GI18、19、20、21、23、24、25
3	Rambler	<i>Medicago sativa</i> L.	GI46、47、48、49、62
4	Zhunguer	<i>Medicago sativa</i> L.	GI26、27、28、29、30、31、32、33、34、35、36
5	Gannong No.3	<i>Medicago sativa</i> L.	GI37、38、39、40、42、44、45、69
6	Aohan	<i>Medicago sativa</i> L.	GI6、7、9、10、11、12

Table 2. Characteristics of representative strain of each group from alfalfa silage

Characteristics	Group A	Group B	Group C	Group D	Group E	Group F	GroupG
	GI36	GI69	GI6	GI30	GI14	GI62	GI44
Shape	Coccus	Coccus	Coccus	Coccus	Coccus	Coccus	Rod
Gram stain	+	+	+	+	+	+	+
Catalase activity	—	—	—	—	—	—	—
Fermentation type	Homo	Homo	Homo	Homo	Homo	Homo	Homo
5℃	—	+	—	+	+	+	+
10℃	+	+	+	+	+	+	+
40℃	+	+	+	+	+	+	w
45℃	+	+	+	+	+	+	—
3.0% NaCl	+	+	+	+	+	+	+
6.5% NaCl	+	+	+	+	+	+	+
PH3.0	w	—	w	w	w	w	—
PH3.5	w	w	w	+	+	+	w
PH4.0	—	+	w	+	+	+	—
PH4.5	+	+	w	+	+	+	w
PH5.0	+	+	+	+	+	+	+
PH7.5	+	+	+	+	+	+	+
PH8.0	+	+	+	+	+	+	—

+: positive reaction, -: negative reaction, w: weakly positive reaction.

Diversity of prokaryotes associated with sugarcane silage treated with chemical and biological additives using PCR-DGGE

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Introduction The calcium oxide promotes a reduction in levels of fiber in sugar cane silage, which improves the nutritional quality (Santos et al., 2008). However silages treated with calcium oxide have a high final pH value, which might promote the growth of undesirable microorganisms. The association between calcium oxide and other additives that can induce rapidly decrease in sugar cane silage pH value could be interesting to reduce the fiber content without the risk of undesirable fermentation. In this sense, the use of microbial inoculants or weak organic acids may be an option to increase the speed and amplitude of the drop in pH of silage treated with calcium oxide. The PCR-DGGE (Electrophoresis in denaturing gradient gel) allows analyzing PCR products, according to their nucleotide sequences and size of products and has been widely used in studies of microbial communities in diverse environments. The objective of this study was to evaluate, using molecular techniques, the diversity of prokaryotes in sugar cane silage treated with selected *Lactobacillus* sp., calcium oxide, and propionic acid

Materials and Methods As experimental silos were used plastic buckets (20L). The additives tested were: calcium oxide (quicklime), propionic acid, both at a dosage of 1% based on fresh forage mass (FF) and a wild strain of *Lactobacillus* sp., (8.2 log cfu/kg forage) previously isolated from sugar cane silage (Ávila et al., 2009). Combinations of these additives were also evaluated and the silos were opened after 170 days of storage. To assess the diversity of prokaryotes were prepared samples of inoculant *Lactobacillus* sp. (UFLA SIL72) (A); of fresh sugar cane (B); of sugar cane silage without additives (control) (C); with microbial inoculant (D), with propionic acid (E), with calcium oxide (F), with the combination of the inoculant and propionic acid (G), with the combination of the inoculant and calcium oxide (H), with the combination of the propionic acid and calcium oxide (I) and with the combination of three additives (J). Total DNA was extracted from samples using the kit PSP Spin Stool DNA (Invitec ®) and for the amplification of the same the 518r and 338f primers of rDNA V3 region were used. Was used for PCR Master Mix kit (Quiagen) and 4 mL of DNA template. The amplicons were separated by DGGE (BioRad Universal Dcode Mutation Detection System, USA) in gel 8% polyacrylamide with denaturing gradient from 20 to 50%. Electrophoresis was performed in a constant voltage of 85 V for 16 h with a constant temperature of 60 ° C. The amplicons were excised, purified, re-amplified and sequenced.

Results and Discussion The results showed a heterogeneous profile of the samples analyzed. It was found eleven different bacteria species, and the respective bands excised: 1, 7, 17= *Lactobacillus hilgardii*; 2, 8 = *L. diolivorans*; 3, 10 = *L. parabuchneri*; 4= *L. acetotolerans*; 5, 6, 11, 20, 21= *L. kefir*; 9= *L. brevis*; 10= *L. parabuchneri*; 12= *L. rhamnosus*; 13, 14, 18, 23= uncultivable microorganism; 15= *L. amylophilus*; 16, 22= *Proteus mirabilis*; 19= *L. parafarraginis*. The silage treated only with calcium oxide showed the highest number of amplicons, suggesting greater bacterial diversity in this silage. This treatment may have favored the growth of microorganisms due to the buffering of acids produced during fermentation

(Klosterman et al., 1960). The sample of fresh forage showed no amplicon, probably due to the limited number of copies of the 16S rRNA in the sample, might due to low number of cells. The bacteria (A) inoculated on silage, amplicons numbered as 1, 2, 7, 8 and 17, remained during whole fermentation, as observed in the samples with inoculant (D) and with association of inoculant and calcium oxide (H). In silage where both *Lactobacillus* sp and propionic acid were added (samples G and I) the amplicons were not observed. Probably the addition of propionic acid had a negative effect on the bacteria. The presence of amplicons of non-cultivable microorganisms (13, 14, 18 and 23) showed the necessity of more detailed studies about the diversity of microorganisms associated with sugar cane silage.

Conclusion The PCR-DGGE technique was able to differentiate the bacterial profile of different samples of silage, therefore it is a good tool for monitoring bacteria species during the evaluation of inoculants in silage.

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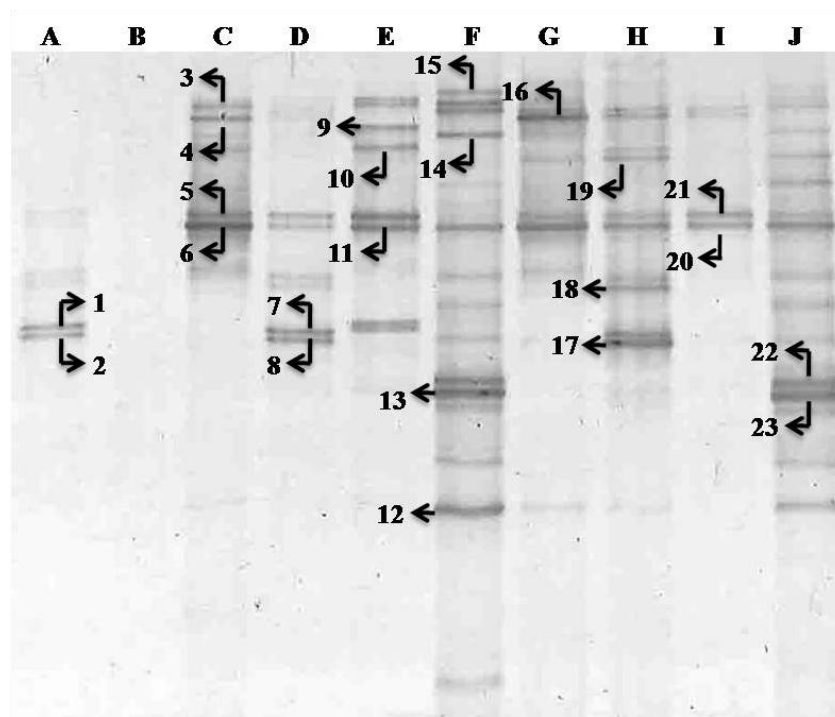


Figure 1 Bacterial communities associated with sugar cane silage treated with additives.

Influence of wilting and additives on the quality and safety of *Festulolium pabulare* silages

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Introduction *Festulolium pabulare* is a hybrid from the crossing of *Festuca arundinacea* and *Lolium multiflorum* (Wacker et al., 1984), thanks to *Festuca arundinacea* is resistant to fungal diseases and dry weather conditions (Holubek et al., 2007). Thanks to the suitable selection of lactic acid bacteria, biological inoculants have a beneficial effect on the improvement of the fermentation quality of silages (Jatkauskas et al., 2010). Prerequisite for high-quality silage are not only the bacteria of lactic fermentation but also a clean and healthy phytomass (Holubek et al., 2007). Development of microscopic fungi may lead to the formation of mycotoxins (Opitz von Boberfeld et al., 2006).

Material and methods The experiment was carried out in triplicates. The cut of *Festulolium pabulare* (cv. Felina) was end of May (stage of earing). The plots (1.5 x 10 m) were harvested by the self-propelled mowing machine. Stubble height was 0.07 m. The assessed grasses were wilted 24 hours (DM 40 %) resp. 12 hours (DM 20 %). Use preservatives were probiotic inoculant (*Enterococcus faecium*, *Lactobacillus lactis*, *Pediococcus pentosaceus*, *Lactobacillus plantarum*, *Lactobacillus casei*), (30×10^9 CFU; 5 g t⁻¹), probioenzymatic inoculant 1 (the same bacteria and cellulase a hemicellulase), (40×10^9 CFU; 15 g t⁻¹), chemical ingredient (43 % formic acid, 10 % propionic acid, 30 % ammonium formate, 2,0 benzoic acid), (4 l t⁻¹) and probioenzymatic inoculant 2 (*Enterococcus faecium*, *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Lactobacillus salivarius*, cellulase, hemicellulase, and amylase), (1×10^{11} CFU; 10 g t⁻¹). Biomass was ensilaged in micro experimental silos. Silages sampled 60 days after the beginning of conservation were assessed for pH, acidity of water extract (AWE), contents of lactic acid (LA), acetic acid (AA) and content of ergosterol.

Results and Discussion Use of silage additives and dry matter influenced quality of water extract silages (Table 1). Probiotic and probioenzymatic inoculants contributed to lower ($P < 0.05$) pH and higher ($P < 0.05$) acidity water extract (AWE). Silage additives significant ($P < 0.05$) contributed to lower contents of ethanol and NH₃. Significant ($P < 0.05$) was interaction between preservatives (PR) and dry matter (DM). At dry matter 20 % organic acids decreased pH and increased AWE while at dry matter 40 % decreased pH and increased AWE rather probiotic and probioenzymatic inoculants. Silage preservations influenced content of ergosterol. The lowest content of ergosterol and this probably lower contamination with fungal shown silages preservation with probioenzymatic inoculants and organic acids.

Conclusion Silage additives decreased content of ethanol and NH₃ in the grass silage water extract. Influence of additives on the pH and AWE depended on the dry matter of silage grass. Additives especially probioenzymatic inoculants and organic acids influenced content of ergosterol.

The study was conducted within the framework of Grant QH81040 'The quality of grass stands and its use in the ruminant nutrition', funded by the National Agency for Agricultural Research (NAZV) and from Grant MSM6215648905 'Biological and technological aspects of sustainability of controlled ecosystems and their adaptability to climate change', funded by the Ministry of Education, Youth and Sports of the Czech Republic.

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Table 1 Effect of preservative (PR) and dry matter (DM) on pH, acidity of water extract (AWE), contents of lactic acid (LA), acidic acid (AA), ethanol and NH₃ in grass silages

Factor	pH	AWE mg 100 g ⁻¹	LA % of DM	AA % of DM	Ethanol % of DM	NH ₃ % of DM
Preservatives (PR)						
Control	4.54 ^a	1194.5 ^a	13.5 ^{ab}	1.35 ^{ab}	4.02 ^a	0.24 ^a
Probiotic	3.98 ^b	1819.0 ^b	14.1 ^a	1.86 ^b	2.72 ^b	0.17 ^{bc}
Probioenz. 1	3.96 ^b	1717.7 ^b	14.0 ^a	1.65 ^{ab}	1.93 ^{cd}	0.16 ^c
Organic acids	4.22 ^{ab}	1346.9 ^a	10.8 ^c	1.14 ^a	1.43 ^d	0.18 ^b
Probioenz. 2	4.51 ^a	1266.3 ^a	11.3 ^{bc}	1.30 ^{ab}	2.31 ^{bc}	0.20 ^d
Dry matter (DM)						
20	4.19	1276.4 ^a	17.8 ^a	2.18 ^a	1.06 ^a	0.29 ^a
40	4.30	1661.4 ^b	7.7 ^b	0.73 ^b	3.91 ^b	0.09 ^b
PRxDM	0.0068	0.0006	0.0495	0.0044	0.0000	0.0000

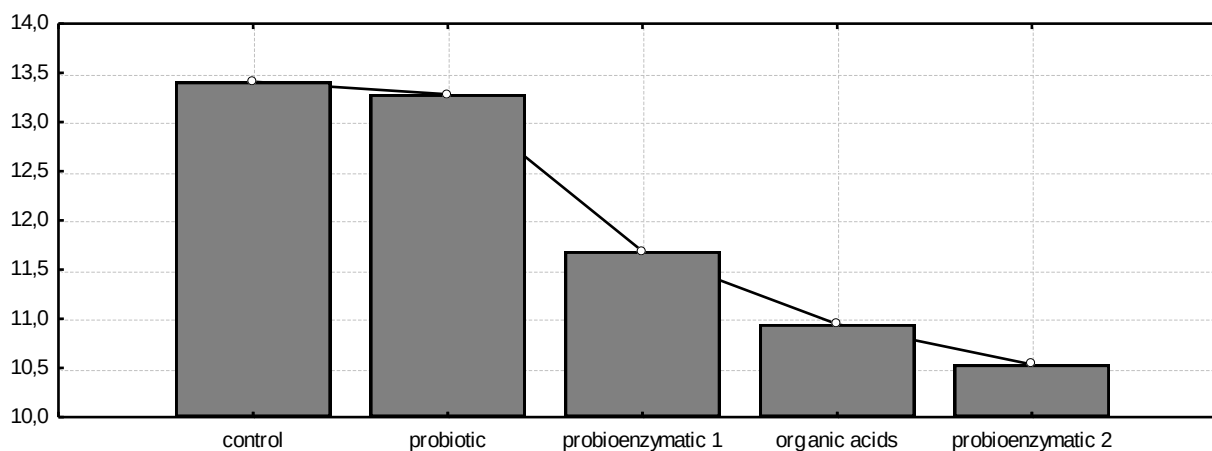


Figure 1 Effect of preservative on content of ergosterol (mg kg⁻¹ DM) in grass silages

Influence of the species and conservation additives on the content of nutrients and grass forage safety

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Introduction Winter feed ration is based on the conserved fodder from the first cut of grassland. As compared with haymaking, ensiling represents a much lower weather risk, which reflects favourably in working costs and low conservation losses (Achilles et al., 2002). Honig and Pahlow (1986) claim that the application of ensiling inoculants to partly wilted grasses with the DM content higher than 26% and lower than 45% improves the course of the fermentation process and decreases the total loss of nutrients. Prerequisite for high-quality silage is a clean and healthy phytomass (Holubek et al., 2007). Development of microscopic fungi may lead to the formation of mycotoxins (Opitz von Boberfeld et al., 2006). These metabolites can cause economic losses in animal production and decrease meat quality (Opitz von Boberfeld, 1996). There are considerable differences among species. Interspecific hybrids of *Festulolium* ssp. combine the resistance of the *Festuca* sp. family with the high quality of the *Lolium* sp. family (Casler et al., 2002).

Material and methods The experiment was carried out in triplicates. The harvesting of grasses *Lolium perenne* (cv. Kentaur), *Festulolium braunii* (cv. Perseus) and *Festulolium pabulare* (cv. Felina) was on begin of June (stage of end earing). The plots (1.5 x 10 m) were harvested by a self-propelled mowing machine. Stubble height was 0.07 m. The assessed grasses were wilted 30 hours. Use preservatives were organic acids (43 % formic acid, 10 % propionic acid, 30 % ammonium formate, 2,0 % benzoic acid), (4 l t⁻¹) and probioenzymatic inoculant (*Enterococcus faecium*, *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Lactobacillus salivarius*, cellulase, hemicellulase, and amylase), (1x10¹¹ CFU; 10 g t⁻¹). Biomass was ensiled in micro experimental silos. Silages sampled 60 days after the beginning of conservation were assessed for digestibility of organic matter (DOM) via pepsin-cellulase method, contents of crude protein (CP), crude fiber (CF) and net energy lactation (NEL). ELISA method was applied to estimate the content of mycotoxins zearalenone (ZEA), fumonisin (FUM).

Results and Discussion While the surveyed fodder species differed in the digestibility of organic matter ($P < 0.05$), the application of conservative additives did not affect the content of organic nutrients (Table 1). *Festulolium pabulare* apparently exhibited a higher content ($P < 0.05$) of crude fiber (CF) and lower contents ($P < 0.05$) of net energy of lactation (NEL). The lower quality of *Festulolium pabulare* as compared with *Lolium perenne* and *Festulolium braunii* is documented also by other works. Interesting is the look at the occurrence of mycotoxins. There was zearalenone detected in the silages (Table 1). Fumonisin was detected in *Festulolium braunii*. None of the studied factors had an influence on the zearalenone content but a tendency to the higher occurrence of zearalenone apparently existed in *Lolium perenne*. As to the preservatives, lower zearalenone content in the silage was apparently found with the use of the biological inoculant or organic acids. Statistical significance was not demonstrated namely with respect to a higher standard error of the mean.

Conclusion *Festulolium pabulare* (*Festuca arundinacea* x *Lolium multiflorum*) unlike *Festulolium braunii* (*Lolium multiflorum* x *Festuca pratensis*) and *Lolium perenne* had lower

quality of forage. However had higher forage safety which was demonstrated with lower content of zearalenone.

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Table 1. Effect of species (SP) and preservative (PR) digestibility of organic matter (DOM), crude protein (CP), crude fiber (CF), net energy lactation (NEL), fumonisin (FUM) and zearalenone (ZEA)

Factor	DOM % DM	CP % DM	CF % DM	NEL MJ kg ⁻¹ DM	FUM ppb	ZEA ppb
Species (SP)						
<i>Lolium perenne</i>	83.8 ^a	10.96	27.70 ^a	5.97 ^a	<LOQ	66.07
<i>Festulolium braunii</i>	82.0 ^a	10.07	30.08 ^{ab}	5.79 ^{ab}	6.07	46.34
<i>Festulolium pabulare</i>	74.6 ^b	9.73	32.56 ^b	5.34 ^b	<LOQ	47.92
Preservatives (PR)						
Control	79.9	9.95	30.52	5.68	<LOQ	62.92
Organic acids	78.9	10.33	30.45	5.72	6.07	48.52
Probioenzymatic	81.6	10.48	29.37	5.69	<LOQ	48.89
SPxPR	0.2512	0.9999	0.9986	0.990	0.4349	0.4177

The study was conducted within the framework of Grant QH71041 ‘The influence of pathogenic microorganisms and secondary metabolites on the quality and hygienic character of fodders’, funded by the National Agency for Agricultural Research (NAZV) and from Grant MSM6215648905 ‘Biological and technological aspects of sustainability of controlled ecosystems and their adaptability to climate change’, funded by the Ministry of Education, Youth and Sports of the Czech Republic.

Effect of additive on content of mycotoxins of grass silage

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Introduction. Silage is an increasingly important forage for cattle and horses. The quality of silage depends on fermentation of the material, but the ensiling process can be optimised with the use of additives. Several different additives can be used for making silage. Mycotoxins are poisons produced by mold and occur frequently in a silage. Mycotoxins are frequently found in forage (silage) at low levels causing subclinical losses in performance. Sometimes concentrations of mycotoxins are encountered in silage which are high enough to cause several performance and health problems. Aflatoxin (AF), deoxynivalenol (DON), zearalenone (ZEN), fumonisin (FB) and T-2 toxins are of greatest concern in animal production. Effect of additives on silage fermentation, chemical composition and content of mycotoxins were also investigated.

Material and methods. Tested silages were prepared from the mixture of the first cut of red clover (*Trifolium pratense* L.) and timothy (*Phleum pratense* L.) 50% + 50%. The one part of silage material was wilted for 24 hours. The silage material was chopped into 2 cm pieces, mixed and conserved in 3 litre glass jars. The silage additives were biological starters (*L. plantarum* + *L. fermentum* - 10^7 cfu g⁻¹) and chemical additive AIV Pro (5 litres/ton). In 90 days the jars were opened for analysis. For the silage, fermentation quality was estimated based on the contents of mycotoxins. Samples were analysed for the content of DM, crude protein, ash and fibre (AOAC, 2005). Crude protein was analysed by Kjeldahl method with Kjeldrec 2300 analyser. The NDF and ADF in the samples were determined with a fibre analyser ANKOM 220. The mycotoxin content was determined by the ELISA method with Ridascreen r FAST kits. Statistical analysis was performed for directly and wilted silage with the generalized linear model procedure of SAS.

Results and discussion. Chemical composition of silages is in the Table 1. In the first trial DM of silages was very low, but the second trial was the better. The fermentation characteristics indicated a good ensiling. The Table 2 shows the effect of several additives of silage on the mycotoxins content. The wilting of silage material increased the content of toxins (AF, ZEN, T-2 toxin and FB) (Table 2). The biological additive of silage increased the content of ZEN and DON. The drying of material on the field contaminated it.

Conclusions. The wilted silage material improved the DM concentration, and quality of fermentation, but increased the content of mycotoxins.

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Table 1. Average chemical composition of tested silages, g kg⁻¹

Silage treatment	Dry matter	Crude protein	Ash	NDF	ADF
Directly ensiling					
Control	140	153	109	485	263
Biological additive	171	161	86	409	266
Chemical additive	162	172	91	452	259
Wilted 24 hours					
Control	256	178	110	414	260
Biological additive	274	172	96	408	256
Chemical additive	295	172	94	409	242

Table 2. Mycotoxins content in silage, ppb

Treatment	Aflatoxin	Zearalenone	Deoxynivalenol	T-2 toxin	Fumonisin
Directly ensiling					
Control	0.8	97.2	317.7	1.6	30.8
Biol.additive	2.6	148.1	570.8	4.4	30.0
Chem.additive	1.7	128.2	356.1	4.3	38.8
24 h wilted					
Control	3.1	171.1	355.5	22.7	89.1
Biol.additive	4.1	245.4	457.3	24.7	84.7
Chem.additive	2.5	296.8	372.1	7.4	87.1
<i>P</i>					
C vs Biol	<0.001	0.015	<0.001	0.052	0.449
C vs Chem	0.012	0.015	0.286	0.136	0.269
CW vs Biol.W	0.394	0.009	0.003	0.427	0.370
CW vs Chem.W	0.132	0.003	0.356	0.029	0.478
C vs CW	<0.001	0.009	0.137	0.011	0.003
Biol, vs Biol.W	0.050	0.002	<0.001	0.034	0.001
Chem.vs Chem.W	0.054	<0.001	0.414	0.104	0.115

Safety issues in managing large-scale bunker silos and drive-over piles

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Introduction Few farming operations invite as many different opportunities for injury or fatality as a silage program. From harvesting forage in the field, transporting it to the farm, placing it into storage, and then removing the silage at feedout, employees are exposed to numerous serious risks. Silage-related tragedy knows no age boundary as workers and bystanders of all ages have been injured or killed during crop harvest and silage feedout (Murphy and Harshman, 2006). Increasingly, stories involve bunker silos and drive-over piles (Bolsen and Bolsen, 2010). Consistently protecting employees, equipment, and property throughout harvesting, filling, and feeding does not occur without thought, preparation, and training. Presented here are several major hazards involved with managing silage in bunkers and piles, and ways these hazards can be eliminated, reduced, or controlled.

Materials and Methods The three hazards presented and discussed include: 1) fall from height, 2) avalanche or collapsing silage, and 3) complacency.

Results and Discussion *Fall from height* It is easy to slip on plastic when covering or uncovering a bunker or pile, especially in wet weather. Standard guardrails should be installed on all above ground level walls. Use caution when removing plastic, tires, or pea gravel bags near the edge of the feedout face, and never stand on top of a silage overhang, as a person's weight can cause it to collapse. Where necessary, use equipment operating from the ground to remove spoiled silage from the surface of bunker silos and drive-over piles. Never allow a person to ride in the bucket of a front end loader!

Crushed by an avalanche/collapsing silage. A major factor contributing to injury or fatality from silage avalanche/collapsing silage is over-filled bunkers and piles (Holin, 2010a). A nutritionist had the following near miss, "I was taking a core sample at one of our large dairy customers and had just moved away from the face when a large section just fell off. This was a very well packed silo and had immaculate face management" (Bolsen and Bolsen, 2009).

It started out as a typical day for dairy nutritionist Doug DeGroff of Tulare, CA (Holin, 2010b). He pulled up to a client's corn silage pile for a forage sample, bucket and pitchfork in hand. After filling the bucket, he turned to walk back to his pickup to mix and core a sample. "The sun basically went out – I could not see any light and the feed hit me on my head and covered me completely," says DeGroff. "I knew what was happening before I hit the ground. The entire face fell on me ... about 20 tons broke away." DeGroff, who had celebrated his 36th birthday with his wife and two toddlers two days before August 27 last summer, was caught in a silage avalanche. DeGroff offered these additional comments, "This particular pile did not look unsafe at all. It was only 11 to 12 feet tall at the time that I sampled it and was mechanically shaven. I personally have taken feed samples from piles where I should not have been. I knew they were not safe, but I took the risk. This pile looked safe from any angle you looked at it from. I feel very blessed to be here and that everything still works. Yes, it was a broken back, but it could have been so much more. I am not on pain medication, and I don't think there are going to be long-term issues."

Avalanche/collapsing silage does not have to happen. Bunkers and piles should not be filled higher than the unloading equipment can reach safely, and typically, an unloader can reach a height of 3.5 to 4.5 meters. Use proper unloading technique that includes shaving silage down the feedout face and never “dig” the bucket into the bottom of the silage. Undercutting, a situation that is quite common when the unloader bucket cannot reach the top of an over-filled bunker or pile, creates an overhang of silage that can loosen and tumble to the floor. Never allow people to stand near the feedout face, and a rule-of-thumb is never stand closer to the face than three times its height. When sampling silage, take samples from a front-end loader bucket after it is moved to a safe distance from the feedout face.

Complacency. Always pay attention to your surroundings and be alert! A dairy nutritionist almost lost his life the day he took silage samples from a bunker silo with a 9-m high feedout face (Schoonmaker, 2000). “Even though I was standing 20 ft from the feedout face, 12 tonnes of silage collapsed on me. I did not see or hear anything. I had been in silage pits hundreds of times, and you just become kind of complacent because nothing ever happens. It just took that one time”.

“The accident happened on June 14, 1974 while making silage at Kansas State University’s Beef Cattle Research Farm. The blower pipe plugged for about the eighth time that afternoon, and I started to dig the forage out from the throat of the blower. The PTO shaft made one more revolution. Zap! The blower blade cut the ends off three fingers on my right hand”. The injured person, Keith Bolsen, said later, “I was complacent and did something pretty stupid” (cited by Bolsen and Bolsen, 2010).

Conclusions Even the best employee can become frustrated with malfunctioning equipment and poor weather conditions and take a hazardous shortcut, or misjudge a situation and take a risky action. It is best to take steps to eliminate or control hazards in advance than to rely upon yourself or others to make the correct decision or execute the perfect response when a hazard is encountered. Only experienced people should be permitted to operate equipment associated with harvesting, filling, packing, sealing and feeding in a silage program. The correct sizing of bunkers and piles can reduce the risk of an accident. Spreadsheet software is available to assist producers and their silage team to better design and manage bunker silos and drive-over piles (Holmes and Bolsen, 2009). Think safety first. The silage industry has nothing to lose by practicing safety: it has everything to lose by not practicing it.

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Economics of sealing maize and alfalfa silages in bunker silos and drive-over piles: an Excel spreadsheet

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Introduction Between 2006 and 2010, the USA produced an average of 97.65 million tonnes of whole-crop maize silage (United States Department of Agriculture, 2011). Approximately 82 to 84 % of this silage was made in bunker silos and drive-over piles. However, the failure to implement proper silage management practices, especially proper sealing technique, resulted in the unnecessary loss of approximately 18 to 20 million tonnes. Standard polyethylene, weighted with discarded full-casing tires or tire sidewalls, has been the most common method used to seal bunkers and piles, but organic matter (OM) losses in the original 0.91 metres can exceed 30.0 % (Berger and Bolsen, 2006).

The use of an oxygen barrier (OB) film (www.silostop.com) as an alternative to standard polyethylene for sealing bunker silos and piles was introduced at the XII International Silage Conference held in Uppsala, Sweden (Degano, 1999). The author stated that the permeability of OB film was 0.025 that of standard polyethylene film of the same thickness. Oxygen transmission rate (OTR) through standard polyethylene film using 100 % oxygen is 1812 cm³/m²/24 h (American Society for Testing Materials, ASTM D3985), while OTR through Silostop film using 100% oxygen is 65.5 cm³/m²/24 h (ASTM D3985). Thus, the permeability of OB film was 0.036 that of the std. polyethylene.

This paper presents an Excel spreadsheet, which estimates the economic benefit of sealing ensiled forage or high moisture grain in bunker silos and drive-over piles, and compares two sealing methods, std. polyethylene and OB film.

Materials and Methods The spreadsheet was developed from research conducted at Kansas State University from 1989 to 1995, and equations published by Huck et al. (1997). In the first section of the spreadsheet, the user enters values for the following: depth from the original surface to be evaluated; silage price; as-fed silage densities; bunker or pile dimensions; percent of the silage in the original depth lost during the storage and feedout phases; and cost of the sealing materials. The results are calculated and reported in the second section. Two examples from the spreadsheet, which compare bunker silos and drive-over piles sealed with either standard (std.) plastic or OB film, are presented in Table 1.

In a large, 18.3 m wide x 76.2 m long, bunker silo of whole-crop maize silage, which has an average depth of 3.66 m, sealing would prevent the loss of 7.33 and 9.94 % of the original 3,637 tonnes of crop ensiled for the std. plastic-sealed and OB film-sealed bunkers, respectively. The OB film (bunker silo 2) would save an additional \$3,765 of maize silage in the original top 0.91 m compared to std. plastic (bunker silo 1).

In a 27.7 m wide x 62.0 m long drive-over pile of alfalfa silage, which has an average depth of 1.98 m, sealing would prevent the loss of 11.5 and 15.7 % of the original 2,054 tonnes of crop ensiled for the std. plastic-sealed and OB film-sealed piles, respectively. The OB film (pile 2) would save an additional \$4,050 of alfalfa silage in the original top 0.91 m compared to std. plastic (pile 1).

Conclusions The economics of properly sealing bunker silos and drive-over piles makes it clear that farmers should pay close attention to the details of this troublesome task. Sealing with OB film has a greater economic benefit than sealing with std. polyethylene.

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Table 1 Profitability of sealing maize silage in bunker silos and alfalfa silage in piles with either std. plastic or OB film (maize and alfalfa silages: \$60 and \$75 per tonne, respectively)¹

Inputs and calculations	Bunker 1 maize std. plastic	Bunker 2 maize OB film	Pile 1 alfalfa std plastic	Pile 2 alfalfa OB film
Density in top 0.91 m, kg fresh wt. per m ³	600	600	550	550
Density below top 0.91 m, kg fresh wt per m ³	750	750	650	650
Silo depth, m	3.66	3.66	1.98	1.98
Silo width, m	18.3	18.3	27.7	27.7
Silo length, m	76.2	76.2	62.0	62.0
Silage lost in original top 0.91 m if sealed, % of the crop ensiled ²	25	12.5	22.5	12.5
Cost of covering sheet, ¢ per sq m	50	140	50	140
Total silage in the silo, tonnes	3,637	3,637	2,054	2,054
Total value of silage in the silo, \$	218,247	218,247	154,051	154,051
Silage in the orig. top 0.91 m, tonnes	761	761	860	860
Silage below the orig. top 0.91 m, tonnes	2,876	2,876	1,194	1,194
Total silage lost in the silo, % of crop ensiled	13.14	10.52	14.07	9.89
Silage lost if sealed, \$	11,421	5,710	14,505	8,058
Silage saved by sealing, \$	15,989	21,699	17,728	24,174
Sealing cost, \$	2,379	4,325	2,930	5,326
Net value of silage saved by sealing, \$	13,610	17,374	14,798	18,849
Net benefit from OB film, \$	---	3,765	---	4,050

¹Numbers in **bold** are user inputs.

²Values from data by Bolsen et al. (1993) and Kuber et al (2008).

PCR-DGGE analysis of the dynamic diversity of lactic acid bacteria in alfalfa silage

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Introduction Most regions of China are affected by monsoons. Since the harvesting period for alfalfa often occurs in the rainy season, alfalfa crops are at a high risk of losses due to deterioration during the drying process. This leads to a lack of forage resources in the winter and early spring (Sheperd et al., 1995). The present study investigated the effects of different water content on the fermentation quality of alfalfa silage. A PCR-DGGE method was used to study the dynamic diversity of lactic acid fermenting bacteria in alfalfa silage and to track changes in the population structure of lactic acid bacteria strains. The study lays a solid foundation for the development of new inoculant products.

Materials and Methods Alfalfa (*Medicago sativa*) silage materials were collected from China Agricultural University and divided into three groups based on moisture content: low (60.7%), medium (71.2%), and high (80.3%) moisture content. They were ensiled for 0, 1, 5, 10, 20, 30, and 45 days and then the genomic DNA from silage microbes was extracted by the phenol-chloroform method. The target fragment was amplified by using the following primers: 357F (5'CCTACGGGAGGCAGCAG-3',3')-GC (5'-CGCCCGCCGCGCGCGGGCGGGGCGGGGGCACGGGGGG) and 517R (5'-GTGCCAGC(A/C)GCCGCGG-3'). The dynamic fermentation diversity of the bacterial populations in the alfalfa silage was studied by PCR-DGGE (Han et al., 2009).

Results and Discussion Figure1 shows amplification bands that were located around 300bp, in agreement with a target fragment length of 280bp. The bands were sufficiently intense to be used for DGGE analysis, with the following results:

- (1) A significant difference was found between the DNA bands among alfalfa silage samples ensiled at different durations. The numbers of bands revealed the diversity of the bacterial population in silage and the intensity of the bands indicated the relative numbers of bacteria.
- (2) Almost all of the DNA samples, except for high moisture silage samples stored for 30 and 45 days, showed similar bands, which included No.1 (*Lactobacillus plantarum*), No.5 (*Lactobacillus farciminis*), No.7 (*Lactobacillus pentosus*), and No.10 (*Lactobacillus plantarum*). However, the bands varied in intensity, which indicated that the moisture content had a slight effect on the population sizes of these bacteria (Figure 2).
- (3) Band No. 10 was consistently intense, suggesting that it came from a dominant bacterial population. Band No.4 showed a similar persistence, but its intensity was not as strong as Band 10 (Figure 2).
- (4) The intensity of Band No. 1 increased during the later periods of fermentation, which may reflect the establishment of an improved environment for reproduction during storage. In contrast, other bands, such as Band No. 2, disappeared over time, perhaps due to the decrease in pH

occurring in the middle and late periods of silage fermentation, which may have led to declines in some aerobic bacterial populations (Figure.2)

Conclusion The 357F-GC and 517R primers were able to amplify genomic fragments from alfalfa silage bacteria and the DNA fragments were effectively separated using the DGGE method. The moisture content had little effect on the size or distribution of the bacterial population. Alfalfa silage contained great numbers of lactic acid bacteria, primarily *Lactobacillus* that showed as 7 bands, and small numbers of cocci.

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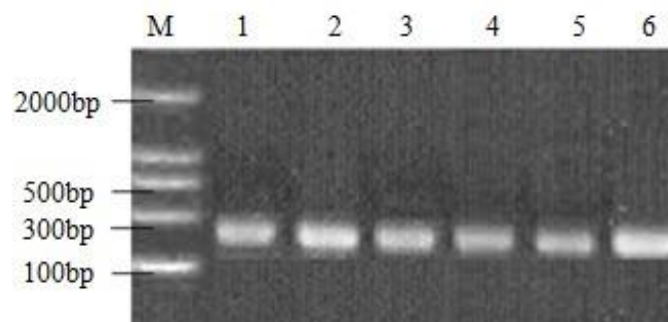


Figure 1. PCR results for microorganisms in alfalfa silage at low and high moisture content. M, 100bp marker; Lane 1-3, alfalfa silage at high, medium, low moisture, respectively. All samples were obtained after 1 day of ensilage; lane 4-6, alfalfa silage in high, medium, low moisture. All samples were obtained after 45 days of ensilage.

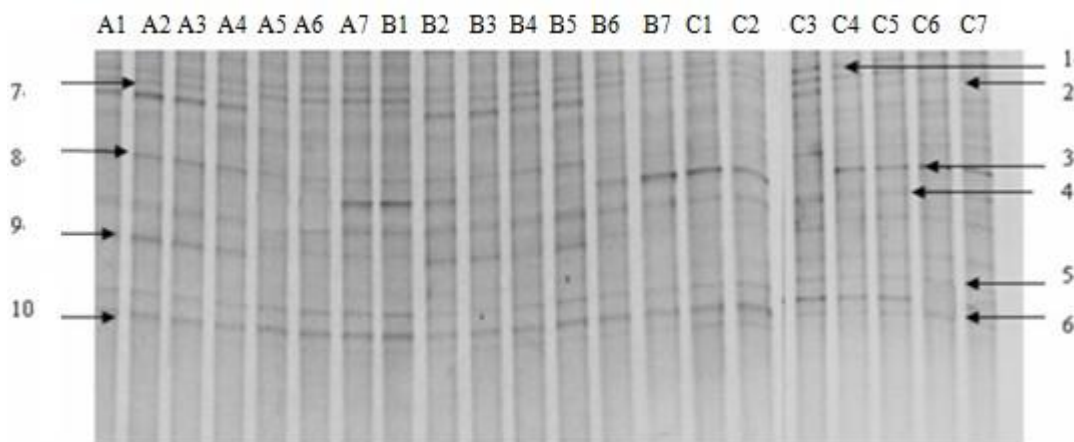


Figure 2. Electrophoretic DNA profiles of microorganisms in alfalfa silage. Lanes A1-A7: low moisture alfalfa silage after 0,1,5,10,20,30, and 45 days of ensilage; lanes B1-B7: medium moisture alfalfa silage after 0,1,5,10,20,30, and 45 days of ensilage; Lanes C1-C7: high moisture alfalfa silage after 0,1,5,10,20,30, and 45 days of ensilage.

Evaluation of four groups of sorghum hybrids for silage.

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Introduction In Argentina, due to advancement of agriculture in the area called *pampa húmeda*, has caused livestock to move toward the north, such as the province of Formosa. This raises the need to adopt new production strategies and feeding, in order to enhance the activity and make it sustainable over time. Sorghum is the most important cereal in many parts of the world for its resistance to drought and high temperatures. It is a culture of excellent behavior in good environments and versatile adaptation to soil and climatic characteristics committed to other crops, developing a wide range of soil conditions. In our country the importance of sorghum as part of a production system is the use as grain and forage for animal feed, as well as an essential part of a rotation system to maintain productivity and soil structural stability. The whole plant silage sorghum is one of the possibilities for increasing forage availability in northeastern Argentina, mainly as a reserve (silo whole plant), being used in the months in which the forage production curve decreases (usually during June, July, August and part September). The aim of this study was to evaluate the agronomic performance of four genetic groups of sorghum: dry matter production (MS) and its components (leaf, stem, panicle).

Materials and Methods The trial was carried out in the Agricultural Experimental Station El Colorado, located in the SE of the province of Formosa, 78 meters above sea level, 26 ° 18 S and 59 ° 23' W. The average annual rainfall is 1170.2 mm and average annual temperature is 22 ° C. The soil is from the series El Colorado (Haplustol oxic, alluvial silt loam). Were evaluated 42 sorghum hybrids with fitness siler, classified into 4 genetic groups (GG): 18 grain sorghum hybrids (G) 2 forage (F), 12 dual-purpose (DP) and 10 siler (S). The crop management was as follows: chemical fallow 3 months prior to planting and applying Sulfosate Touchdown® and Tordon® 24K, in pre-sowing Sulfosate Touchdown® and Dual Gold, atrazine 50% in pre-emergence, Concep III® in the hybrids that came with this antidote cured, 50 kg/ha of diammonium phosphate at planting and 100 kg/ha of urea when plant had 6 leaves. Planting was done with a pilot drill four grooves on October 29, 2010 with a seed density of 16.5 seeds per linear meter of furrow, and 0.52 m between rows, the production target was 200 000 plants per hectare at harvest. The phenological stage of the crop was cut for forage materials (F) by looking at 4 to 6 dead basal leaves for the rest (G, DP and S) when the grain was in a pasty state drive, for all materials between 30 - 35% of DM, the height of cut was 20 cm. The variables were evaluated by groups: kg DM/ha, % DM, and total percentage of the plant is partitioned to leaf (% L), stem (% S) and panicle (% P). The design was randomized complete block with three replications, randomized groups within each block, plots were 10 m long and 1.56 m wide (15.6 m²). The data were analyzed with statistical software version INFOSTAT 2011 and subsequent multiple range test of Duncan.

Results and Discussion Significant differences were observed across groups for all variables studied (Table 1). The group of grain sorghum was smaller for DM production/ha and % S, as well as higher % P, no differences between other groups for these variables. The reduced

production of DM could be due to lower participation of stem fraction, which is characteristic of this genetic group. The group of foragers was the one who showed the lowest percentage of DM and the highest percentage of L, not significant differences for these variables in other groups, this could be due to the high production of green matter, added to that, the parameter used to harvest 4 to 6 dead basal leaves, could have been harvested before reaching the minimum value of DM to be harvested.

Conclusion The variety of genetic materials of sorghum available in our area shows great potential for obtaining high performance silos, making this a key resource within the production systems. The distribution of dry matter is dependent on the genetic group that we use and gives us an idea of the nutritional quality than we get. Grain sorghum cultivars have a higher percentage of grain (more digestible fraction and quality) and feed a greater percentage of leaves (fraction of lower digestibility).

Table 1. Maximum, minimum, mean and coefficient of variation for study variables of four genetic groups of sorghum

Variable	Genetic groups			
	Siler	Grain	Forage	Dual Purpose
Kg DM/ha				
maximum	75550	40087	43197	49726
minimum	3073	14802	28484	29420
mean	44555 _b	24009 _a	35840 _b	40854 _b
CV	43.42	29.73	29.03	15.50
% DM				
maximum	49	37	36	45
minimum	27	31	20	30
mean	38 _b	34 _b	28 _a	37 _b
CV	18.73	6.52	40.41	12.51
% leaf				
maximum	25	30	37	30
minimum	9.47	13	15	12
mean	17.09 _a	17.71 _a	26 _b	17.91 _a
CV	27.82	21.01	59.83	27.80
% stem				
maximum	78.08	65	63	75
minimum	33.00	31	63	44
mean	59.10 _b	43.20 _a	63 _b	54.81 _b
CV	25.12	21.25	0	16.13
% panicle				
maximum	43.00	50	22	40
minimum	8.19	22	22	10
mean	23.81 _a	39.02 _b	22 _a	26.61 _a
CV	48.35	20.01	0	38.27

a-b Means within a row with different subscripts differ according to Duncan's test ($P < 0.05$).
CV = coefficient of variation

Fermentative capacity of soybean varieties harvested in two stages of development

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Introduction Soybean forage is considered a legume with high protein content. With soybean is possible to reduce protein concentrates participation in animal feed, once this nutrient is largely used in diets and is the most expensive on formulation allowing the decrease of total diet cost. This forage can be utilized as silage, making it a promising option since it has some favorable characteristics such as seed availability in the market, production capacity in different climates, erect to facilitate mechanization in harvesting, good yield and high protein concentration (Rigueira et al., 2007). The objective of this work was to evaluate the effect of soybean varieties cultivated for grain production, harvested at two stages of development, on fermentative capacity, dry matter, crude protein, soluble carbohydrates and buffering capacity.

Materials and Methods The trial was carried out in Ilha Solteira, SP, Brazil (22 ° 22'S, 51 ° 22'W) and the evaluated treatments consisted of two factors: varieties (Valiosa RR, A7002 and BRSMG 790) and harvesting stage (R6 and R7). The plants were chopped into stationary machine for a particle size between 3-8 mm and then two samples were taken of each variety (3), harvesting stage (2) and repetition (4), ensiled in plastic buckets (15 kg), which contained 2,5 kg of sand in the deep and lids adapted with Bunsen valves. Samples for carbohydrates (SC) and buffering capacity (BC) (Campos et al. (2004)) were taken immediately and stored in a freezer (-8 °C). For dry matter (DM) determination, samples were dried at air oven on 55 °C for 72 hours. After, they were ground at 1 mm and dried at 105 °C for 12 hours for determination of second DM. Crude protein (CP) was adjusted for DM on 105 °C. The fermentative capacity (FC) was estimated as suggested by Weissbach & Honig (1996): $FC = DM(\%) + 8 \times (SC:BC)$.

Results and Discussion There was effect of harvesting stage, variety and interaction between stage x variety in most parameters evaluated (Table 1). In Table 2, there was difference ($P < 0.01$) in DM for harvesting stage. The R7 had the highest results and the variety BRSMG 790 showed higher DM content, followed by the variety A7002 and Valiosa. At the R7 stage production and forage quality were higher because the higher content of grains in the total mass, so CP was also high (Hoffman et al., 2004). For BC, there was no difference between harvesting stages, just for varieties, with the BRSMG 790 showing the lower value. In addition to the DM and SC, the BC which defines the resistance of the pH variation and it is another factor that influences the conservation of forage as silage. The values of SC differed between the stages and the varieties Valiosa and A7002 showed the highest results. In stage R7 the variety BRSMG 790 showed higher FC, than the varieties A7002 and Valiosa. The FC of all varieties in R6 stage was below the minimum value of 35, while varieties in R7 stage were above it. According to Weissbach and Honig (1996) forages with FC less than 35 pass undesirable fermentation in the silo. The lower content of SC observed in R7 was compensated by the higher content of DM and provided FC value above 35. The same was observed in the variety BRSMG 790.

Conclusion Soybeans harvested at R7 stage provide highest values of silage fermentation process. The varieties analyzed provide potential for silage.

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Table 1. General mean, treatments effects and interactions, in the parameters of dry matter (DM), crude protein (CP), buffering capacity (BC), soluble carbohydrates (SC) and fermentative capacity (FC)

Item (%)	Variety	Stage	V x S	CV (%)
DM	**	**	**	4.36
CP	*	**	**	6.00
BC	*	NS	**	7.27
SC	**	**	**	13.81
FC	**	**	**	4.57

NS P > 0.05; *P < 0.05; **P < 0.01

Table 2. Dry matter (DM), soluble carbohydrates (SC), buffering capacity (BC) (emg HCl/100g DM) and fermentative capacity (FC) of soybean forage varieties harvested at two stages (R6, R7)

Item (%)	Stage		Varieties		
	R6	R7	Valiosa	A7002	BRSMG 790
DM	31.6a	44.35b	33.65c	36.27b	44.01a
CP	19.43b	21.77a	19.94b	20.27ab	21.59a
BC	32.65a	31.88a	34.45a	35.41a	26.94b
SC	6.37a	4.95b	6.25a	6.82a	3.91b
FC	33.14b	45.36a	35.09c	37.46b	45.20a

Means followed by different letters in lines differ by Tukey test (P<0.05)

Quality of maize silage fermentation process infected with *Ustilago maydis*

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Introduction Maize smut (*Ustilago maydis*) can create greyish bulging galls with sticky and badly smelling, later strongly dusting mass of teliospores which can infect the stands, seeds and soil. Bochowiak and Skorupska (2006) state that the distinctive temperature changes were the main reason behind the incidence of maize smut in Poland. Many studies have shown that silages made from the infected maize by smut *Ustilago maydis* had low mycotoxins content (Potkanski et al., 2010) and silages did not have a harmful effect on the production and health of cattle. On the other hand, Richter et al. (1994) state that plants infected with maize smut had lower DM content, lower nutrients content and *in sacco* DM degradability and OM did not differ from healthy plants. However, sheep ate 28% less DM from sick plants than from healthy plants.

Materials and Methods In the model experiment there was used ensiled maize from healthy stand with 358.95 g.kg⁻¹ DM of original mass (A) and maize plants naturally infected with smut (*Ustilago maydis*) from the same land (B). Established were two experimental variants (A, B) in three repetitions. Model silages were stored at average laboratory temperature of 25–27°C for 185 days. Parameters assessed to establish the quality of the fermentation process were as follows: Dry matter (DM) content of silage, pH, water extract acidity (AWE), amounts of lactic acid (LA), acetic acid (AA), sum of acids in DM and ethanol contents. Analytical procedures were described in our earlier work (Dolezal, 2002). Results were statistically processed by using the analysis of variance and differences between individual groups were analyzed by Scheffe-test in program Statistica 8. Data in the text are presented as average ± standard deviation.

Results and Discussion From the outcome of the model experiment in which the influence of smut (*Ustilago maydis*) infection of maize on the quality of fermentation (Table 1) was studied, it is apparent that the course of fermentation was different due to the differing DM content of silage biomass. The silage infected with maize smut had, in comparison with control silage, higher ($P<0.01$) concentration of fermentation acids in 1kg of DM (12,15 %) as compared to 9.37 % and that was reflected in the higher value of AWE (1,744.3 mg KOH) as compared to 1498.3 mg KOH in 100 g of silage. The higher value of titrate acidity corresponded statistically lower ($P<0.05$) with the lower average pH value of silage. In the experimental silage even considering the statistically lower ($P<0.01$) DM content (249.63 g.kg⁻¹) it was found lower average content of lactic acid and higher ($P<0.05$) average content of acetic acid (0.838 %) in comparison to control silage (2.388 %, resp. 0.692 %), which had a higher DM content (328.70 g.kg⁻¹). Richter et al. (1994) also stated lower DM content in infected silage as opposed to the silage from healthy maize plants. This confirmed that the infection of maize stand with smut (*Ustilago maydis*) results in lower DM content ($P<0.01$) of infected plants. It is obvious from the results as shown in Table 2 that the smut (*Ustilago maydis*) infection of maize plants also influenced the change in microbial composition of silages. The silage from infected plants also contained higher counts of lactic fermentation bacteria (63.6×10^6 cfu/g) in comparison with control silage (57.1×10^6

cfu/g). From the Table 2 is apparent that the infection of maize plants with smut leads to the overall increase in the amount of microorganisms but at the same time it does not lead to the increase in the amount of micromycetes. This hypothesis can be also supported by the lower DM content in infected plants which is more convenient for bacterial microflora.

Conclusion The results of the experiment indicated that the used sick plants with *Ustilago maydis* have different effect on the contents of lactic acid bacteria and the quality of fermentation process. Ethanol fermentation was significantly ($P<0.01$) reduced in silage from sick plants in comparison to control. There was found a significantly lower content of moulds and yeast fungi, but on the other hand significantly higher total amount of microorganisms and higher fermentation loss (4.30 %) in the experimental silage from sick plants in comparison to control.

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Table 1. Average characteristics of maize silage from healthy and sick plants of *Ustilago maydis*

Maize silage	DM (g/kg)	pH	AWE mg KOH	LA %	AA %	Σ acids in DM %	LA/AA	Ethanol %	Ammonia %
Healthy plants	328.70 ±19.738 ^A	3.865 ±0.04 ^a	1,498.3 ±37.333	2.388 ±0.00	0.692 ±0.261 ^b	9.370 ±0.755 ^B	3.605 ±0.936	2.385 ±0.203 ^A	0.040 ±0.00
Sick plants	249.63 ±5.797 ^B	3.692 ±0.124 ^b	1,744.3 ±253.8	2.195 ±0.219	0.838 ±0.084 ^a	12.250 ±1.39 ^A	2.628 ±0.237	0.87 ±0.511 ^B	0.035 ±0.005

AWE - water extract acidity; LA - lactic acid; AA - acetic acid; *Variants in capitals differ* ($P<0.01$); *variants in lowercase differ* ($P<0.05$).

Table 2. Average content of micromycetes in maize silages (in 1 g)

Maize silage	TAM	LAB	Total	Micromycetes		
				Yeast fungi	Moulds	
					in Total	Geotrichum
A- Control	34,063,636	57,100,000	46,182	45,955	227	0
B - Sick plants	54,500,000	63,600,000	11,272	11.227	45	0

TAM - total amount of microorganisms; LAB - lactic acid bacteria

Acknowledgement: The work was funded from the Projects NAZV QH 71041 and VZ MSM 6215648905.

Greenhouse gas emissions during the fermentation of sugarcane silages

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Introduction In recent years, agricultural systems have been presented as major source of greenhouse gas (GHG) emissions, mainly due to the use of fertilizers, deforestation and enteric fermentation from ruminants. In Brazil, according to the National Inventory of Greenhouse Gases Emissions (MCT, 2009) 71% of all methane came from the agriculture. Emissions of methane (CH₄) and nitrous oxide (N₂O) contribute considerably to the greenhouse effect, with potentials of global warming of 23 and 296 times higher than the carbon dioxide (CO₂) (IPCC, 2001). In Brazil, there is a lack of detailed information and research about the pollutant potential of industrial activities, energy and mainly agriculture. The last one is of fundamental importance due to the international role of Brazil as a food-producing power. Also, the pollutant potential of silage production is poorly understood in comparison with the quantitative importance of this food in animal production systems. The aim of this paper was to evaluate the GHG emission during the fermentation of sugarcane silages and the potential for mitigation using additives.

Materials and Methods The trial was carried out at the Centro de Pesquisa em Forragicultura (CPFOR) of Federal University of Paraná, in Curitiba, PR, Brazil. The sugarcane variety RB 72-454 was harvested at 32.8% of dry matter (DM) and the following treatments were applied: CONTROL – no additives; Chemical additive within LOW (4 g t⁻¹) or HIGH (8 g t⁻¹) dosages (wet basis), with five replicates for each treatment. The treated forage was ensiled in plastic buckets (20 L), equipped with a mobile apparatus for recovery and measurement of the volume of gas produced. The bulk density of the silos was 600 kg m⁻³, and they were stored for 66 days. Using a pipeline and a collection chamber made of low density polyethylene, the total amount of gas of each silo was registered daily. On days 5, 33 and 61, samples were collected for determination of the concentration of carbon dioxide (CO₂), nitrous oxide (N₂O) and methane (CH₄). For gas sampling, 20 mL polypropylene syringes with closure valve were used. After homogenization, gas samples were collected and syringes were kept in a styrofoam box with ice (below 10 °C) and were sent to Laboratory of Soils of Federal University of Rio Grande do Sul for analysis using Gas Chromatography (GC Shimadzu 14-A). Data was analyzed for a completely randomized design using PROC GLM of SAS.

Results and discussion No treatment effect was detected for any variable, probably due to the high coefficient of variation. A higher gas production has occurred during the first seven days of fermentation, and a strong influence of temperature over the gas production was noticed (Figure 1). The room temperature influences the metabolism of microbial population reflecting over gas production. However, the temperature has strong effect on the physical volume of the gases, which can lead to errors in analysis of results. The average daily gas production for each silo was 138.3 mL. The total amount of 2.08 L of gas was produced for each kg of DM ensiled. The gases were mainly composed by carbon dioxide (23054 ppmv – 99.9%) with low levels of methane (2 ppmv) and nitrous oxide (937 ppbv). Probably, the high concentration of CO₂ is related to the yeast metabolism, which converts sucrose to ethanol, producing 2 mols of CO₂ (McDonald et al., 1991).

A poor correlation (0.146) between volume of gas measured (mL) and disappearance of dry matter as gas (% of dry matter) was detected (Table 1). This sounds alarming once the traditional measuring of gases could be inaccurate considering as gases losses the volatile compounds lost during oven drying. The average GHG emission was 36.4 g of CO₂ eq per ton of forage (wet basis). This values are much lower than the estimates of GHG emission from feedlot cattle (5.6 kg CO₂ eq per kg of live weight gain) or dairy cattle (1.1 kg CO₂ eq per kg of milk) (Phetteplace et al., 2008). Although no statistical difference was observed due to the high coefficient of variation, the high dosage of additive showed a production of gases 7.5 times lower than control (27.5 vs 208.9 ml day⁻¹ in control) and the emissions of GHG was reduced by 8 times when compared with untreated silage (7.0 vs 56.1 g CO₂ eq t⁻¹ of forage for untreated silage).

Conclusion Carbon dioxide is the main gas produced during sugarcane ensiling process. The use of additives on silages can be an important strategy for GHG mitigation.

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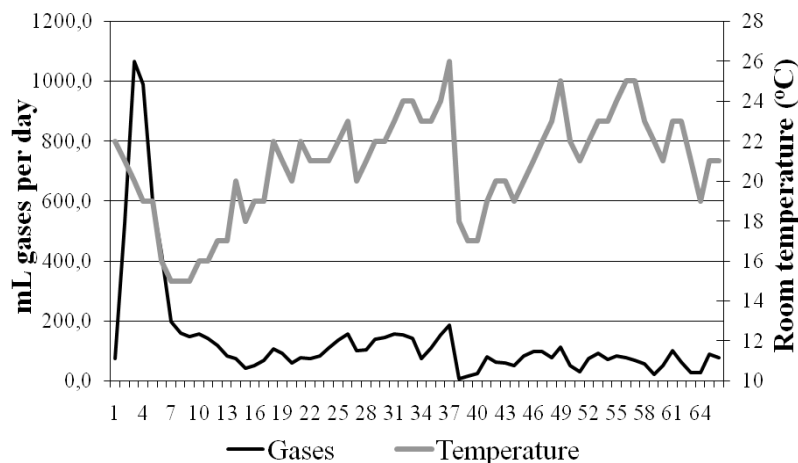


Figure 1 Gas production of sugarcane silages and room temperature during 66 days of storage.

Table 1. Greenhouse gas emissions during ensiling of sugarcane silage

Variable	Treatment ¹			Mean	CV (%)
	Control	Low	High		
Gas production, % of DM	6.7	6.2	9.1	7.3	34.5
Gas production, mL per day	208.9	178.4	27.5	138.3	214.8
GHG, g CO ₂ eq t ⁻¹ forage	56.1	46.1	7.0	36.4	214.7

¹Control, no additives; Low, 4 g of chemical additive per ton; High, 8 g of chemical additive per ton.

Characterization of the sugarcane silage effluent and the potential of environmental pollution

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Introduction Brazil is a major supplier of animal protein for the world. Recent estimative by FAO (2010) have projected a 29% growth in Brazilian meat exports until 2019. This growth brings with it several issues that must be studied, especially environmental issues. The use of sugarcane silage has increased in recent years, because of several advantages over other types of grasses, mainly the productivity of more than 35 t DM ha⁻¹. However, the inadequate utilization of this forage as silage may lead to the formation of effluent during the ensiling process, with unknown pollution potential. Thus, this study aimed to quantify and evaluate the pollutant potential of sugarcane silage effluents.

Materials and Methods The trial was carried out at the Centro de Pesquisa em Forragicultura (CPFOR) of Universidade Federal do Paraná, in Curitiba, PR, Brazil. The sugarcane variety RB 72-454 was harvested at 328 g kg⁻¹ of dry matter (DM) and the following treatments were applied: CONTROL – no additives; ADD – chemical additive within LOW (4 g t⁻¹) or HIGH (8 g t⁻¹) dosages (wet basis), with five replicates for each treatment. The treated forage was ensiled in plastic buckets (20 L) equipped with a 2 cm tall plastic pallet on the bottom for recovery the effluent. The bulk density of the silos was 600 kg m⁻³. Samples were collected for determination of DM, brix and pH (Table 1). Immediately after ensiling, a vacuum pump was used to remove the air in bottom of the silo. After 66 days of storage, the silos were opened and the effluents were collected from 10 silos, and five showed only a small and insufficient quantity of effluent for analysis. The effluent production was measured by differences in the weight of the empty silos before and after ensiling.

Soluble solids (brix) were measured immediately from sugarcane juice and in the effluent using an optical refractometer. Another sample of effluent was collected and stored in a freezer and used for determination of chemical oxygen demand (COD) as described by Peralta-Zamora et al. (2005), pH and DM content, under 55°C forced air oven. The density of the effluent was measured by weighing one mL of effluent using a high precision scale.

Results and Discussion There were no effects of treatments over the tested variables. The average amount of effluent production was 13.3 kg per ton of silage (wet basis). It is equivalent to 12.8 L t⁻¹ (Table 2) and it is below the most common values found in literature, which is between 30 and 90 kg t⁻¹ for sugarcane silages. Probably, this low effluent production could be related to the high DM content (328 g kg⁻¹) of good quality sugarcane used in this trial (brix 19.8%). Thus, the effluent production of sugarcane silages could be reduced by harvesting the plant at high maturity stages.

The effluent showed 568,497.7 mg L⁻¹ average COD. This value is extremely higher than the mean value of 38,344.0 mg L⁻¹ reported by Bernardino et al. (2005) for effluent of Elephant Grass silages. This shows the importance of avoiding silage effluent production because it is hundreds times more pollutant than other agents considered of great environmental importance, such as landfill leachate (Peralta-Zamora et al. 2005).

A strong correlation (R: 0.984) between brix and DM content of effluent suggests that large amounts of soluble solids, mainly sucrose, are lost by leaching. In this study it was

observed losses of 1.83 kg of soluble dry matter as effluent for each ton of ensiled forage. The high DM content of the effluent caused a great demand for oxygen and resulted in the oxidation of all carbohydrates in this component. Likewise, the low pH indicates high concentration of organic acids that could be used by ruminants, but they were lost in this residue. When released into water sources, these components are readily metabolized by aquatic aerobic microorganisms, which reduce the oxygen content of water and generate large environmental impact.

Conclusion The effluent of sugarcane silages is rich in nutrients and shows a great potential for pollution of aquatic environments and therefore must be prevented. The brix can be used as a tool to estimate the amount of total soluble solids that are in the effluent of sugarcane silages.

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Table 1- Dry matter content, brix and pH of sugarcane before ensiling

Variables	Mean	Standard-deviation
Dry matter, g kg ⁻¹	328	4.2
Brix, %	19.8	1.65
pH	6.10	0.06

Table 2- Chemical oxygen demand (COD), brix, pH, DM content, density and total production (kg t⁻¹) of the effluent from sugarcane silage

Variable	Mean	Standard-deviation
COD, mg O ₂ L ⁻¹	568,497.7	106,553.8
Brix, %	14.4	0.78
pH	3.17	0.26
Dry Matter, g kg ⁻¹	138	7.8
Density	1.0465	0.02
Effluent, kg t ⁻¹	13.3	9.34

Improvement of aerobic stability and reduction of aerobic dry matter losses in different silage materials by biological inoculants containing *Lactobacillus brevis*

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Introduction Aerobically instable silages are typically characterized by heating caused by yeast growth, which is unfavourable in many aspects. Spoilage by yeasts is an energy consuming process which results in high energy losses due to ethanol fermentation but also in degradation of dry matter. In addition aerobic spoilage favours mould growth and consequent production of mycotoxins. Therefore aerobic instability in silage is usually not only an animal health issue, but also an economic challenge. This challenge was now addressed by inoculating silage with inoculants containing, among others, *Lactobacillus brevis*, a specie of heterofermentative lactic acid bacteria. Heterofermentative lactobacilli are able to produce acetic acid in addition to lactic acid. Since acetic acid is a known inhibitor of fungi, the intention of adding *L. brevis* is to ameliorate aerobic stability and reduce aerobic surface losses.

Materials and Methods Five different lab scale silage trials were conducted in different materials (grass, clover-grass mix, field beans (whole plant), maize (whole plant and mashed grains) between 2008 and 2010. Trials compared untreated silages with silages treated with either Biomin® BioStabil Plus (2×10^5 cfu/g silage) or Biomin® BioStabil Mays (1×10^5 cfu/g silage), both of which contain *Lactobacillus plantarum*, *Enterococcus faecium* and *Lactobacillus brevis* in different ratios. Sample silos were prepared in triplicates per trial group in sizes of about 2 kg. The trial time was 90 (± 3) days after which the silos were sampled and analysed for aerobic stability by differential temperature measurement according to Honig (1990). Silages were considered unstable if the temperature in the silage increased to more than 3°C above ambient temperature (23 ± 2 °C). At the end of stability testing surface losses were determined by drying (105°C, 24 h) and differential weighing of the dry matter. Statistics were carried out with SPSS 10.0 using T-test and ANOVA for meta-analysis preceded by tests for normal distribution.

Results and Discussion It was found that aerobic stability was improved in most silages (with the exception of clover grass) by 1.5 – 3.0 days with addition of the inoculant (Figure 1). The stability improvement was statistically significant ($P < 0.05$ in whole plant maize and $P < 0.1$ in meta-analysis over all trials). Aerobic losses of dry matter were markedly decreased in most trials, especially within grass and maize silages they were reduced by up to 6.0 % ($P > 0.05$). Even in the clover grass trial where there was no difference in duration of aerobic stability aerobic dry matter loss was reduced by 0.4% (Figure 2).

Conclusions The inoculants Biomin® BioStabil Plus and Biomin® BioStabil Mays were both found suitable to improve aerobic stability of green forage and maize silages. Both additives cause longer inhibition periods of moulds and yeasts than can be expected in untreated silages. Furthermore the economic loss of dry matter upon the exposure of the silage surface to air is diminished.

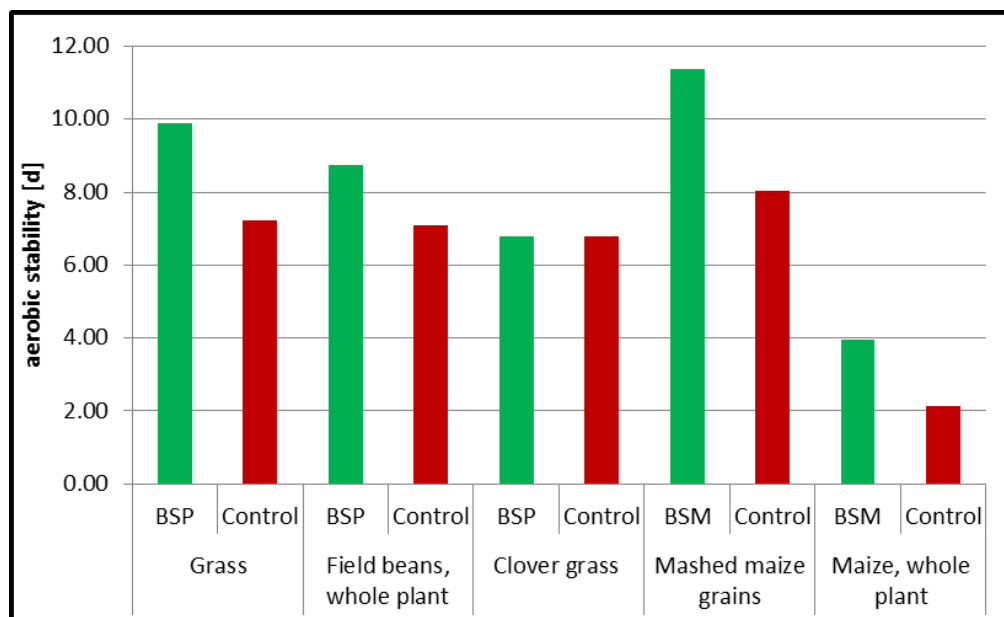


Figure 1: Aerobic stability of treated vs. untreated samples (n =3)

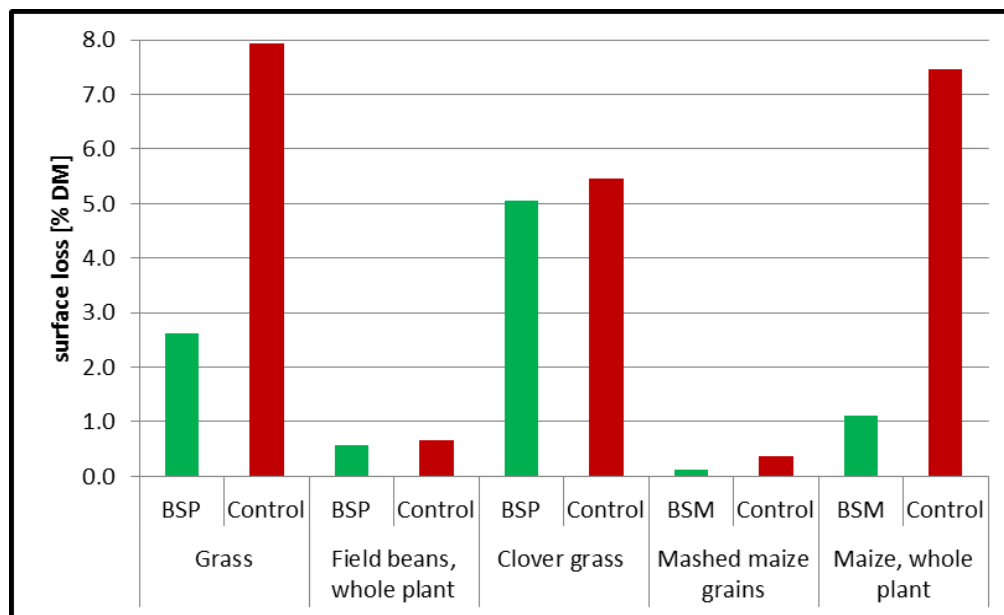


Figure 2: Aerobic dry matter loss in treated vs. untreated samples (n =3)

Inhibition of biogenic amines in silage by *L. plantarum* and *E. faecium*

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Introduction Occurrence of biogenic amines in silage is an indicator of poor silage quality. Putrescine, cadaverine, tyramine and histamine belong to the most prevalent amines found in silage (Křížek, 1993; Olt et al., 2005). These compounds can be produced by contaminants like Enterobacteria and Clostridia (Oude Elferink et al., 1999) and have been found to cause feed intake depression and impair digestive functions (Phuntsok et al., 1998). This is why a trial was conducted to find out about the influence of biological silage inoculants on the formation or inhibition of biogenic amines in laboratory scale silage trials.

Materials and Methods A lab scale silage trial in wilted alfalfa (~43% dry matter (DM)) was conducted over three months. Silages were either untreated (control) or inoculated with one of three strains of lactic acid bacteria (*Lactobacillus amylovorus*, *Lactobacillus plantarum*, *Enterococcus faecium*) at a dosage of 5×10^4 cfu/g silage. Sample silos were prepared in triplicate for each day of analysis. Silages were sampled 3, 7, 44 and 90 days after ensiling. In addition to various in-house investigations including DM loss, organic acids and carbohydrates (HPLC), aerobic stability (according to Honig, 1990), microbial counts and the content of biogenic amines were analysed via HPLC at the University of Natural Resources and Life Sciences, Vienna. Statistics were carried out in SPSS 10.0 using ANOVA and post-hoc Tukey HSD or Tamhane T2 test preceded by tests for normal distribution and homogeneity of variances.

Results and Discussion The prevalence of the four biogenic amines measured decreased from histamine > tyramine > cadaverine > putrescine. Levels of cadaverine were lowered to a certain extent by all lactic acid bacteria (Figure 1), while putrescine and tyramine levels were lowered most effectively in trial groups with *L. plantarum* treatment (Figure 2 and 3). Histamine levels were rather high in all groups (1500 mg/kg DM and above) with only small reductions in the treated groups vs. Control (Figure 4). Amine levels remained essentially unchanged during an 8 days aerobic exposure phase, with the exception for histamine, where levels were partly visibly lowered.

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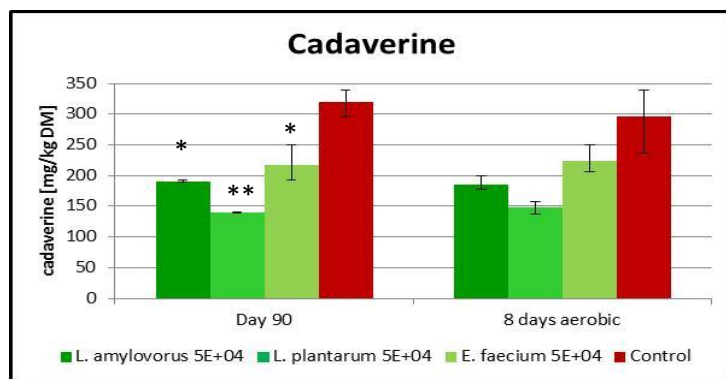


Figure 1 – Cadaverine levels in alfalfa silage (n=3)⁽¹⁾

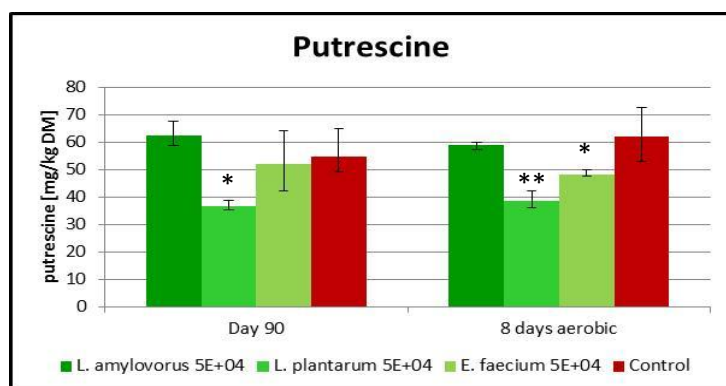


Figure 2 – Putrescine levels in alfalfa silage (n=3)⁽¹⁾

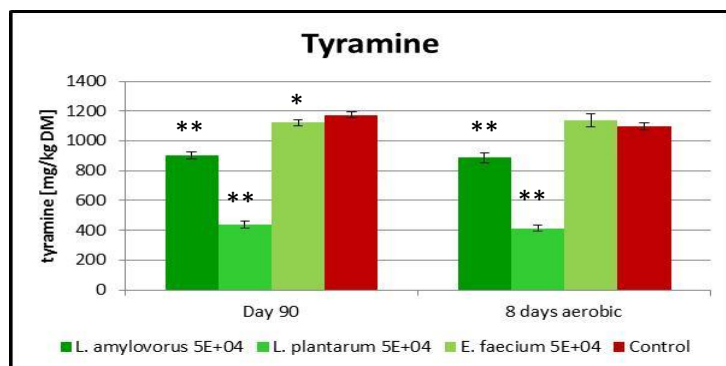


Figure 3 – Tyramine levels in alfalfa silage (n=3)⁽¹⁾

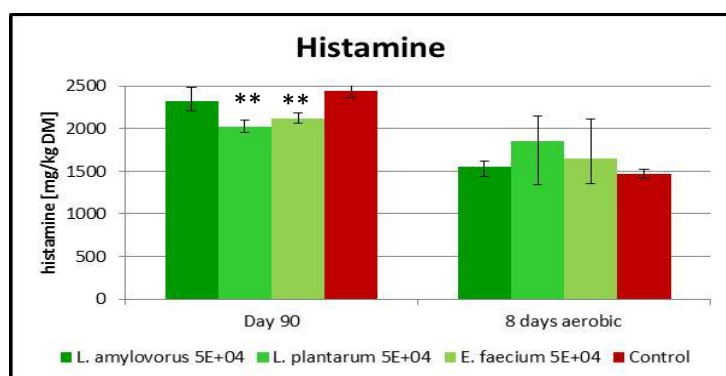


Figure 4 – Histamine levels in alfalfa silage (n=3)⁽¹⁾

⁽¹⁾ *P<0.1 **P<0.05

Low doses of *Lactobacillus brevis* strongly improve quality of grass silage

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Introduction *Lactobacillus brevis* strains are frequently found in fermented foods and silage (Teixera, 1999), and are occasionally also used as inoculants for silage (Tobía et al., 2008; Burghardi et al., 1980). As a heterofermentative species of lactic acid bacteria *Lactobacillus brevis*, when used as a silage inoculant, would be expected to improve aerobic stability of silages. In this trial the properties of one *L. brevis* strain (DSM 23231) to improve the quality of fermentation with respect to acidification, dry matter loss and suppression of undesired microorganisms was investigated in a lab scale silage trial.

Materials and Methods In a lab scale silage trial with wilted grass (Austrian meadow grass, ~38% dry matter (DM)) *Lactobacillus brevis* was used as an inoculant at concentrations of 5×10^4 and 2×10^5 cfu/g. Treated silages were compared to untreated control silages. For each treatment group sample silos were prepared in triplicate per sampling day. Silos were sampled 3, 7, 48 and 92 days after ensiling using silos of 0.5 kg (day 3, day 7) and 2 kg (day 48, day 92), for different days of analysis. The set of analyses carried out at each sampling day comprised determination of Clostridia spores via MPN method, analysis of carbohydrates and organic acids via HPLC, pH measurement via single rod measuring cell, determination of the dry matter (DM) loss via drying (105°C, 24 h) differential weighing and calculation and statistical evaluation (SPSS 19.0, ANOVA plus Tukey HSD or Tamhane T2, U-tests for Clostridia).

Results and Discussion The use of *L. brevis* significantly reduced DM losses by day 92. (loss reduction of 1.0 - 1.5% independently of dosage (Figure 1)) Acidification was strongly facilitated by inoculation with *L. brevis* (Figure 2) as pH values were significantly lower in the treated groups than in the untreated control at all points of time. As a consequence of the low pH value spoilage by clostridia was reduced - the amount of clostridia spores was lowered by up to 3 orders of magnitude by both concentrations of *L. brevis* (Figure 3), which led to a difference in butyric acid fermentation of about 20 – 30 g butyric acid/kg DM between treated and untreated samples. The effect was statistically significant on day 92 independently of dosage. (Figure 4)

Conclusion It is concluded that *Lactobacillus brevis* DSM 23231, even at low dosages of 5×10^4 cfu/g silage, considerably improves fermentation quality of grass silages.

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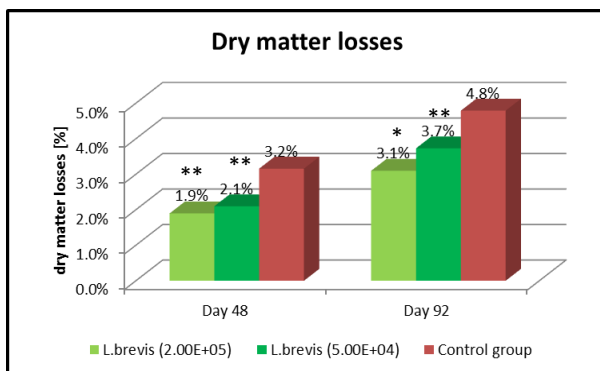


Figure 1 – DM losses in treated and untreated silage samples (n=3)⁽¹⁾

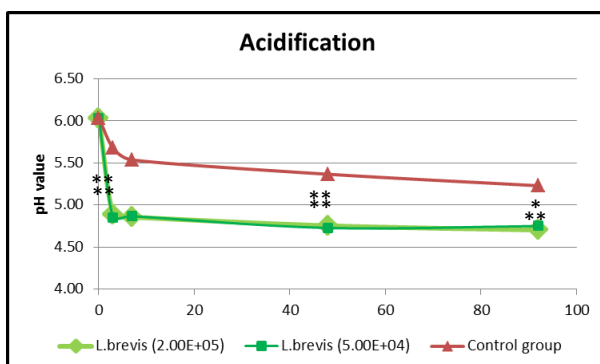


Figure 2 – Acidification of treated and untreated silage samples (n=3)⁽¹⁾

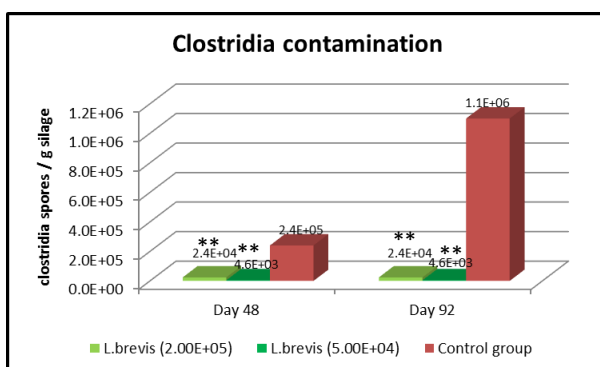


Figure 3 – Amount of clostridia spores in treated and untreated silage samples (n=3)⁽¹⁾

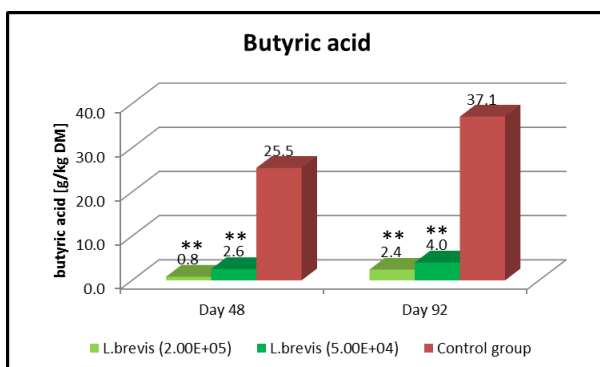


Figure 4 – Butyric acid in treated and untreated silage samples (n=3)⁽¹⁾

⁽¹⁾ *P<0.1, **P<0.05

Stability enhancing effects of *Lactobacillus brevis* on maize silages

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Introduction Upon exposure of silage to air the fungal population starts to propagate. This includes the growth of yeasts, which can be observed as heating on the one hand and visible colonies on the other hand, leading to large economic losses. Such losses can be related to health issues of animals fed contaminated silage, to energy losses of up to 40 % by ethanol fermentation, to shrinkage of the dry matter amount or to portions of the silo which have to be discarded. They can, however, be counteracted if silage is well-preserved and contains anti-fungal substances like inhibiting organic acids. Among these, acetic acid is known as a potent inhibitor, and it can be quite easily produced by heterofermentative species of lactic acid bacteria added a silage inoculant. Therefore, *L. brevis*, a heterofermentative lactobacillus, is expected to improve aerobic stability of silages. In two recent lab scale silage trials the properties of *L. brevis* to improve fermentation quality and prolong the aerobic phase were investigated in maize silages, which are traditionally especially susceptible to aerobic spoilage due to a high sugar content.

Materials and Methods Maize was ensiled in two lab scale trials. One trial was conducted with whole maize plants (MWP), the other with crushed maize grains (CMG). As inoculant, *Lactobacillus brevis* was used at a dosage of 5×10^4 cfu/g silage. Untreated silages served as control. The size of the sample silos was 0.5 or 2.0 kg, depending on the day they were analysed. There were 4 sampling days: 2 and 7 days after ensiling small (0.5 kg) silos were opened, 45 (± 1) and 91 days after ensiling large (2.0 kg) silos were opened. For each treatment group triplicate silos were prepared for each sampling day. The total trial time was 3 months. Analyses comprised aerobic stability testing by differential temperature measurement according to Honig (1990). Aerobic stability was evaluated as the time during which temperature in the silage samples did not increase to more than 3°C above ambient temperature. The test was carried out at an ambient temperature of 23 (± 2) °C. Further analyses included determination of acidification by pH measurement via single rod measuring cell and analysis of anaerobic dry matter (DM) loss by drying (105°C, 24 h), differential weighing and calculation. Statistics were determined via SPSS 10.0 using U-tests or ANOVA plus post-hoc Tukey HSD test.

Results and Discussion It was found that aerobic stability was improved in both whole plant maize silage and crushed maize grains silage by up to 7 days in comparison to the untreated control (Figure 1). Furthermore it was found that fermentation quality of both silages was improved. The dry matter loss was reduced by ~0.5% in both silages in comparison to control (Figure 2). Acidification in both silages was significantly faster ($P < 0.05$ on day 7) in the samples treated with *Lactobacillus* (Figure 3).

Conclusion The addition of *Lactobacillus brevis* successfully improved both the aerobic and anaerobic quality of maize silages. The prolonged aerobic stability grants a longer feedout phase and less mould and yeast spoilage. Quicker acidification results in anaerobically stable silage and prevents growth of anaerobic undesirable microorganisms. The reduction of dry matter loss is an additional economic advantage.

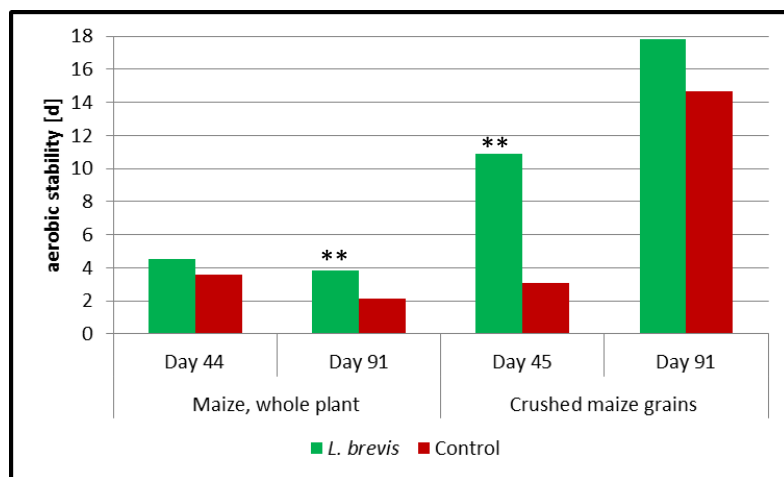


Figure 1. Aerobic stability in *L. brevis*-treated and untreated maize silages (n=3)⁽¹⁾

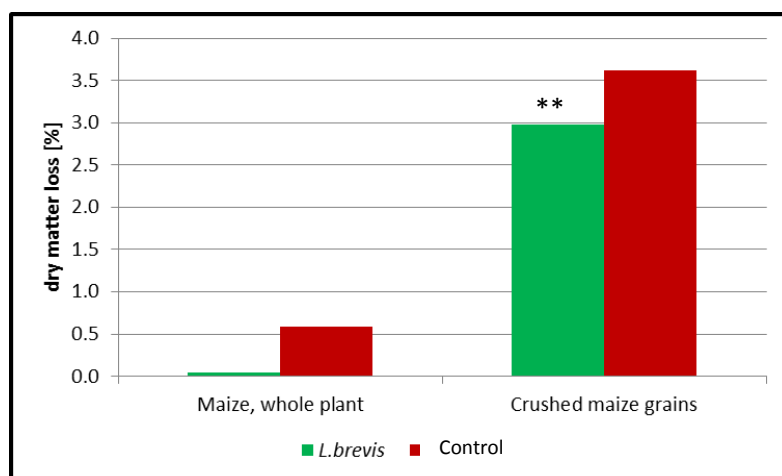


Figure 2. DM loss in *L. brevis*-treated and untreated maize silages (n=3)⁽¹⁾

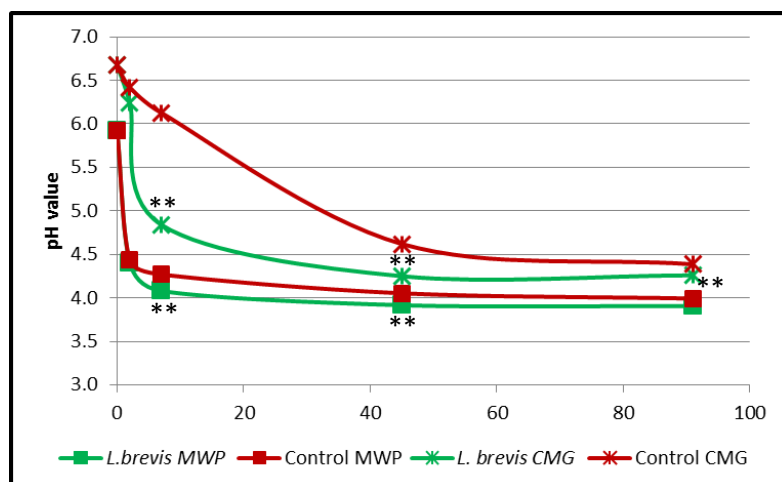


Figure 3. Acidification in *L. brevis*-treated and untreated maize silages (n=3)⁽¹⁾
⁽¹⁾ **P<0.05

Effect of an oxygen barrier film on composition and losses from the upper layers of grass/clover crops ensiled in farm-scale bunker silos

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Introduction. The upper layer of silage in bunker silos covered with conventional polyethylene film can deteriorate if oxygen ingress occurs by permeation through the top polyethylene film during the storage period. Borreani et al. (2007) found, with untreated maize silage stored in a split single farm-scale bunker silo under either oxygen barrier (OB) film or under conventional film (C), that loss of DM from bags buried in the upper 40 cm layer was 37% to 10% for OB and C, respectively ($P=0.04$). The reduction in loss of DM was attributed to a reduction in mould count and was also associated with a reduction in butyric acid spore count (Borreani and Tabacco, 2008). The two studies described here were undertaken with grass/clover crops ensiled in farm-scale bunker silos to compare the effect on silage preservation of a silo sealing system based on OB film compared to a conventional sealing system based on polyethylene film.

Materials and methods. Wilted crops of mixed grass and red clover were harvested with inoculation and ensiled in farm-scale bunker silos. One half of the top surface of each silo was covered lengthways with OB film ("Silostop", S), 45µm thickness, on which was placed a woven polypropylene net. The other half was covered with a single layer of conventional white-on-black plastic film (150µm, C). The conventional film was protected by car tyres. Divisions between sheets and the edges of the silo were weighed down with 20 kg bags of woven polypropylene containing gravel. After 120-day storage periods, four samples of the silage were taken at random to a depth of 30 cm from the top surface of both halves of each silo for analysis. Single pooled sub-samples per treatment were subjected to microbial analysis. In Experiment 1, four gauze cloth bags were filled with the crop during the silo filling period and placed within the top 30 cm of each side of the silo to calculate DM loss. In Experiment 2, losses were calculated by weighing the harvested crop as it was ensiled and by weighing all the material removed from the silo during the feed-out period.

Results and discussion. In both experiments pH and concentrations of $\text{NH}_3\text{-N}$ and butyric acid were lower, whilst concentrations of lactic and acetic acids were higher for silage stored under S than for silage under C ($P<0.05$, Table 1). The higher lactic acid in silage stored under S probably reflected restricted development during the storage period of *Clostridium tyrobutyricum* (Table 2). Losses of DM from bags buried in the top 30 cm of the silo in Experiment 1 averaged 5.0% and 2.5% for C and S, respectively. The total quantity of silage fresh weight discarded in Experiment 2 from the top 30 cm layer because it was judged to be inedible by livestock was 1.7 tonnes (0.1%) and 100 tonnes (5.9% of the total 1700 tonnes fresh weight ensiled) for S and C, respectively. The high mould count found in the crop at harvest in Experiment 2 (Table 2) may have been a contributory factor to the poor fermentation quality of the material stored under C. Counts of yeasts, moulds and clostridial spores were numerically lower in both experiments for silages stored under S than under C, in agreement with Borreani and Tabacco (2008). These

results illustrate the importance of placing an effective protective layer on top of conventional polyethylene film to restrict oxygen permeation as Bernades et al. (2009) also found, or alternatively to use an oxygen barrier film.

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Table 1 Composition of the upper 30cm layers of silages covered with either conventional polyethylene (C) or oxygen barrier film (S).

Type of covering	<i>Experiment 1</i>			<i>Experiment 2</i>		
	C	S	s.e.d.	C	S	s.e.d.
DM (g/kg fresh weight)	310	294	8.86	207	221	9.93
pH	4.5	4.2	0.08	8.5	3.7	1.00
Crude protein (g/kg DM)	151	156	2.25	110	142	5.68
NH ₃ -N (g/kg total N)	59	48	3.40	145	69	3.57
Lactic acid (g/kg DM)	44.0	94.0	5.75	0.60	17.1	6.30
Acetic acid (g/kg DM)	17.5	19.5	0.89	3.10	12.6	1.72
Butyric acid (g/kg DM)	6.20	0.45	0.51	6.10	0.0	0.54

Table 2 Microbiological characteristics (cfu/g) of the crops at harvest upper layer of the upper 30 cm layers of silages covered with either conventional polyethylene (C) or oxygen barrier film (S).

Type of covering	<i>Experiment 1</i>			<i>Experiment 2</i>		
	Crop at harvest	Silage		Crop at harvest	Silage	
		C	S		C	S
Yeasts	4.5 x 10 ⁴	1.3 x 10 ⁴	6.8 x 10 ²	4 x 10 ⁴	2.2 x 10 ⁷	2 x 10 ⁵
Moulds	6.3 x 10 ⁵	6.2 x 10 ³	4.4 x 10 ²	8.85 x 10 ⁸	8 x 10 ⁴	4 x 10 ²
<i>C.tyrobutyricum</i> vegetative cells	-	-	-	ND	1.95 x 10 ⁶	ND
<i>C. tyrobutyricum</i> spores	ND	1.8 x 10 ⁴	6.8 x 10 ²	ND	1 x 10 ²	ND

ND = Not detected

The effect of different types of inoculants on DM losses, fermentation pattern, volatile organic compounds (VOC) and aerobic stability of sorghum silage

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Introduction There has been evidence that volatile organic compounds, e.g. alcohols, organic acids and esters thereof, which can be detected in silages, may detrimentally affect feed intake by dairy cattle (Weiss *et al.*, 2009). In addition, those substances have been discussed in relation to climate-damaging ozone formation, and it was reported that silages on dairy farms may be a significant source of VOC emission (Mitloehner *et al.*, 2009). As the knowledge of the formation of VOC in silages is still very limited, it was the aim of this study to test the effects of different silage additive types on fermentation pattern, production of VOC and aerobic stability. Sorghum was chosen as this silage type is often characterized by excessive ethanolic fermentation so that high production of VOC could be expected.

Material and Methods Sorghum (*Sorghum bicolor*, varieties: Maya and Goliath) was harvested on a dairy farm in Trebbin, State of Brandenburg, Germany on October 13th 2009 by a Class Jaguar, chopped to a theoretical particle size of 20 mm and subsequently filled into 1.5 L glass jars. Silages were anaerobically stored at 25 °C for 105 days, and three replicates per treatment were prepared. The following treatments were tested: C - Control, LP - *L. plantarum* DSM 3676/*L. plantarum* DSM 3677 (50%/50%, 1×10^5 cfu/g forage), LB1 - *L. buchneri* DSM 13573 (1×10^5 cfu/g forage), LB2 - *L. buchneri* DSM 13573 (2.5×10^5 cfu/g forage), LB3 - *L. buchneri* DSM 13573 (5×10^5 cfu/g forage), LP+LB1 (2×10^5 cfu/g forage) and BS – 500 g/t sodium benzoate + 300 g/t potassium sorbate (applied in 2 L/t as aqueous solution). All additives were diluted with tap water to give an application rate of 10 mL/kg fresh forage. The control treatment received 10 mL of tap water per kg fresh forage.

Chemical analysis of the fresh crop was performed according to official German standards for feed evaluation. DM of silages was measured and corrected for the loss of volatiles during drying according to Weissbach and Strubelt (2008). Determination of pH was done potentiometrically using a calibrated pH electrode. Lactic acid was analyzed by HPLC; volatile fatty acids, alcohols and VOC were determined by GC. Ammonia concentration was analysed photometrically. Losses of DM during fermentation were calculated according to Weissbach (2005). Aerobic stability was measured for 14 days by the temperature method (Honig, 1990). Data were statistically evaluated by employing the procedure MIXED of SAS. Differences among means were tested by Tukey test, and significance declared at $P \leq 0.05$.

Results and Discussion Results are only given on the variety Maya. Fresh forage contained 26.1 % DM, 4.6% crude ash, 5.5% crude protein, 29.1% crude fibre and 27.1% water-soluble carbohydrates (all nutrient based on DM).

There was no effect of treatment on DM and ammonia concentration, and all silages remained stable for 14 days (data not shown). The range in final pH was between 3.72 and 3.82, with significant differences between treatments (data not given), but those were without practical relevance.

All parameters presented in table 1 were significantly affected by treatment. Inoculants had only marginal effects on DM losses as compared with control silages, whereas the chemical additive reduced it by >30%. Lactic acid was reduced by all additives containing *L. buchneri* DSM 13573 and by the chemical mix. According to its special mode of action, the single application of the heterofermentative LAB strain increased acetic acid and 1,2-propanediol contents, and reduced ethanol levels. However, the most significant reduction in ethanol was caused by the chemical additive. As reported by Weiss *et al.* (2009), concentration of VOC (ethyl esters) clearly depended on the concentration of ethanol and the respective organic acids. Formation of ethyl lactate was lowest in silages treated either solely with *L. buchneri* DSM 13573 or the chemical mix, whereas a low level of ethyl acetate level could only be ensured by the chemical product.

Data on the Goliath variety were affected by treatment in a similar way as found for the Maya variety (data not shown). VOC were lowest in silages treated with the chemical additive.

Conclusion Silage additives affected DM losses and fermentation pattern, but no effect was found on aerobic stability. Regardless of variety, formation of VOC depended on the level of ethanol, lactic acid and acetic acid, and thus can be modified by silage additive type. The mix of sodium benzoate and potassium sorbate was superior to all other treatments in reducing VOC.

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Table 1. Effects of silage additives on DM losses, fermentation pattern, volatile organic compounds and aerobic stability of sorghum silage

Parameter	Treatment ¹⁾							SED	P level
	C	LP	LB1	LB2	LB3	LB1+ LP	BS		
DM loss (% DM)	9.0 ^c	8.7 ^{bc}	8.7 ^b	9.0 ^{cd}	9.2 ^{cde}	9.5 ^e	6.1 ^a	0.09	<0.001
Lactic acid ²⁾	4.03 ^b	3.83 ^b	2.28 ^a	2.46 ^a	1.78 ^a	2.69 ^a	2.44 ^a	0.11	<0.001
Acetic acid ²⁾	2.73 ^a	2.20 ^a	3.70 ^b	4.55 ^c	5.18 ^c	2.28 ^a	2.79 ^a	0.08	<0.001
Ethanol ²⁾	3.42 ^{cd}	2.88 ^c	1.95 ^b	1.83 ^b	1.96 ^b	3.95 ^d	0.78 ^a	0.10	<0.001
1,2-propanediol ²⁾	1.34 ^b	0.29 ^a	2.47 ^c	3.57 ^d	5.11 ^e	1.22 ^b	1.21 ^b	0.05	<0.001
Ethyl lactate ³⁾	467 ^b	463 ^b	294 ^{ab}	237 ^a	193 ^a	453 ^b	195 ^a	51.6	<0.001
Ethyl acetate ³⁾	120 ^b	123 ^b	119 ^b	128 ^b	106 ^{ab}	105 ^{ab}	44 ^a	17.9	<0.05
Total ethyl esters ³⁾	587 ^b	586 ^b	414 ^{ab}	365 ^{ab}	299 ^a	559 ^b	239 ^a	67.5	<0.001

¹⁾ for description see Materials and Methods; ²⁾ % of DM; ³⁾ mg/kg DM; means in columns with unlike superscripts differ significantly

The effect of different types of inoculants on DM losses, fermentation pattern and aerobic stability of sorghum silage

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Introduction Sorghum silages play an important role in the feeding of ruminants, particularly in semi-arid climate zones. Also in continental Europe, e.g. Germany, this crop has recently attracted significant attention due to its higher tolerance to drought stress than corn. In many areas, especially those with light, sandy soil and low precipitation, sorghum may be an alternative source of silage for ruminants, and also a substrate for biogas production. However, information has been scarce on ensilability, DM losses during fermentation, fermentation pattern and aerobic stability on sorghum grown in temperate climates. Therefore, this study aimed to evaluate the effects of different inoculant types on quality of sorghum silage.

Material and Methods Sorghum (*Sorghum bicolor*, variety: SuperSile 18) was grown on the research farm of Humboldt Universität in Thyrow, State of Brandenburg, Germany and harvested on October 1st, 2007 by a Pöttinger plot harvester. The crop was chopped to a theoretical particle size of 40 mm. The material was packed into either 1.5 L glass jars for strict anaerobic storage for 98 days, or into 1.5 L jars which had a hole (diameter: 5 mm) in the lid of the jar and one at 5 cm above the bottom. These holes were closed by rubber stoppers. On day 28 and 50 of fermentation, the rubber stoppers were removed to allow free air ingress for 24 hours. The length of this type of experiment was 57 days. All jars were stored at constant temperature of 25 °C, and three replicates per treatment were prepared.

The following treatments were tested: C - Control, LP - *L. plantarum* DSM 3676/*L. plantarum* DSM 3677 (50%/50%, 1×10^5 cfu/g forage), LB - *L. buchneri* DSM 13573 (1×10^5 cfu/g forage), LP+LB (2×10^5 cfu/g forage). All additives were diluted with tap water to give an application rate of 10 mL per kg fresh forage. Ten mL of tap water per kg fresh forage were added to the control treatment.

Chemical analysis of the fresh crop was performed according to official German standards for feed evaluation. DM of silages was measured and corrected for the loss of volatiles during drying according to Weissbach and Kuhla (1995). Determination of pH was done potentiometrically using a calibrated pH electrode. Lactic acid was analysed by HPLC; volatile fatty acids and alcohols were determined by GC. Ammonia concentration was analysed photometrically. Losses of DM during fermentation were calculated according to Weissbach (2005). Aerobic stability was measured by the temperature method (Honig, 1990). Data were statistically evaluated by employing the procedure MIXED of SAS. Differences among means were tested by Tukey test, and significance declared at $P \leq 0.05$.

Results and Discussion Fresh forage contained 33.5 % DM, 3.6% crude ash, 3.6% crude protein, 33.1% crude fibre and 18.7% water-soluble carbohydrates (all nutrient based on DM).

Treatment had no effect on DM concentration of silages, and butyric acid was not found in any of the experimental silages (data not shown). Data presented in table 1 indicate pronounced effects of silage additives on DM loss, fermentation pattern and aerobic stability. All inoculant types increased DM losses during fermentation, which can be explained by the observed fermentation pattern. Homofermentative LP increased ethanol content, whereas the heterofermentative *L.*

buchneri-containing additives produced higher amounts of acetic acid (and 1,2-propanediol). Both fermentation pathways result in the production of CO₂, which escapes from the silo. Silages stored for 57 days were more susceptible to aerobic deterioration than was seen in silages after 98 days of fermentation. There was no effect of homofermentative inoculation on aerobic stability. The single use of *L. buchneri* DSM 13573 as well as its application in combination with the homofermentative *L. plantarum* strains significantly improved stability upon exposure to air so that silages remained stable throughout the entire experimental period.

Conclusions Regardless of treatment, all silages were of good fermentation quality as butyric acid was not found and ammonia levels were low. Inoculation of sorghum with different inoculant types resulted in modified fermentation pattern which had the most prominent effect on aerobic stability: The sole use of *L. buchneri* DSM 13573 and its combination with *L. plantarum* DSM 3676 and DSM 3677 significantly improved stability upon exposure to air. Increased anaerobic DM losses associated with the use of *L. buchneri* DSM 13573 are overcompensated by the reduction in aerobic DM losses. The use of this heterofermentative LAB alone or in combination with homofermentative strains is therefore highly recommended.

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Table 1. Effects of inoculants on DM losses, fermentation pattern and aerobic stability of sorghum silage

Parameter	Treatment ¹⁾				SED	P level
	Control	LP	LB	LP+LB		
DM loss (% DM)	6.3 ^a	6.8 ^b	7.5 ^c	7.4 ^c	0.11	<0.001
pH	3.78 ^b	3.76 ^{ab}	3.75 ^a	3.78 ^b	0.01	<0.01
NH ₃ -N (% total N)	7.7 ^b	7.5 ^b	6.7 ^a	6.5 ^a	0.18	<0.001
Lactic acid (% of DM)	4.63 ^b	5.27 ^c	4.20 ^{ab}	3.91 ^a	0.20	<0.001
Acetic acid (% of DM)	1.33 ^a	1.28 ^a	2.73 ^b	2.91 ^b	0.07	<0.001
Ethanol (% of DM)	0.83 ^b	1.33 ^c	0.43 ^a	0.40 ^a	0.06	<0.001
1,2-propanediol (% of DM)	0.13 ^a	0 ^a	3.81 ^c	4.26 ^d	0.10	<0.001
ASTA ²⁾ (hours)	101 ^a	83 ^a	168 ^b	168 ^b	11.48	<0.001
ASTA ³⁾ (hours)	199 ^a	194 ^a	336 ^b	336 ^b	3.13	<0.001

¹⁾ for description see Materials and Methods; ²⁾ aerobic stability measured after 57 days of fermentation for 168 hours; ³⁾ aerobic stability measured after 98 days of fermentation for 336 hours; means in columns with unlike superscripts differ significantly

The effect of inoculants on DM losses, fermentation pattern and aerobic stability of high-moisture corn

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Introduction Inoculants are widely used in high-moisture corn (HMC) to improve its stability upon exposure to air. Numerous commercial products are available in different market places, and frequently it is claimed that the number of different lactic acid bacteria strains as well inoculation rate influence the result of additive application. Therefore, the aim of the study was to compare different commercially available inoculants (pure heterofermentative products and combinations of homo and heterofermentative bacteria) regarding their effects on DM losses, fermentation pattern and aerobic stability of HMC.

Material and Methods HMC was grown on a dairy farm in Trebbin, State of Brandenburg, Germany and harvested on 18 October 2010 by Class Jaguar and subsequently crimped. The material was packed into either 1.5 L glass jars for strict anaerobic storage for 97 days, or into 1.5 L jars which had a hole (diameter: 5 mm) in the lid of the jar and one at 5 cm above the bottom. These holes were closed by rubber stoppers. On day 28 and 56 of fermentation, the rubber stoppers were removed to allow free air ingress for 24 hours. The length of this type of experiment was 63 days. All jars were stored at constant temperature (25 °C), and three replicates per treatment were prepared.

All inoculant products were purchased from authorized dealers in Germany or Sweden. The following treatments and inoculation rates (IR, cfu/g fresh forage) were tested: T1 – no additive, T2 – *L. plantarum* DSM 12837, *L. buchneri* DSM 12856, *P. pentosaceus* DSM 12834: IR 2.5×10^5 cfu/g; T3 – *L. buchneri* NCIMB 40788: IR 5.0×10^5 cfu/g; T4 – *L. plantarum* DSM 3676, *L. plantarum* DSM 3677; *L. buchneri* DSM 13573: IR 2.0×10^5 cfu/g; T5 – *L. buchneri* DSM 13573: IR 1.0×10^5 cfu/g; T6 – *L. buchneri* CCM 1819: IR 1.5×10^5 cfu/g; T7 – *L. buchneri* LN 4637 ATCC PTA-2494: IR 1.1×10^5 cfu/g. All additives were diluted with tap water to give an application rate of 10 ml per kg fresh forage, and the control treatment received 10 ml of tap water per kg fresh forage.

Chemical analysis of the fresh crop was performed according to official German standards for feed evaluation. DM of silages was measured and corrected for the loss of volatiles during drying according to Weissbach and Strubelt (2008). Determination of pH was done potentiometrically using a calibrated pH electrode. Lactic acid was analyzed by HPLC; volatile fatty acids, alcohols were determined by GC. Ammonia concentration was analysed photometrically. Losses of DM during fermentation were calculated according to Weissbach (2005). Aerobic stability was measured for 17 days by the temperature method (Honig, 1990). Data were statistically evaluated by employing the procedure MIXED of SAS. Differences among means were tested by Tukey test, and significance declared at $P \leq 0.05$.

Results and Discussion Fresh HMC contained 63.5 % DM, 1.3% crude ash, 8.0% crude protein, 75.7% starch and 3.5% water-soluble carbohydrates (all nutrients based on DM). Epiphytic microbial counts (per g fresh matter) were as follows: lactic acid bacteria: 3.7×10^5 cfu/g, total yeasts: 5.2×10^5 cfu/g, lactate-assimilating yeasts: 2.5×10^5 cfu/g, moulds: 5.7×10^4 cfu/g.

With the exception of T4, all treatments increased DM losses if compared with untreated silages. This observation was reflected by the typical shift in fermentation pattern, caused by *L. buchneri*-inoculation. Lactic acid is partially degraded into acetic acid and 1,2-propanediol, thereby generating CO₂, which escapes from the silo (Oude-Elferink *et al.*, 2001). Aerobic stability after 63 days of fermentation was improved only by products which solely contained *L. buchneri*. Increasing the fermentation length not only produced more stable untreated silages, but also excellent results on aerobic stability for all other treatments. Higher aerobic stability is associated with a marked reduction in DM loss upon exposure to air during feed-out so that elevated anaerobic DM losses by heterofermentative inoculation are acceptable. Even more importantly, the use of heterofermentative additives results in a net saving of DM from ensiling to feeding (Weissbach, 2011).

Conclusions Regardless of composition and inoculation rate, all products were shown to be suitable additives to improve aerobic stability of HMC. However, combinations of homo- and heterofermentative lactic acid bacteria should only be used if fermentation length exceeds 3 months, whereas products only containing *L. buchneri* showed their full effects already after 9 weeks even under challenging test conditions.

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Table 1. Effects of silage additives on DM losses, fermentation pattern and aerobic stability of high-moisture corn after 97 days of fermentation (unless otherwise stated)

Treatment ¹⁾	DML ²⁾ (% DM)	pH	LA ³⁾	AA ⁴⁾	EtOH ⁵⁾	1,2PD ⁶⁾	ASTA ⁷⁾	ASTA ⁸⁾
			(% of DM)				(days)	
T1	3.3 ^b	3.98 ^b	1.71 ^{cd}	0.30 ^a	0.32 ^{ab}	0 ^a	0.8 ^a	6.6 ^a
T2	3.6 ^c	3.98 ^b	1.71 ^{cd}	0.80 ^c	0.31 ^{ab}	0.77 ^d	3.0 ^a	15.9 ^c
T3	4.3 ^f	4.44 ^e	0.61 ^a	1.41 ^e	0.56 ^c	1.43 ^f	16.8 ^c	17.0 ^c
T4	3.2 ^a	3.94 ^a	2.01 ^d	0.49 ^b	0.26 ^a	0.13 ^b	1.2 ^a	10.7 ^b
T5	4.4 ^g	4.48 ^f	0.72 ^{ab}	1.39 ^e	0.72 ^d	1.48 ^f	15.0 ^c	16.7 ^c
T6	3.7 ^d	4.06 ^c	1.46 ^c	0.77 ^c	0.38 ^b	0.63 ^c	8.5 ^b	16.6 ^c
T7	4.1 ^e	4.29 ^d	1.01 ^b	1.12 ^d	0.51 ^c	1.09 ^e	9.3 ^b	17.0 ^c
SED	0.02	0.011	0.098	0.047	0.030	0.026	0.89	0.13
Significance	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

¹⁾ for description see Materials and Methods; ²⁾ DM loss; ³⁾ lactic acid; ⁴⁾ acetic acid; ⁵⁾ ethanol; ⁶⁾ 1,2-propanediol;

⁷⁾ aerobic stability after 63 days of fermentation; ⁸⁾ aerobic stability;

means in columns with unlike superscripts differ significantly

Minimum temperature for successful silage making

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Introduction For farmers, who produce silage in climatic zones, in which low temperatures (below 0°C) are common at the end of the growing season, information on the minimum temperature for the successful ensilage of forage corn would be helpful. However, this kind of information is hard to find in literature. If ambient temperature at ensilage is too low, the fermentation process is impeded. As long as temperatures stay sub zero, the unfermented forage might not markedly deteriorate. However, when temperatures rise again, maybe months later, there is an apparent risk that the forage might not properly ferment but heat up and eventually mould. The goal with this project was to get an indication on the minimal temperature for the successful fermentation of corn silage.

Materials and Methods During two successive years, fresh whole crop corn was obtained from local farmers (N 59° 58', E 17° 31'). The forage was chopped in a precision-chop harvester and ensiled in laboratory silos (1.7 L glass jars with water-filled air locks mounted on lids). The forage was kept at a low temperature (2-6°C) until all silos were filled. After homogenizing and sampling of the fresh forage, 10 or 14 silos per storage temperature were filled and moved into climate chambers or ventilated refrigerators set at 3 or 4 different temperatures. During the first year, mean storage temperatures were 6°, 12° and 18°C and during the second year, mean silage temperatures were 2.6°, 6.7°, 13.6° and 21.1°C. In the first year, 16 silos were moved from 6°C to 18°C after 45 days to see which effect that would have on silage pH compared to silages that had been stored at 18°C from the beginning. To study the pH course during silage fermentation, 2 silos from each storage temperature were opened and sampled at 5 or 7 occasions during a storage period of approx. 60 days. Aerobic stability was determined only in the first year (according to Honig, 1990).

Results and Discussion The composition of the fresh corn material is shown in Table 1. Cobs were more mature during the second than the first year and the epiphytic LAB counts were very high compared to grass-based forages. In the first year silages were well fermented with the exception of high ethanol levels (Table 2). Aerobic stability was high (>7 days) in all these silages irrespective of storage temperature. From the course of the pH decrease in silages (Figure 1) it became evident that even at temperatures as low as 2.6° an acid formation and pH-decrease occurred. However, pH values of these silages leveled out at a much higher level as compared to silages stored at higher temperatures. When silages were moved after 45 days from 6° to 18°C ambient temperature, pH values dropped to approximately the same level as in silages stored at 18°C from the beginning. The initial pH increase in silages stored at 2.6° and 6°C might be explained by a longer aerobic phase because plant respiration was restricted by low temperatures. First after oxygen is depleted, lactic acid producing bacteria may be able to grow readily and decrease silage pH.

Conclusions This experiment indicates that a fermentation process (pH decrease) occurs even at temperatures as low as 2°C. The pH-decrease was, however, much slower and final pH values leveled out at considerably higher values than in silages stored at common temperatures.

Considering the limited data on fermentation quality and aerobic stability, the minimum temperature lays probably between 2° - 6°C.

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Table 1. Composition of the fresh corn material. Values in g/kg DM if not stated otherwise.

Year	Lot	DM	Ash	WSC	Starch	NDF	CP	pH	BC ^a	FC ^b	LAB ^c
2008	1	213	52	61	156	511	100	5.00	2.50	41	1.3x10 ⁷
2009	2	293	39	75	199	475	86	4.54	1.93	60	2.2x10 ⁷

^aBC = buffering capacity (g lactic acid to reduce pH in 100 g DM from 6.0 to 4.0); ^bFC = fermentability coefficient = DM/10 + (0.8 x WSC/BC) (Weissbach 1996); ^cLAB = lactic acid bacteria in the fresh crop (cfu/g FM).

Table 2. Composition of silages from the first year after 61 days. Silos which were moved from 6° to 18°C after 45 days (6°→18°C) were analyzed after 106 days (= 45+61).

Storage temp.	DM	N-NH3 ^a % of N	Lactic	Acetic	Butyric	Propionic	2,3-Butanediol	Ethanol
			g/kg DM					
6°C	219	6.1 ^a	34 ^d	16 ^d	<0.6	0.3 ^c	3.2 ^b	19.7 ^b
12°C	224	6.7 ^b	50 ^c	2.0 ^c	<0.6	0.3 ^c	3.2 ^b	13.7 ^c
18°C	218	7.0 ^c	59 ^b	29 ^b	<0.6	1.3 ^b	2.9 ^b	10.4 ^d
6°→18°C	215	7.5 ^c	61 ^a	34 ^a	<0.6	2.3 ^a	4.6 ^a	22.6 ^a
Mean	219	6.8	51	25	<0.6	1.1	3.5	16.6
Prob. ^c	NS	**	***	***	NS	***	**	***
MSD ^b	183	0.81	1.8	1.8	-	0.6	0.7	2.8

^aN-NH3 = ammonia-N in % of total N; ^bMSD = minimum significant difference between two means ($p = 0.05$);

^cProb. = Probability that all treatment means are equal; NS = not significant.

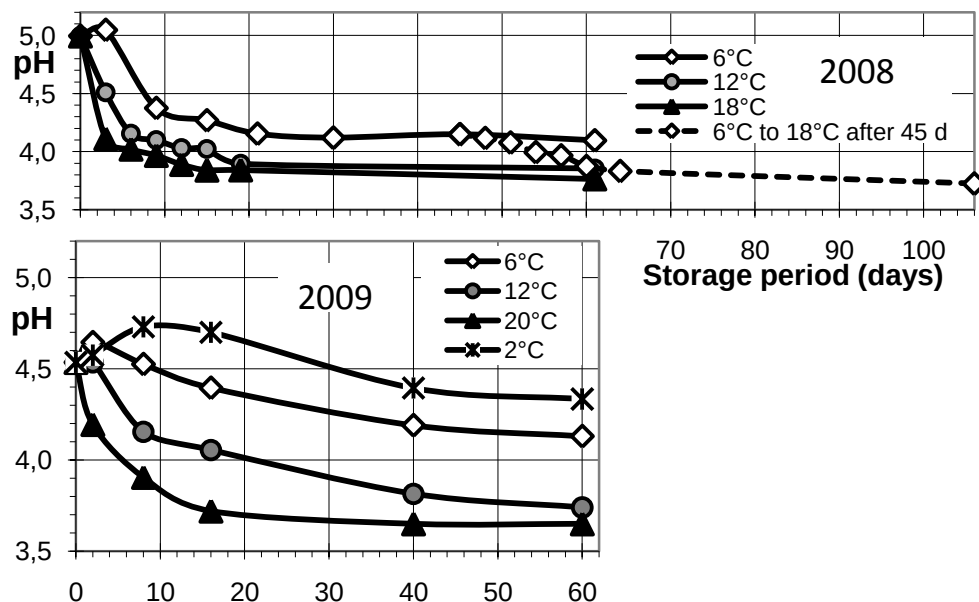


Figure 1. Course of pH drop in silages stored at different temperatures (year 2008 + 2009).

Chemical composition of elephant grass clones (*Pennisetum purpureum* Schum) silage

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Introduction It is recommended to cut the elephant grass around 50 to 60 days for silage, but at this age the dry matter content is about 15 to 20 %, which limits its use at silage process (Lavezzo, 1985). This problem has stimulated research activities aiming at the selection of new genotypes with higher DM content (Freitas et al., 2004). In this perspective, through the agreement between IPA/UFRPE surveys have been conducted to evaluate *Pennisetum* sp. with higher DM content. The purpose of this experiment was to evaluate the chemical composition of five elephant grass clones (*Pennisetum purpureum* Schum) silages.

Materials and Methods Three clones of short height (Mott, IPA/UFRPE Taiwan A-146 2.27 and IPA/UFRPE Taiwan A-146 2.37) and two of high height (Elephant B and IRI 381) from Experimental Station of IPA – Itambé (PE) were evaluated. The crops were harvest at 70 days of growing, samples were collected to analysis (Table 1), and ensiled in PVC silos with four replications, that remained closed for 120 days. The relation between WSC and buffer capacity, considering the DM content, was used to characterize the Fermentation Coefficient, as cited by Oude Elferink et al. (2000). At the silos opening, samples were collected for DM, CP, NDF, ADF, pH, NH₃-N and WSC analysis. Furthermore, total carbohydrate and non-fiber carbohydrates were estimated (Hall, 1999). A completely randomized design with four replicates was used and data were submitted to analysis of variance and means were compared by Tukey test at 5%.

Results and Discussion In general, looking at Tables 1 and 2, it can be seen that the ensiling process promoted small change in chemical composition. However, this statement is based only on numerical values, because it was not possible to perform statistical analysis between chemical compositions of the crops and the silages, since the crops were not distributed on the field in a way that permitted replications. Short height clones (Table 2), even mentioned as those with lower chance of success fermentation by applying FC equation, were those that resulted in silages with the highest ($P<0,05$) levels of residual WSC. It is pertinent to emphasize that the percentage of WSC consumption during the fermentation process for silages made with tall clones were higher than those obtained in short height clones silages (values of 97.9, 90.3, 90.3, 82.9 and 69.8% for Elephant B, IRI 381, Taiwan 2.37, Taiwan 2.27 and Mott, respectively). Furthermore, this result contributed to protein preservation that can be confirmed by the lower NH₃-H values (Table 2) of short height clones silages. These results enhance the idea that it is not necessary the use of additive or wilting to achieve success in the ensiling of the short height clones silages evaluated in this study.

Conclusions Mott, IPA/UFRPE Taiwan A-146 2.27 and IPA/UFRPE Taiwan A-146 2.37 silages, made at 70 days of growing, have higher CP and residual WSC fractions than Elephant B and IRI 381 silages.

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Table 1. Chemical composition of five clones from elephant grass, grown at Itambé (PE)

Item	Elephant B	IRI 381	Taiwan	Taiwan	Mott
			A-146 2.27	A-146 2.37	
DM, %	30.48	34.61	25.74	23.82	23.95
CP, % DM	4.22	3.35	6.41	5.87	6.55
NDF, % DM	76.46	78.50	71.72	73.11	69.46
ADF, % DM	49.81	52.15	43.58	46.74	43.39
BF ¹ , eq mg NaOH/100g DM	13.07	10.88	18.00	20.41	23.29
WSC, % DM	18.90	15.86	19.60	15.08	10.26
FC ³	42.08	46.27	34.45	29.73	27.47
TC ⁴	89.67	84.56	84.67	84.56	87.26
NFC ⁵	13.21	12.66	12.95	11.45	13.76
WSC: CP ratio	4.50	4.70	3.10	2.60	1.56

¹Buffer capacity, ² Water soluble carbohydrate, ³Fermentation coefficient, ⁴Total carbohydrate, ⁵ Non-fiber carbohydrates

Table 2. Chemical composition of elephant grass clones silage

Item	Elephant B	IRI 381	Taiwan	Taiwan	Mott	CV (%)
			A -2.27	A - 2.37		
DM. %	31.92 b	35.82 a	26.59 c	27.35 c	24.09 d	2.59
CP. % DM	4.23 b	3.20 c	5.68 a	5.69 a	6.31 a	6.23
NDF. % DM	74.26 a	75.23 a	68.73 ab	68.66 ab	67.13 b	4.42
ADF. % DM	52.00 a	42.28 b	55.88 a	41.76 b	41.02 b	9.09
pH	4.10 ab	3.52 c	3.87 b	4.12 a	4.02 ab	2.86
NH ₃ -N ¹ . % total N	2.58 b	4.08 b	7.00 a	6.33 a	3.73 b	17.09
WSC ² . % DM	0.39 c	1.22 bc	1.89 ab	2.58 ab	3.10 a	35.83

¹Ammonia nitrogen. ² Water soluble carbohydrate

Effect of ensilage on kinetic of ruminal degradation of elephant grass clones (*Pennisetum purpureum* Schum.)

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Introduction Elephant grass (*Pennisetum* sp.) stands as one of the most promising species for silage making. However, the low DM content at the age indicated to harvest may limit its use to silage making. The amount of WSC necessary to obtain adequate fermentation depends on the DM content and the buffer capacity of the crop (Oude Elferink et al., 2000). Ensiling of forages with low DM content leads to high WSC consumption by the silage microflora. In addition to the WSC loss, the ensilage may decrease protein by extensive proteolysis (McDonald, et al 1991). The aim of this work was to evaluate the effect of ensilage on kinetic of DM and NDF ruminal degradation of elephant grass clones (*Pennisetum purpureum* Schum.).

Materials and Methods Three clones of short height (Mott, IPA/UFRPE Taiwan A-146 2.27 and IPA/UFRPE Taiwan A-146 2.37) and two of high height (Elephant B and IRI 381) from Experimental Station of IPA – Itambé (PE) were evaluated. The crops were harvest at 70 days of growing, ensiled in PVC silos and stored for 120 days. To evaluate the effect of ensiling on DM *in vivo* ruminal degradation of the five clones, before and after ensiling, four adult sheep (40 kg of LW) fitted with permanent rumen fistula were used. Samples were weighed into 7 x 14 cm nylon fiber bags (28.6 mg DM/cm²) and were suspended, in duplicate, for periods of 3, 6, 12, 24, 48, 72 and 96 hours in the rumen. An additional two bags per treatment, washed by procedure outline previously, were used for zero-hour. Washed nylon bags were dried (65°C for 72 h) and the content of bag was subjected to NDF analysis (Van Soest et al., 1991). The exponential model of Ørskov and McDonald (1979) was used to compute the kinetic of DM and NDF degradation. A completely randomized design in a split-plot scheme with four replicates was used (clone on the plots and ensilage on subplot). Data were submitted to analysis of variance and means were compared by Tukey test at 5%.

Results and discussion The soluble fraction represents the fraction of the plant that is readily available to the rumen microorganisms, but the process of silage can change it. Elephant B and IRI 381 clones had decrease this fraction for DM (Table 1). IPA/UFRPE-Taiwan A-146 2.27 and IPA/UFRPE Taiwan A-2.37 clones did not alter the soluble fraction after ensilage. It is noteworthy that the ensiling can increase the soluble fraction, as occurred in this study with Mott clone. The rise in this fraction can be assigned to the higher CP, lower NH₃-N and residual soluble carbohydrates content shown by Mott silage. There was no significant difference between the rates of degradation of *b* fraction (*c* parameter), for clones and ensiling effect. This fraction ranged from 1.16 to 4.15 for NDF and from 1.59 to 4.09 % per hour. According to Sampaio et al. (1995), rates of dry matter degradation less than 2% per hour indicate low quality food, since they require long time to be degraded in the rumen. For Mertens (1993), NDF degradation rates between 2% and 6% per hour are considered as good for forage of appreciated quality.

Conclusion The ensilage process causes greater impact on the soluble fraction in the rumen (*a* fraction) in Elephant B and IRI 381 clones (high stature). However, the potentially degradable fraction in the rumen did not differ between the clones, regardless of stature, nor suffer action of the ensilage (when the process is well done).

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Table 1 Kinetic of DM and NDF rumen degradation of elephant grass clones and its silages

Item	a ¹ (%)		b ² (%)		c ³ (%/h)	
	DM	NDF	DM	NDF	DM	NDF
Elephant B						
<i>Fresh forage</i>	31.28 Aa	7.48 Aa	46.71 Aa	66.38 Aa	2.22 Aa	2.19 Aa
<i>Silage</i>	16.90 Bb	0.92 Bc	49.61 Aa	57.61 Aa	1.59 Aa	1.51 Aa
IRI 381						
<i>Fresh forage</i>	25.68 Ab	7.48 Aa	49.25 Aa	50.46 Aa	4.09 Aa	1.94 Aa
<i>Silage</i>	22.20 Ba	5.47 Ab	42.02 Aa	66.69 Aa	3.49 Aa	1.16 Aa
IPA/UFRPE Taiwan A-146 2.27						
<i>Fresh forage</i>	23.23 Abc	4.04 Ab	41.23 Aa	49.55 Aa	3.51 Aa	3.78 Aa
<i>Silage</i>	24.53 Aa	1.76 Ac	47.58 Aa	53.60 Aa	3.69 Aa	3.08 Aa
IPA/UFRPE Taiwan A-146 2.37						
<i>Fresh forage</i>	22.68 Abc	8.97 Aa	43.44 Aa	58.93 Aa	2.02 Aa	2.62 Aa
<i>Silage</i>	22.15 Aa	11.46 Aa	41.36 Aa	62.86 Aa	2.63 Aa	1.98 Aa
Mott						
<i>Fresh forage</i>	19.67 Bc	2.54 Ab	48.92 Aa	66.38 Aa	1.69 Aa	4.15 Aa
<i>Silage</i>	25.35 Aa	4.87 Ab	42.23 Aa	57.61 Aa	3.99 Aa	2.45 Aa
CV(%) clones	10.75	25.32	23.98	21.76	23.76	29.95
CV(%) ensiling	6.10	21.42	28.05	20.02	34.03	24.54

¹Wash loss. ²Degradability of water insoluble fractions. ³Degradation rate of b fraction.

Values with different letters to the same variable, lowercase for clones and uppercase for ensiling, differ by Tukey test (5%)

Microbiological evaluation of wet brewer's grain silage at different opening periods

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Introduction The searching for alternative foods in order to assure the efficiency in animal diets through the periods of weather changes (such as winter or a dry season) is relevant. Nowadays, the wet brewer's grain (WBG) plays an important role because of its high nutritional quality and availability. On the other hand, there are limitations on the use of this byproduct in animal feed, which are mainly related to the typical low dry matter (DM) content, compromising the transport and storage. In general, given good management and the improved equipment available today, silage fermentation offers the best means to maximize and preserve the nutritive value of a given material (Pahlow et al., 2003). It must be noted that the microbial population of a particular product is not the same as that found during the ensiling process and depends, among other factors, on pH and dry matter content found in silage. The present study aimed to perform microbiological evaluations of wet brewer's grain (WBG) submitted to different ensiling periods, through the counts of aerobic bacteria, filamentous fungi, *Bacillus cereus* and *Escherichia coli*.

Materials and Methods The trial was carried out in Manaus, AM, Brazil and consisted of five treatments (10, 17, 24, 31 and 38 days of ensiling) with three replications. The material was ensiled in PVC experimental silos (10 cm diameter x 50 cm) with capacity of approximately 4 kg of fresh weight. The samples were taken of WBG *in natura* and at the time the silos were opened according to the different storage periods to evaluate the fresh and ensiled microbial quality of the WBG. In the sample collection it was considered only the middle portion of the silos. The partial dry matter (DM) values were determined in comparison to the DM amounts obtained with the microwave oven method. The DM content, total DM losses (Schmidt, 2006) and pH in silages were also evaluated. The microbial population has been shown to vary with the pH range. Data obtained from DM, pH and DM losses of WBG were submitted to variance analysis using a completely randomized design and the means were compared by the Tukey test.

Results and Discussion Mean DM content (Table 1) are not appropriated for silage, however they are between the variations (9% to 30%) found for this product. Probably the behavior seen, about the pH values, can be attributed to insufficient production of lactic acid, since this may not have been produced at inhibitory levels to keep the pH low. However, the initial pH value (5.65) was suitable for the material ensiling. After 10 days, mean DM losses reached 7.45% which indicates a trend of increased losses strongly linked to the low DM content (23.99 %). It was considered appropriate to use the microwave oven to determine the DM content of WBG silages, due to the similarity of values obtained with oven drying (Table 1). The effect of opening day ($P < 0.05$) was observed at 38 days when the results have shown satisfactory silage quality. Silage fermentation was able to reduce the population of aerobic bacteria and filamentous fungi in silages at 10 days (Figure 1), which explains the importance of the rapid decline in pH. Between 31 and 38 days of ensiling the counts observed were close to those found in the original samples for *Bacillus cereus* and *Escherichia coli* that can be related to the growth of lactic acid bacteria (LAB) and decrease in pH.

Conclusion The microwave oven can be used to obtain the DM content of WBG silage in 12 minutes. The DM losses are considered normal at the level of experimental silos. The silages in T5 (38 days) presented better quality. Silage promoted a significant reduction of mesophilic aerobic bacteria, filamentous fungi, *Bacillus cereus* and *Escherichia coli* compared to the fresh material.

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Table 1. WBG silage fermentation variables according to different days of ensiling

Days of ensiling	Variable ¹				
	DM, %	DMmw, %	DMo, %	pH	DM losses, %
0*	23.93 ^b	24.01 ^d	25.77 ^{ab}	5.64 ^a	0.0 ^d
10	23.99 ^b	25.20 ^{cd}	25.04 ^b	3.56 ^c	7.45 ^c
17	23.16 ^{ab}	27.76 ^{ab}	26.51 ^{ab}	3.99 ^b	8.37 ^c
24	25.08 ^{ab}	26.67 ^{bc}	26.17 ^{ab}	4.34 ^b	10.70 ^b
31	25.07 ^{ab}	27.02 ^{abc}	26.72 ^a	4.33 ^b	13.22 ^a
38	25.29 ^a	28.86 ^a	27.18 ^a	4.0 ^b	12.03 ^{ab}
CV, % ²	1.90	2.88	2.29	3.32	8.90

Means followed by different letters in the same column differ (P<0.05) by Tukey test.

*WBG *in natura*.

¹DMmw = dry matter obtained with microwave oven; DMo = dry matter determined with oven drying.

²CV = coefficient of variation.

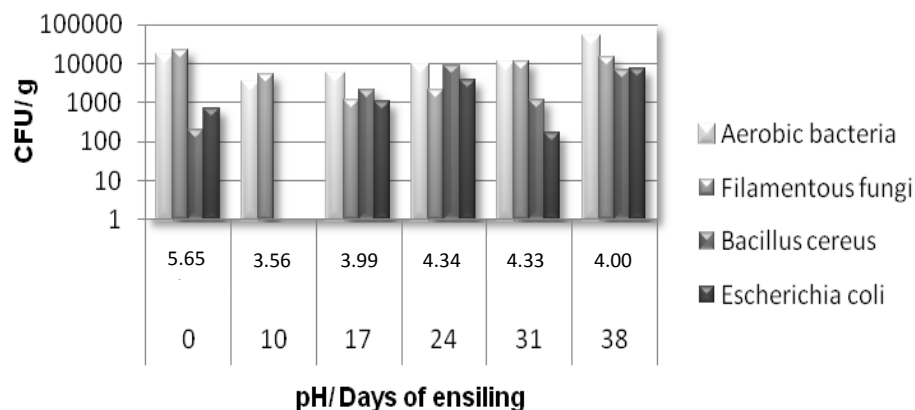


Figure 1. Colony forming units (CFU/g) of aerobic bacteria, filamentous fungi, *B. cereus* and *E. coli* found in the WBG silages related to the pH range and days of ensiling.

Bromatological and chemical composition during the silage fermentation of moist grape residue

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Introduction The production of the wine industry is growing in Brazil, generating large amounts of waste. Use of this product in animal feed could come to assist in environmentally friendly destination of waste. However, studies are needed to evaluate the addition of this material in animal feed. Despite the seasonality in the availability of this by-product, silage could increase the supply of food in periods of scarcity, and reduce the costs of animal production. The purpose of this study was to evaluate the efficiency of silage fermentation of moist grape residue (MGR) through the bromatological and chemical composition on different days of opening.

Materials and Methods For this study, 36 polyethylene buckets (4 liters) were used as experimental. The MGR was placed, compacted and then the silos were closed and sealed. Two samples were collected from *in natura* material to represent the time zero, the remaining silos were opened in the following days: 1, 3, 6, 9, 12, 24, 36, 48, 62, 75, 88 and 96. The samples were subjected to pH, dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and *in vitro* dry matter digestibility (IVDMD) analysis according to procedures recommended by Mizubuti et al. (2009). It was used a completely randomized design with three replications. The results were submitted to ANOVA and Tukey test at 5% probability.

Results and Discussion The statistical analysis showed no difference ($P > 0.05$) for the parameters: DM, OM, NDF, ADF and ADL, across different opening days. The DM value of the *in natura* material was 33.73%, which is within the recommended range for making silage. The average OM content found in MGR silage was 98.06%. Rotava (2007) found the value of 97.4% of OM, working with moist grape marc. The cell wall constituents: NDF, ADF and ADL, showed average values of 67.51%, 56.55% and 41.68%, respectively. Those values can be explained by the high content of seeds (61% in DM). The average CP of silage was 12.20%, with significant differences only between the days 1 and 62 (Table 1). However, Larwence et al. (1984) said that this protein has low digestibility, and that together with the levels of cell wall components and the condensed tannins, impairs the digestibility of this by-product. In this work we observed an average of 51.98% for IVDMD, possibly indicating that the constituents, except for lignin and tannins, have a high digestibility. Dantas et al. (2008) found the value of 46.02% working with dehydrated residue of grapes. The average EE content of the silage was 7.91%. In the literature values range from 5.63% (Dantas et al., 2008) to 16.20% (Rotava, 2007). Wastes from the grape are very variable due to, among other factors, managements and climates in which they were grown and to the harvesting point, which explains some of those variations in the constituents. Regarding some differences in the pH, the values for the silages remained within a range of 4.21 (day 1) to 4.57 (day 96), and the value of *in natura* material was 4.63. In general, values below 4.2 are considered ideal for pH. However, Jobim et al. (2007) argued that this variable should be used with discretion, as unconventional materials can have pH values above 4.2. Woolford (1984) showed that inhibition of secondary fermentation depend mainly on the speed of pH drop,

ion concentration and humidity of the environment. Given the values of day zero and day one, it is possible that a very rapid decrease in pH had occurred, early in the fermentation period.

Conclusion The process of ensiling was efficient in preserving the material, preventing losses in the bromatological and chemical composition of silages studied. MGR silage showed characteristics that enable its use for animal feed.

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Table 1. Bromatological and chemical composition of silage of moist grape residue on different days of opening

Days of Opening	DM, %	OM	CP	EE	NDF	ADF	ADL	IVDMD	pH
% of DM									
0	33.73	97.97	11.61 ^{ab}	7.64 ^{abc}	68.82	57.25	41.41	52.38 ^{abc}	4.63 ^c
1	33.71	98.01	11.13 ^b	7.38 ^{abc}	70.11	57.89	43.51	50.88 ^{abc}	4.21 ^a
3	33.23	97.97	11.66 ^{ab}	6.98 ^{abc}	65.74	55.39	42.05	52.16 ^{abc}	4.43 ^{bc}
6	33.25	97.92	11.58 ^{ab}	7.94 ^{abc}	70.08	56.72	42.27	50.63 ^{abc}	4.42 ^{bc}
9	33.24	98.06	11.91 ^{ab}	6.57 ^b	64.93	59.57	41.70	48.10 ^c	4.32 ^{ab}
12	33.45	97.99	12.03 ^{ab}	6.06 ^c	66.81	56.36	41.90	50.51 ^{abc}	4.41 ^{bc}
24	33.12	98.01	12.70 ^{ab}	7.18 ^{abc}	66.59	54.99	40.22	51.42 ^{abc}	4.45 ^{bc}
36	32.40	97.92	12.35 ^{ab}	7.45 ^{abc}	68.94	56.34	40.40	50.38 ^{bc}	4.44 ^{bc}
48	35.02	97.78	12.30 ^{ab}	9.57 ^a	66.89	54.60	39.22	55.40 ^a	4.54 ^c
62	33.99	98.04	13.16 ^a	9.08 ^{ab}	67.93	56.58	42.75	54.40 ^{ab}	4.51 ^{bc}
75	34.40	97.90	12.42 ^{ab}	9.14 ^{ab}	68.23	56.86	42.03	52.29 ^{abc}	4.54 ^c
88	32.45	97.80	12.60 ^{ab}	8.15 ^{abc}	69.52	57.45	41.25	53.57 ^{ab}	4.52 ^{bc}
96	34.57	97.75	12.55 ^{ab}	9.38 ^a	64.32	55.83	42.86	54.16 ^{ab}	4.57 ^c

^{a-c} means within a column with different superscripts differ (P < 0.05)

Dehydration curve of Tifton 85 hay dried in the sun and in the shed

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Introduction The negative consequences for a hay that wasn't dried enough, due to unfavorable environmental conditions are associated with reduced nutritional value, primarily by microbial activity, followed by heat generation, which favors the oxidation of non-structural carbohydrates, increase of fibrous components, protein degradation and the growth of fungi, which may be associated with the production of mycotoxins. These changes may decrease animal performance (Coblentz et al., 2000). The main factors affecting the rate and extension of hay-making are the initial water content, the age of re-growth and environmental factors such as: solar radiation, temperature, air humidity, soil moisture, rainfall and plant characteristics associated with the management of hay-making process, influencing both the process of drying and wetting (Rotz, 1995). The aim of this study was to access the dehydration curve of Tifton 85 hay dried in the field under the sun or in the shed.

Materials and Methods The experiment took place between October 2010 and January 2011, in a field of hay production implanted with Tifton 85 in Marechal Cândido Rondon, Paraná, Brazil (24 ° 33'40"S, 54 ° 04'12"W, elevation 420 m). The experimental plots were five nitrogen rates (0, 25, 50, 75 and 100 kg ha⁻¹), two ages of re-growth (28 and 35 days) and four replications. For each age of re-growth there were two ways of drying: the field under the sun and in the shed. There were performed four sampling times (0, 8, 23 and 32 hours after harvesting) for drying in the sun and twelve sampling times (0, 3, 18, 27, 42, 51, 66, 75, 90, 99, 114 and 123 hours after harvesting) for drying in shed, with turning twice a day. The curves of dehydration of both ages and shapes of dehydration were determined.

Results and Discussion For harvesting with re-growth ages of 28 and 35 days, which drying was performed at field under the sun, there was an adjustment period of dehydration by polynomial regression model of degree 3 (Figure 1). In the step until about 8 hours after the cuts, it was observed a rapid loss of water, more than 6 g g⁻¹ h⁻¹ of dry matter (DM). This result was favored by high moisture content of the forage, by the stomata opening in the first hour after harvesting and the deficit of steam pressure between air and dehydrating forage (Collins and Coblentz, 2007) and favorable weather conditions, resulting in an average rate of dehydration until 32 hours after harvesting of 2 g g⁻¹ h⁻¹ of DM. In the period between 8 and 23 hours, the reduction of dehydration intensity was attributed to the dew, the drop in temperature, reduction of radiation and the increase in average and minimum air humidity during the night. The dehydration curves of dried fodder in the shed (Figure 2) were obtained after 123 h of dehydration and adjusted to the linear regression, although the appropriate content (> 85%) of DM for the age of re-growth in 35 days were not achieved due to the maintenance of higher humidity, in consequence of rain. These environmental conditions and the way to make dehydration resulted in dehydration rates of 0.4983 and 0.3942 g g⁻¹ h⁻¹ DM for 28 and 35 days re-growth, respectively. The dehydration rates can achieve progressive rates near zero, due to the balance between the steam water pressure contained in the plant and the surrounding environment and may remain unchanged in moisture content indefinitely, if the environment is favorable to it (Rotz, 1995). There were no re-wetting

cycles of grass in the process of Tifton 85 hay production in the shed because it was protected from dew and moisture of the soil and this is consistent with that reported by Neres et al. (2010). A possible explanation for the long period of dehydration may have not been utilization of the solar radiation in the shade of the shed. According to Wright et al. (2000), with ryegrass in controlled environmental conditions, it was found that the radiation was more important than the pressure deficit between plant and environment and wind speed, due to the effect on the evaporation of internal moisture content of the plant.

Conclusion In tropical climate, it is possible to produce hay of Tifton 85 grass with overgrowth age of 28 and 35 days in field conditions under the sun, in a cut-off time smaller than 40 hours, with rapid drying in the initial period even if there is a little re-wetting by the dew at night and with a daily revolving. The time required for the production of hay in shed is more than 120 hours and is difficult to achieve proper levels of dry matter.

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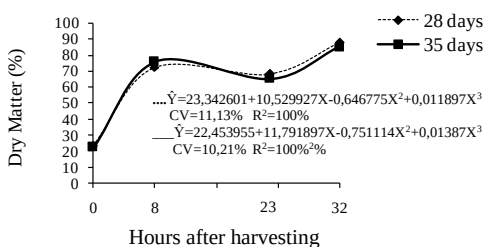


Figure 1. Dehydration curves of Tifton 85 hay drying in the sun with two ages of re-growth and two cuts. The regression coefficients were significant at 1% probability by t test.

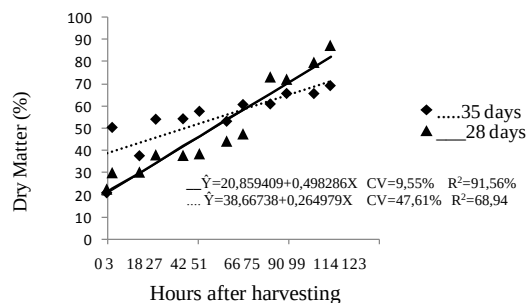


Figure 2. Dehydration curves of Tifton 85 hay dried in shed with two ages of re-growth and two cuts. The regression coefficients were significant at 1% probability by t test.

Development of fungi in different stages of the production of Tifton-85 hay with N fertilizer

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Introduction The yield of hay of high quality over the summer is important, therefore the efficient form to utilize the yielded forage with the purpose of higher allowance of the bulky is over the dry season (Calixto Júnior, 2007). Moisture content higher than 200 g kg⁻¹ in hays, produce heat spontaneously, fungi growth and undesirable modifications in the nutritive value of forages (Turner et al., 2002). There are little information about the characteristics of storage and the modifications in the nutritive value in hays of tropical grasses. The decrease in the nutritive value of hays is induced by microbial activity, heat, no structural carbohydrates; growth of fungi associated with toxin production and increase in the fibrous components and breaking of protein fraction (Coblentz, 2000). However, several works show that N fertilization improved nutritive value of forage, in special crude protein. The purpose of this work was to evaluate the effects of N doses and three stages in the process of hay-making of Tifton-85 grass on the incidence of fungi.

Materials and Methods The experiment was conducted between October 2010 and December 2010 in a field of hay production established with Tifton-85 grass in Marechal Cândido Rondon, PR, Brazil (24°33'40''S, 54°04'12''W, 420 m altitude). The experimental design was a completely randomized blocks in a factorial scheme 5x3 with five N doses (0; 25; 50; 75 e 100 kg ha⁻¹) applied as urea after forage harvesting and three stages to obtain samples (at harvesting, during packing and after 30 days storage), with four replications. The forage harvesting was realized within 28 days regrowth. The dehydration time was of 6 days, until reach 85% dry matter. The forage was turned twice a day. After packaging the forage was stored for 30 days. The samples, approximate 100 g, were reduced in particles of 5 mm and diluted in distilled water (1,000 mL) and sowing in resource means of cultivation with 200 g of potato, 20 g of dextrose, 15 g of agar e 1,000 mol of distilled water. The genus of fungi was identified with a microscope (Guarro et al., 1999) and specific keys. The statistical analysis was submitted to the variance analysis and means were compared by Tukey test with 5% probability.

Results and Discussion There was effect of N x stages in populations of *Fusarium* and *Penicillium* (Figure 1). Without N application, the *Fusarium* population (46 CFUg⁻¹) was higher in the packing stage. In the application of 25, 50 and 75 kg ha⁻¹ of N, the harvesting stage presented the lower *Fusarium* populations, 5, 8 and 5 CFU.g⁻¹, respectively. There was no difference in the *Fusarium* population between harvesting, packing and storage stages in the higher N dose. The population of *Penicillium* (15 CFU g⁻¹) was higher in the packing stage, with application of 50 kg ha⁻¹ N, and in the application of 100 kg ha⁻¹ N, the population of *Penicillium* in the storage stage was higher than the harvesting and packing stages, which presented lower populations but did not differ. In the 0 and 25 kg ha⁻¹ N doses there was no difference between stages for *Penicillium* population. There was no significant difference for *Aspergillus* populations. The *Fusarium* is a fungi typically found in the field and can result in losses during packing and storage, which was not observed in this study, because both *Fusarium* and *Penicillium* were the largest populations in these stages and *Aspergillus* rarely develops in less than 15% moisture. During the dehydration period, the average relative humidity and temperature

above 80% and 22.9°C, respectively, may have facilitated the proliferation of conidia that contaminated fodder. Nascimento et al. (2000) studying methods of natural drying and storage periods in alfalfa hay, found a higher occurrence of *Aspergillus* and *Penicillium* at the packing day. At 15 and 30 days storage the predominance was for *Penicillium*, with the occurrence of *Fusarium* and also, *Aspergillus* in the hay pile (partially dried in the sun and after in the shadow). Séguin et al. (2010), studying a permanent field of ‘hay production harvested in two periods and two different agricultural practices, identified several species of fungi and attributed this diversity to the level of low-cut (3 cm) bale with 75% dry and rain after harvesting. The predominance was of fourteen species of *Aspergillus*, nine species of *Penicillium* and two species of *Fusarium*.

Conclusion The hay packing and storage stages provided higher population of fungi *Fusarium* and *Penicillium*. Further studies are needed on the conditions of production of Tifton-85 hay to understand the dynamics population of fungi.

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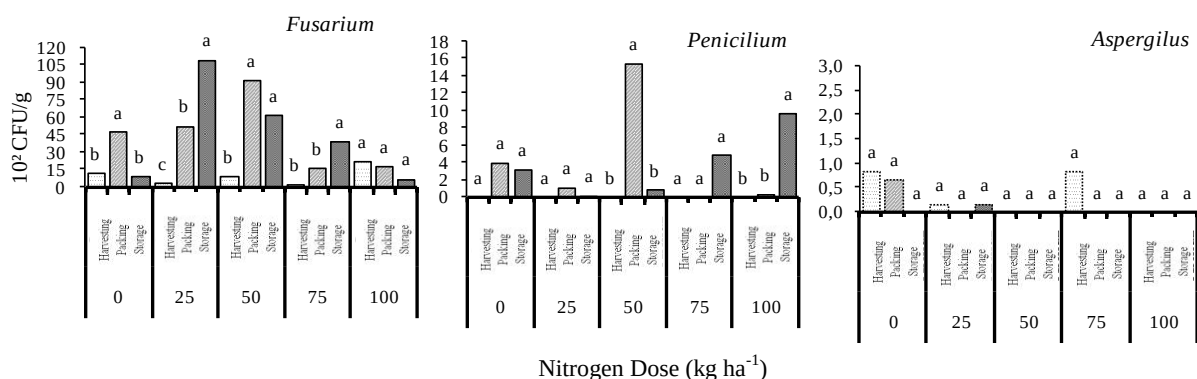


Figure 1. Colony forming unit (cfu g⁻¹) of fungi in Tifton-85 hay at different stages of the hay-making process and fertilizer N doses. Different letters between stages differ by Tukey test (P<0.05) into of each N fertilization dose.

Mycotoxin production in Tifton 85 hay dried in the sun and at shed

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Introduction This research was carried out in a permanent field in two harvesting periods and in two different agricultural practices. It was identified several species of fungi (14 *Aspergillus*, 9 *Fusarium* and 2 *Penicillium* species) and such diversity contributed to the low cutting level (3 cm), baling with 75% dry matter content and rain after harvesting. These conditions led to mycotoxin production by fungi (Séguin et al., 2010). Mirocha et al. (1968) reported problems with fertility in dairy cows due to feeding contaminated hay with mycotoxins from *Fusarium* fungi. Studies reported the presence of mycotoxins in hay which affected horses (Raymond et al., 2000) and food for ruminants (Fink-Gremmels, 2008). The aim of this study was to evaluate the presence of the mycotoxins (aflatoxin, fumonisin and zearalenone) in hay dried in the sun or in shed.

Materials and Methods The experiment took place between October 2010 and January 2011, in a field of hay production located in Marechal Cândido Rondon, PR, Brazil (24 ° 33'40"S, 54 ° 04'12"W, 420 m elevation). The hay was from Tifton 85 grass, with 28 and 35 days regrowth and subjected to five levels of nitrogen (N) fertilization. The dehydration process was under field in the sun and in shed, in the shade. Composite samples were performed, one for each replication of N dose and age, resulting in 20 samples, half for each dehydration condition. Data was analyzed using the Mann-Whitney and means compared by Tukey test.

Results and Discussion For aflatoxins, the average of sun-dried samples ($5.38 \pm 1.73 \mu\text{g kg}^{-1}$) were higher than the average of samples dried in the shade ($3.07 \pm 1.67 \mu\text{g kg}^{-1}$) and although the levels of aflatoxin were low, the results were significant ($P = 0.0071$) with higher presence in the hay dried in the sun. Aflatoxin is produced by *Aspergillus flavus* and *Aspergillus parasiticus* and lingers long after formed in the food and it is heat resistant (up to 220°C). The more known aflatoxins are B₁, B₂, G₁ and G₂, being the B₁ aflatoxin the more carcinogenic substance (Domingues, 2006). Mean level of fumonisin in the samples from sun-dried hay was $90.00 \mu\text{g kg}^{-1}$ and for hay dried in the shed $60.00 \mu\text{g kg}^{-1}$ and there was not significant effect ($P = 0.8038$) in the comparison (Table 1). One possible explanation is that a mycotoxin, fumonisin, produced by *Fusarium* fungi, can produce toxins in the pre-harvesting stage, and it is regarded as the flora of grains and remains in dead plants which serve as substrate for their stay in the field and infected a subsequent crop, producing toxins (Bullermann and Tsai, 1994). The fumonisin B₁ in horses is associated with leukoencephalomalacia, and lung disease in pigs (Cast, 2003). For zearalenone, the average of sun-dried samples was $79.89 \mu\text{g kg}^{-1}$ and for the ones dried at shadow was $40.33 \mu\text{g kg}^{-1}$ and the difference was significant ($P = 0.0257$). Zearalenone (or RAL and F-2 toxin) is a potent estrogen metabolite that causes infertility, miscarriage and other reproductive problems in dairy cows, heifers and sows and it is produced by many species of *Fusarium*, especially *F. graminearum* and *F. culmorum* (Milicevic et al., 2010). One hypothesis for the highest level of mycotoxins in the dry hay in the sun may be the largest temperature range (daytime 32.4 °C x

night 17.3 °C) which may have stimulated the fungi to produce mycotoxins before the grass has reached moisture content below 15% (Cast, 2003). It is also speculated that the cutting height (5 cm from soil) has facilitated the contact and contamination with this remaining culture.

Conclusion It is likely that the environmental conditions and management for hay production during the harvesting and drying process are as important as the dry matter content being suitable for hay storage, in order to reduce fungi contamination and provide reduced conditions that lead the mycotoxins production. The suggestion is to conduct studies with different cutting heights from the soil with tropical grasses and consider factors such as soil moisture, conditioning at harvesting time and turning after cutting.

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Table 1. Results of tests for the presence of aflatoxin, fumonisin and zearelenone performed by ELISA.

Variable*	Treatment	n	($\mu\text{g kg}^{-1}$)	P-value	C.V.(%)
Aflatoxin	Field drying (sun)	10	5.38	0.0071	48.23
	Drying in the shed (shadow)	10	3.07		
Fumonisin	Field drying (sun)	10	90.00	0.8030	128.87
	Drying in the shed (shadow)	10	60.00		
Zearalenone	Field drying (sun)	10	79.89	0.0257**	66.17
	Drying in the shed (shadow)	10	40.33		

*Statistics held by Mann-Whitney test. **Significant at 5% probability by Tukey test. Analyses carried out in the NUTRILAB - Nutrifarma Laboratory Analysis - Taió - Santa Catarina - Brazil.

Effect of tannin addition on losses and fermentation profile of soybean silage¹

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Introduction Silage of legumes is an alternative to increase the protein level in animal diets. Although presenting high nutritive value, legumes generally have bad characteristics for an adequate fermentation (Tabacco et al., 2006). A common practice is the use of additives to improve the quality of the ensiled material. So, the objective of this work is to evaluate the effect of commercial tannin addition on fermentation characteristics and losses during the soybean ensiling process.

Material and Methods The experiment was carried out at Santa Monica Experimental Station, of Embrapa Dairy Cattle, and the legume used was Glycine Max (L.) cv. Juliana. Plants were harvested at R6 and R7 stages and chopped using a stationary chopper. After that a sample was collected for bromatological analysis and the rest of forage used to make silages with 0, 2, 4, 6 and 8% of tannin. In the bottom of each silo, a dry sand bag previously weighted was placed to absorb the effluent and the superior cap was fitted with a Bunsen valve to allow gas escape. The content of each silo was manually compacted up the equivalent to 600 kg/m³ (original matter). Fifty six days after the ensiling the silos were opened and gas and effluent losses were calculated. Total loss was considered as the sum of gas and effluent losses. Dry matter recovery was calculated based as the percentage of total dry matter ensiled. Crude protein was determined in the silage and ammonia nitrogen in the silage extracted juice. Casual experimental design was used with five replications and the results were submitted to variance and regression. The best model choice was based on the determination coefficients and the significancy of regression coefficients using SNK at 5%.

Results and Discussion There was a quadratic effect ($P < 0,05$) for all variables studied (Table 1). In order to reduce losses the best dose was 4% of commercial condensed tannin. Related to pH and ammonia nitrogen related to total nitrogen the 6% dose was more efficient. This result agree with the data of Tabacco et al. (2006), The authors included tannin in alfalfa silage and verified a better ensiling process explained by the complexation between tannin and the alfalfa protein. The progressive reduction in the protein content, however, may be explained by the tannin dilution effect once it presents a lower nitrogen concentration when compared to soybean forage. Although 6% tannin had caused a lower dry matter recovery, and consequently, lower crude protein recovered when compared to 4% addition, other parameters such as pH and ammonia nitrogen indicate that 6% level must be used.. This study, however, demands other evaluation, mainly regarding the availability of crude protein to the animal, once complexation may have occurred. .

Conclusion Soybean silage must be done with 6% of commercial tannin.

Thankfulness To FAPEMIG that supported the attendance to this event

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Table 1 – Regression equations, coefficient of determination (R^2) for effluent loss, gases loss, dry matter recovery (RDM), pH, percent of ammonia nitrogen related to total nitrogen ($\text{NH}_3/\text{TN}\%$) and crude protein (CP) of soybean silage with tannin.

Variable	Percent tannin added to soybean silage					Regression Equation
	0	2	4	6	8	
Gas loss	2.92	1.66	1.48	1.86	1.75	$Y = 2.77 - 0.51X + 0.05X^2$ $R^2 = 80.7$
Effluent loss	0.97	0.50	0.39	0.25	0.46	$Y = 0.96 - 0.25X + 0.024X^2$ $R^2 = 97.1$
Total loss	3.88	2.16	1.88	2.11	2.21	$Y = 3.72 - 0.76X + 0.74X^2$ $R^2 = 90.5$
RDM	97.2	98.3	98.5	98.1	98.2	$Y = 97.23 + 0.51X - 0.051X^2$ $R^2 = 80.7$
pH	5.59	5.56	5.54	5.27	5.27	$Y = 5.63 - 0.067X + 0.008X^2$ $R^2 = 91.5$
$\text{NH}_3/\text{TN}\%$	15.6	12.6	10.7	8.3	9.5	$Y = 15.77 - 1.94X + 0.14X^2$ $R^2 = 96.7$
CP (%)	22.7	21.9	22.3	21.0	19.4	$Y = 22.50 + 0.10X - 0.59X^2$ $R^2 = 92.7$

Temperature evaluation on aerobic stability of corn silage with increasing levels of glycerin

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Introduction Aerobic stability can be defined as the resistance of silage to deterioration after the silo opening (Jobim et al, 2007). After air exposition, the silage deterioration starts resulting in substantial loss of dry matter (Woolford, 1990), due to fungi and bacteria interaction activity. The temperature increase after the silo opening is consequence of the reactions promoted by filamentous fungi, yeast and aerobic bacterias (Bernardes et al. 2007). According to Driehuis et al. (2001) aerobic stability is the time for the silage, after the silo opening, to show a 1°C increase related to ambient temperature. The objective of this work was to evaluate the effect of glycerin inclusion on the aerobic stability of corn silage.

Material and Methods Corn silage was prepared at the experimental station of Embrapa Dairy Cattle 20 experimental silos received corn forage containing 0, 5, 10 or 15% of glycerin based on wet basis. Each silo was 50 cm long, constructed from PVC tubes with 10 cm diameter. After 60 days of fermentation each silo was opened, the silage removed and put in a plastic bucket. The buckets, each one covered with one layer of cheesecloth, were kept in room at ambient temperature. Silage and room temperatures were recorded at 8 and 15 h intervals, respectively, during 8 days (192 hours). Silage temperature was determined using a mercury thermometer put 10 cm depth in the center of silage mass. The methodology of Driehuis (2001) was used to determine the aerobic stability.

Results and Discussion During the first 39 hours there was no change in the temperature of the silos (Figure 1). After 54 hours, 40% of the silage without glycerin showed temperature 1 °C higher than the ambient temperature meaning stability loss according to Driehuis (2001) methodology. After 72 hours, the temperature of 100% of the silages containing 0 and 5% of glycerin, and 20% of those containing 10% of glycerin exceeded 1°C. After 87 hours, 100% of the silages containing 0, 5 and 10% of glycerin and 20% of those containing 15% of glycerin exceeded 1°C. After 102 hours, 40% of the silage containing 15% glycerin remained stable and after 111 hour only 20%. No silage was stable after 125 hours.

Conclusion Corn silage containing 15% glycerin presented aerobic stability longer (111 hours) than silage with no glycerin (63 hours).

Thankfulness To FAPEMIG that supported the attendance to this event

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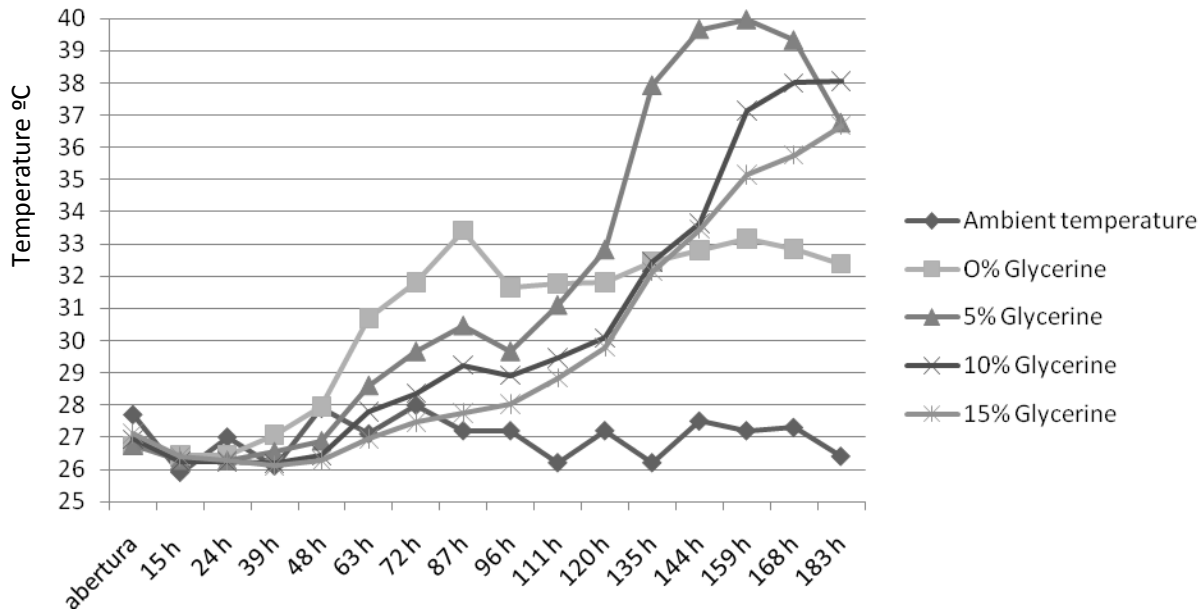


Figure 1. Temperature evolution on aerobic stability of corn silage with increasing levels of glycerin

Microbiological evaluation of aerobic stability of corn silage with increasing levels of glycerin

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Introduction Corn is considered the best forage to be ensiled due to its dry matter content, low buffering capacity and adequate levels of soluble carbohydrates for fermentation (McDonald et al., 1991). The use of crude glycerin as ingredient in ruminant concentrates has been studied in the last years (Gonçalves et al., 2006), but not as silage additive. When used as additive it can improve energy density and decrease total dry matter content. However there is no information about silage stability after silo opening. The objective of this work was to evaluate the changes occurring on microbial populations in corn silage containing different levels of glycerin after air exposition.

Material and Methods Corn silage was prepared at the experimental station of Embrapa Dairy Cattle. Twenty experimental silos were filled with corn forage containing 0, 5, 10 or 15% of glycerin on wet basis. Silos were 50 cm long, constructed with PVC tubes with 10 cm diameter. After 60 days of fermentation each silo was opened, the silage removed and put in a plastic bucket. The buckets, each one covered with one layer of cheesecloth, were kept in a room at ambient temperature. Samples of silages were collected in days 0, 4 and 8 and transferred to the following media: MRS (Himedia) for lactic acid bacteria (LAB) count and MALTE (Himedia) for fungi and yeast count. The number of colony forming units (CFU) was transformed to logarithm. A casual design with four replications was used. Treatment (0, 5, 10 and 15% glycerin) was used as plot and exposure time (0, 4 and 8 days) as sub-plots.

Results and Discussion Silage with 15% glycerin showed less LAB population ($P<0.05$) than silage with 0% glycerin in all times studied. Starting at the fourth day after opening, silage with 0, 5 and 10% glycerin increased the CFU number of fungi and yeast, when compared to 15%. In the eighth day, silage with 0 and 5% glycerin presented a higher number of CFU of filamentous fungi and yeast related to corn ensiled with 15% glycerin.

Conclusion Adding 15% glycerin to corn forage in wet basis during ensiling decrease, in the eighth day after opening, the number of CFU of lactic acid bacteria, fungi and yeast, compared to corn silage without glycerin.

Thankfulness To FAPEMIG that supported the attendance to this event

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Table 1. Colony-forming units (CFU) in lactic acid bacteria growth medium (log10/g silage)

Days	Percentage of glycerin				
	0%		5%	10%	15%
0	0.04	Ba	0.034 Bab	0.018 Bab	0.008 Bb
4	0.114	Aa	0.120 Aa	0.104 Aab	0.076 Ab
8	0.126	Aa	0.128 Aa	0.120 Aa	0.072 Ab

Means followed by same letter, lowercase and uppercase letters on the same line in the same column, * do not differ (P> 0.05).

Table 2. Colony-forming units (CFU) in filamentous fungi and yeasts growth medium (log 10/g silage)

Days	Percentage of glycerin			
	0%	5%	10%	15%
0	0.016 Bb	0.042 Ba	0.022 Bab	0.006 Cb
4	0.136 Aa	0.116 Aa	0.114 Aa	0.070 Bb
8	0.134 Aa	0.130 Aa	0.118 Aab	0.098 Ab

Means followed by same letter, lowercase and up-percase letters on the same line in the same column, * do not differ (P> 0.05).

The effect of chemical preservative supplementation on the nutritive value of lupine silage.

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Introduction Lupine (*Lupinus*, L.) is presently an important non-traditional leguminous crop whose economic meaning is gaining importance worldwide. Typical for lupine as roughage is a low DM content, low concentration of water-soluble carbohydrates (WSC), and therefore, it must be wilted before the ensiling (Jones et al., 1999). Despite a higher content of nitrogen substances, the buffering capacity of the whole ensiled lupine plant is similar as that of some grass varieties, but significantly lower in comparison with other legumes. The aim of this experiment was to evaluate the effect of chemical additive on the nutritive value of lupine silage when ensiling lupine under exactly defined model conditions.

Material and methods In the model experiment, yellow lupine (*Lupinus luteus*) variety Juno was ensiled. The whole lupine plants with a dry matter content of 187.15 g/kg were harvest, wilted and subsequently chopped during collection to a particle length greater than 35–50 mm. This model experiment used fresh crop lupine with 240.4 g/kg DM. One control (A) without preservative and two experimental variants (B and C) with preservative (acids mixture) were used. The chemical preservative composed of formic acid, propionic acid, benzoic acid, and ammonium formate. The preservative was applied at a dose of 3 L/t (B) and 6 L/t (C). This material was ensiled in three replications in special experimental containers with content (volume) of 4 L, was hermetically sealed, and stored at a temperature of 22–25 °C for three months. The analytical procedures were described earlier AOAC, (1980). The pH value was determined electrometrically, and NH₃ by the microdiffusion method according to Conway. Rumen degradability of silages protein was determined by *in sacco* method (Kacarovský et al., 2007). Results were statistically analysed using the method one factorial analysis of variance analysis.

Results and Discussion The chemical composition and nutritive value of the whole ensiled lupine plant differed from other leguminous crops. One kilogram of the original biomass dry weight contained 8.2 g of crude protein, 221.7 g of crude fibre, 290.4 g ADF, 410.9 g NDF, and 140.5 g of starch. Rumen degradability of crude protein was 60.18 %. The content of water-soluble carbohydrates was 31.5 g of 1 kg DM. Dry weight of the whole lupine plant was low and after one-day wilting its value increased only to 18.72 %, which is within the range recommended by Jones et al. (1999). The nutritive value of the control and treated model lupine silages from wilted forage are shown in Table 1.

Conclusion The best nutritive value was found in model silages with the supplement of acid mixtures dosed at 6 L/t since they showed not only a better content of net energy (NEL) and CP but also a significantly ($P<0.01$) lower NH₃, pH value, a more favourable RDP content than the control silage. The positive effect on RDP and starch content was higher in silages treated with the preservative in level of 6L/t as in untreated silage.

The study was supported by the Research Grant MSM6215648905 “Biological and technological aspects of sustainability of controlled ecosystems and their adaptability to climate change”, funded by the Ministry of Education, Youth and Sports of the Czech Republic.

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Table 1 Effect of chemical preservative on nutritive value and digestibility of crude protein of lupine silage

Silage Variant	A	B	C
Specification	Average± s.e.	Average± s.e.	Average± s.e
DM (g/kg)	187.99±0.68 ^A	182.15±2.89 ^B	188.40±3.04 ^A
pH	4.07±0.01 ^C	3.81±0.02 ^A	3.90±0.02 ^B
NH ₃ (mg/kg)	696.18±4.99 ^C	556.95±11.11 ^B	475.87±10.76 ^A
CP (g/kg DM)	202.7	217.3	205.3
RDP (g/kg DM)	699.1	668.2	635.2
Fat (g/kg DM)	20.3	22.0	2.15
CF (g/kg DM)	245.5	269.6	268.0
DF (g/kg DM)	312.8	337.9	339.3
NDF (g/kg DM)	471.7	524.5	523.2
Ash (g/kg DM)	104.1	101.0	92.2
Starch (g/kg DM)	95.7	111.2	127.9
ME (MJ/kg DM)	9.44	9.40	9.59
NEL (MJ/kg DM)	5.56	5.51	5.59
PDIN (g/kg DM)	107.98	109.56	108.25
PDIE (g/kg DM)	76.32	76.71	76.44
WSC (g/kg DM)	2.1	9.5	11.9

DM – dry matter; RDP – rumen degradability of protein; CF – crude fibre; ADF – acidodetergent fibre; NDF – neutral detergent fibre;

Influence of the maturity stage of alfalfa (*Medicago sativa* L.) on the chemical composition and *in sacco* organic matter digestibility

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Introduction Ensiling of wilted alfalfa has increased in Czech Republic during the last years. Alfalfa (*Medicago sativa* L.) is the oldest and largest multi-fodder with the highest content of crude protein. Lucerne has also the highest nutritive value (Mitrik, 2006; Holubek et al., 1995 and other). Mitrik (2006) determined that the leaves contained 18–28 % of NDF, 12–20% of ADF and over 30% of CP, while the stems contented 35–70% of NDF, 30–55% of ADF and only 10–20% of total CP. Digestibility of organic nutrients is affected not only by content, fiber but also by the degree of foliage plants, as well as the secondary metabolites, especially phenolic compounds (Scehovic, 1990). Holubek et al. (1995) noted that the nutritional value of alfalfa decreases during development. Simko (1998) and Homolka and Pavelek (2005) discussed a depressive effect of lignin content of alfalfa on organic matter digestibility. The influence of the stage of maturity of alfalfa on the chemical composition and *in sacco* digestibility was studied in a laboratory experiment.

Material and methods Green forage alfalfa (variety Palava) of first cut was harvested at regular intervals of vegetation: the formatting of buds (1), at the beginning buds (2), full bud (3), at the end bud (4), early bloom (5), full bloom (6), late bloom (7) and after flowering (mature) of alfalfa (8), each of three randomly selected sites (area 1 m²). After cooling and stabilization, the samples were homogenized and over through a sieve with a mesh size 1 mm and then analyzed for organic nutrients (AOAC 1995). Digestibility of organic matter was determined by the modified method *in sacco* described Kacerovsky et al. (1990).

Results and Discussion It was discovered that during vegetation there was a statistically significant increase in the crude fiber content and in the ADF and NDF fractions and a reduction in nitrogenous substances. A strong negative correlation was detected between the stage of maturity and content of crude protein and net energy for lactation ($r = -0.97$ and $r = -0.92$, respectively). In the individual stages of vegetation the differences in the content of nitrogenous substances, fiber and fat were statistically significant ($P < 0.05$). The statistically highest content of crude protein (28.97%) was detected in the first stage before bud setting and the lowest (11.97%) in the stage after the fall of blossoms. Later vegetation also had a stronger depressive effect on the content of fat and NEL. The organic matter digestibility ($P < 0.05$) was the lowest in alfalfa in the last stage of sampling (62.6%) when it reached a mere 85.99% of the digestibility at the beginning of bud setting. The highest organic matter digestibility (72.80%) was detected in a sample of young alfalfa prior to bud setting. In the experiment we confirmed a strong and negative correlation ($r = -0.97$) between the crude fiber content and organic matter digestibility.

Conclusion In the individual vegetation stages not only the content of total fiber increased ($r = 0.99$), but also the individual ADF and NDF fractions ($r = 0.99$ and $r = 0.98$, respectively). As a result of this phenomenon the organic matter digestibility dropped from 71.80 % to 62.60 %. The concentration of NEL energy decreased from 5.46 MJ/kg to 4.47 MJ/kg of dry matter. Due to this

reduction the potential milk production from 1 kg of alfalfa dry matter decreased from 1.74 kg FCM to 1.43 kg FCM and the dependence of energy on the vegetation stages was very high ($r = -0.92$). The benefit of harvesting alfalfa during the recommended vegetation stage is not only its optimal nutritional value, higher digestibility of organic nutrients, but also more effective production.

The study was funded from the Grant MSM6215648905 “*Biological and technological aspects of sustainability of controlled ecosystems and their adaptability to climate change*”, by the Ministry of Education, Youth and Sports of the Czech Republic.

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Table 1 Average chemical composition of alfalfa in different stages of vegetation (1 kg of DM)

Stages of Vegetation	%							MJ/k g DM	MJ/k g DM	MJ/k g DM	%
	CP	Fat	CF	ADF	NDF	Ash	NFE	ME	NEL	NEV	DOM
1	28.97	2.09	21.44	24.46	29.83	13.69	33.81	9.30	5.46	5.28	72.8
2	23.62	1.70	23.26	26.8	32.23	9.86	41.56	9.64	5.68	5.53	71.8
3	23.02	1.64	24.33	28.35	33.32	10.93	40.08	9.08	5.30	5.07	70.3
4	19.59	1.39	24.71	28.94	33.66	9.73	44.58	9.19	5.38	5.18	70.7
5	16.86	1.33	25.61	30.02	34.79	9.82	46.37	8.47	4.89	4.58	67.5
6	14.76	1.13	27.58	32.82	36.99	9.33	47.20	8.06	4.68	4.37	67.1
7	15.6	1.26	28.74	33.66	39.01	9.40	45.00	8.42	4.85	4.53	65.5
8	11.97	1.23	30.12	36.18	39.92	8.91	47.78	7.64	4.47	4.16	62.6

CP = crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber; NFE = N free extractives value; ME = metabolisable energy; NEL = net energy for lactation; NEV = net energy for fattening; DOM = digestibility of organic matter

Diversity of filamentous fungi associated with anaerobic and aerobic phase of sugar cane silage treated with biological additives

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Introduction The ensiling of sugarcane in the farms has operational advantages in relation to daily cutting. However, the fermentation process of sugarcane silage is difficult to control, and the microorganisms are still poorly studied, which can have a significant interference in forage quality. The presence of undesirable microorganisms such as filamentous fungi can significantly affect the nutritional value of silage, their appearance and acceptance by the animals, besides being a risk to people handling these silages. The growth of filamentous fungi can be accompanied by the mycotoxins production. The main genera producers of mycotoxins are *Aspergillus*, *Fusarium* and *Penicillium*. The species belonging to these genera are able to synthesize a number of enzymes that degrade the silage. The objective of this work was to enumerate and identify filamentous fungi that occur in sugarcane silages treated with different microbial inoculants.

Materials and Methods There were used silos of PVC tubes with 10 cm diameter and 60 cm length, which were capped and sealed with special rubber caps for PVC tubes adapted with Bunsen valves. The treatments consisted of 14 microbial inoculants containing strains isolated from sugarcane silage (Avila et al., 2009) and pre-selected in the laboratory. After 126 days storage, the silos were opened and 2 kg of silage were placed in plastic buckets, which were maintained for 10 days at 24°C ($\pm 1.5^\circ\text{C}$). Samples were collected at the silos opening and after 5 and 10 days of exposure to air for pH measurement and counting of filamentous fungi. Twenty five g of sample were placed in 225 mL of sterile peptone water (0.1%) and stirred for 20 minutes and serial decimal dilutions were inoculated in DRBC media (Dichloran Rose Bengal Chloramphenicol) and DG 18 media (Dichloran Glycerol Medium Base). The plates were incubated for 7 days at 25°C. The purification of the fungi was done by successive streaking in the media MEA (Malt Extract Agar). Identification of filamentous fungi was done by macroscopic and microscopic analysis according to Barnett & Hunter (1987), Samson (2004) and Samson & Houbraken (2009).

Results and Discussion Most of the fungi were observed 10 days after the silo opening time, showing high population and a larger number of morphotypes in DRBC media. The media comparison showed that DRBC was the most suitable for fungi isolation from sugarcane silage. The incidence of fungi was lower in silage treated with bacteria strains UFLA SIL 34 and UFLA SIL 46. The species identified in the silages were *Aspergillus foetidus*, *A. fumigatus*, *A. ustus*, *Gymnascella aurantiaca*, *Paecilomyces variotii*, *Penicillium carneum*, *P. citrinum*, *P. paxilli*, *P. roqueforti* and *P. solitum*. The species *Penicillium roqueforti* is quoted silage spoiler and producer of the mycotoxin roquefortine and *P. citrinum* producer of citrinin. The species *P. solitum* and *P. carneum* were detected all sampling. The first is cited as silage spoiler and the second can produce peritrema A, mycophenolic acid and patulin that controls bacterial growth and may affect the development of inocula when the silage is exposed to air. *Aspergillus fumigatus* and *Paecilomyces variotii* are pathogens of humans and animals. Positive correlation

was found between the value of silage pH and the total number of isolated fungi and also between the time of exposure to air and the number of isolates (0.84 and 0.83, respectively). These results allowed us to infer that the fungi growth in silage is directly related to the concentration of acids in silage and also with exposure to air. In practical terms, the silo will be rarely exposed to air for 10 days, but the occurrence of poorly compacted silages, the breaking of the seals and even the opening of the silos for animal feeding allows the entry of oxygen in the silo, which might favor the development of filamentous fungi. It is necessary to protect against air infiltration during storage and feedout process avoiding the aerobic deterioration and, also inhibiting the spreading of microorganisms that produce mycotoxins.

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Table 1. pH values, population and number of morphotypes of filamentous fungi isolated from sugarcane silages treated with 14 novel strains of lactic acid bacteria after 126 days of fermentation

Silages	Population (cfu/g silage) ¹ / Number of morphotypes ² of filamentous fungi								
	Time of exposure to air (days)								
	0 ³			5			10		
	pH	DRBC	DG 18	pH	DRBC	DG 18	pH	DRBC	DG 18
Control	3.51	0 / 1	0 / 0	5.31	0 / 1	0 / 1	5.2	5.72 / 1	6.00 / 2
Strain 17	3.54	0 / 2	0 / 0	4.79	0 / 1	0 / 2	4.81	6.30 / 2	6.00 / 2
Strain 19	3.61	0 / 1	0 / 0	3.63	0 / 2	0 / 1	4.92	5.40 / 3	5.79 / 1
Strain 24	3.51	0 / 1	0 / 1	4.68	0 / 1	0 / 1	4.99	6.00 / 2	7.46 / 1
Strain 25	3.51	0 / 0	0 / 0	5.12	0 / 1	0 / 1	4.81	4.95 / 3	0 / 1
Strain 27	3.52	0 / 0	0 / 2	5.30	0 / 1	0 / 1	4.94	6.00 / 2	6.77 / 1
Strain 32	3.61	0 / 0	0 / 1	4.54	0 / 1	0 / 0	5.26	6.30 / 3	0 / 1
Strain 33	3.55	0 / 0	0 / 0	4.74	0 / 1	0 / 1	4.98	5.07 / 3	0 / 1
Strain 34	3.60	0 / 0	0 / 0	3.59	0 / 0	0 / 0	5.17	0 / 1	0 / 1
Strain 35	3.68	0 / 1	0 / 0	3.88	0 / 0	0 / 1	5.13	5.23 / 3	6.00 / 2
Strain 41	3.55	0 / 0	0 / 0	4.10	0 / 0	0 / 0	5.38	8.19 / 3	6.69 / 3
Strain 42	3.61	0 / 1	0 / 0	4.26	0 / 0	0 / 1	5.19	8.00 / 3	6.60 / 2
Strain 46	3.66	0 / 0	0 / 0	3.35	0 / 0	0 / 0	4.11	0 / 1	0 / 1
Strain 51	3.67	0 / 1	0 / 1	4.49	0 / 1	0 / 1	4.83	6.00 / 2	4.69 / 1
Strain 55	3.80	0 / 1	0 / 1	4.53	0 / 0	0 / 1	4.97	4.47 / 3	4.00 / 1

¹Population quantified by plating surface, ²number of morphotypes isolated from the plating surface and direct,

³after 126 days of fermentation

Intercropping of corn and soybean to make silage: forage yield, silage quality and animal performance

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Introduction The use of corn silage is a strategic tool for the intensification of livestock systems not only due to its high production potential and quality, but also its versatility (De León y Giménez, 2009). However, corn silages have protein deficiency as a main limiting factor, (Díaz et al., 2009) which must be corrected in order to optimize the animal performance by adding a source of this nutrient. Protein concentrates are a frequently used alternative but they are expensive. Soybean crop has become very important in the livestock systems for its conservation as silage, mainly because of its high protein content. This has allowed the possibility of using silages made from mixtures of soybean and corn to increase the protein content of corn silage (Romero et al., 2010). This paper aims to determine the forage productivity, the quality of the whole plant silage made from corn and soybean mixtures and animal performance in comparison with pure silage of each of these crops.

Materials and Methods The following treatments have been evaluated: One hybrid corn (C), one soybean cultivar corresponding to group IV (S), and an intercropping with 50% of each material (C+S). They were sown randomly in plots of 3 ha each and they were harvested in the following grain maturity: in half milk line for (M) and R7 for (S). Before the ensiling, 10 samples of 1 m² of each crop treatment were taken in order to establish the total forage yield (kg DM/ha) and the relative participation of the different fractions of the plant (leaf, stem and grain) and they were later ensiled in plastic bags. After 45 days, at the time of silos opening, 3 samples were taken to determine the nutritive value of the silages through NDF, ADF, CP and lignin content. The digestibility and energy concentration were estimated from the ADF. In order to determine the daily weight gain (DWG) and the feed intake (expressed as kg DM animal-1 day-1 and percentage of BW), 180 kg live body weight Aberdeen Angus steers were taken into consideration. These steers were fed with the silages in pens of four animals each, with two repetitions and in a completely randomized experimental design. Once a day (9:00 am), diets prepared with each of the silages were provided *ad libitum* (10% of orts). The experimental period was 109 days and four determinations of body weight were carried out for calculating DWG through individual linear regressions. The daily voluntary feed intake was determined in two periods of five days each, through the difference between the feed supplied and the orts. In order to perform a statistical analysis of the results, the data was subjected to an analysis of variance (ANOVA), processed by means of Infostat Professional v2007p, whereas the difference between means obtained for the treatments were determined by the LSD Fisher method.

Results and Discussion The forage yield (kg DM/ha) shows significant differences between the treatments with 19.265, 15.279, and 4.054 for (C), (C+S) and (S), respectively. There was a significant decrease in yield as the participation of soybean in the crop increased. On the contrary, there was no significant difference in the proportions of leaves, stems and grains between the treatments. The nutritive values of the silages are presented in Table 1.

The results shown in Table 2 indicate that the DWG was statistically different across treatments, whereas the feed intake was lower in the S treatment, in agreement with its less digestibility and

lower animal performance. However, the magnitude of the poor response in the S treatment could be explained due to fact that the lipids content in the soybean grains makes low digestibility of the NFD and, therefore, restricts the feed intake.

Conclusions

There appears to be no advantage in incorporating soybean in intercropping with corn to make silage because it causes lower total dry matter yield, there is not enough increase in the protein content and it restricts feed intake and animal performance.

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Table 1. Nutritive values of corn, soybean and corn + soybean silages

	Treatments ¹		
	S	C	C+S
DM (%)	34.6 ^a	41.9 ^b	35.9 ^a
CP (%)	20.1 ^c	6.5 ^a	9.0 ^b
NDF (%)	43.9 ^a	50.6 ^b	52.9 ^b
ADF (%)	31.9 ^b	26.9 ^a	29.7 ^{ab}
Lignin (%)	6.9 ^c	2.9 ^a	4.2 ^b
Digestibility (%)	65.5 ^a	68.9 ^b	67.0 ^{ab}
ME (Mcal/kg DM)	2.4 ^a	2.5 ^b	2.4 ^a

^{a-c} Means within a row with different superscripts differ ($P < 0.05$)

¹Treatments: S = soybean silage; C = corn silage; C+S corn + soybean silage

Table 2. Feed intake, animal performance and feed conversion with different silages (corn, soybean and intercropping corn+soybean)

	Treatments ¹		
	S	C	C+S
Voluntary intake (kg DM an ⁻¹ día ⁻¹)	3.6 ^a	7.2 ^c	6.2 ^b
Voluntary intake (% BW)	1.8 ^a	2.6 ^b	2.5 ^b
DWG (kg/steer)	0.2 ^a	1.2 ^c	0.8 ^b
Feed Conversion (kg silage/kg DWG)	24.6 ^b	6.1 ^a	8.3 ^a

^{a-c} Means within a row with different superscripts differ ($P < 0.05$)

¹Treatments: S = soybean silage; C = corn silage; C+S corn + soybean silage

Nutritional values of Tifton 85 grass silage with or without pre-drying under the sun

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Introduction The silage of tropical forages, when correctly carried out, may represent a promising alternative to preserve surplus forages and its supply to animals during shortage terms. However, some factors should be considered in order to produce high quality silage as dry matter (DM) of material to be ensiled, which must vary from 30 to 35% (McDonald et al., 1991). Thus, the work studied the chemical composition of silage obtained from a Tifton 85 grass submitted or not to pre-drying under the sun in order to increase DM contents.

Material and Methods The experiment was carried out in Marechal Cândido Rondon city, Paraná, Brazil (24° 33 '40"S, 54° 04' 12"W). Tifton 85 grass was harvested at 32 days of regrowth in an area for hay and silage production, using a forage harvester tractor-implement, Tarupe model, with the following composition: 15.2% crude protein (CP), 68.4% of neutral detergent fiber (NDF), 36.0% acid detergent fiber (ADF), 32.4% hemicellulose, 9.63% mineral (M), 35.0% acid detergent insoluble protein (ADIP). Plants were harvested at 5cm height from soil and chopped into particles of about 5 cm. For dehydration, part of the chopped forage was put under the sun for two hours on clean canvas, so that a 10cm layer in height was formed and revolved twice at every 45 minutes. During ensilage, the chopped Tifton 85 presented 26% DM, while the Tifton that was pre-dried under the sun presented 32.1% DM. The experimental design was a completely randomized in a 2x3 split-plot design with two conditions submitted to Tifton 85 silage (with and without pre-drying under the sun), and three periods of silo opening (28, 56 and 112 days after ensilage), totaling 36 experimental units, represented by experimental silos made of PVC pipes with 50 cm high and 10 cm diameter. An amount of 1.700 and 1.500 kg of Tifton 85 grass was stored in each silo, with and without pre-drying under the sun, respectively. The material was compacted and silos were lid with caps equipped with Bunsen valves to let gases escape. At silos opening, a sample of the material was taken to determine CP, NDF, ADF, hemicellulose, MM and ADIP.

Results and Discussion Data for CP, NDF and hemicellulose showed a significant effect concerning the periods ($P < 0.05$), but there was no significant answer for the presence or absence of pre-drying ($P > 0.05$). There was a significant interaction among factors for ADF contents ($P < 0.05$), while, MM, ADIP and hemicellulose were not affected by studied sources of variation ($P > 0.05$). CP contents decreased according to the periods of evaluation. Thus, the lowest answers were found in silages at 56 and 112 days of silos opening in relation to the ensiled material (Table 1). The same behavior was observed for NDF and hemicellulose, but with greater reductions. The ADF was higher in silage from Tifton 85 submitted to pre-drying at 56 days after ensiling. The results are consistent when compared to the ones found by Tosi et al. (1995) and Evangelista et al. (2000), who observed no effect of wilting on CP content of tropical grasses. Castro et al. (2006) also observed reductions on NDF levels at late opening of silos. The results are consistent with ADIP for Umaña et al. (1991), who also observed no change on ADIP silage content in relation to pre-wilting. This result shows that there was an overheating of Tifton 85 during dehydration, so, some indigestible compounds were produced due to Maillard reaction (Evangelista et al., 2004).

Conclusion The Tifton 85 grass can be ensiled with or without pre-drying under the sun without causing any problem to the nutritional values of silage. However, it must be consumed before 56 days after ensiling.

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Table 1. Nutritional values of silage prepared with Tifton 85, submitted or not to pre-drying under the sun at the ensiling period and at three periods of silos opening

	Chopped	Pre-dry	Means	Chopped	Pre-dry	Means	Chopped	Pre-dry	Means
Periods	CP			NDF			ADF		
Silage	15.36	14.78	15.07a	65.43	64.72	65.08a	30.11Aa	31.72Ab	30.92
28 days	15.01	13.98	14.49ab	57.78	59.11	58.44b	34.42Aa	33.55Ab	33.99
56 days	13.92	14.30	14.11b	57.54	58.70	58.12b	32.91Ba	38.07Aa	35.49
112 days	14.47	14.09	14.28b	52.14	53.60	52.87c	33.90Aa	33.19Ab	33.54
Means	14.69A	14.29A		58.22A	59.03A		32.84	34.13	
CV1 (%)	9.68			4.8			7.85		
CV2 (%)	4.34			5.92			6.42		
	Hemicellulose			M			ADIP		
Silage	35.32	33.00	34.16a	13.92	14.30	14.11a	22.25	21.97	22.11a
28 days	23.35	25.56	24.46b	14.47	14.09	14.28a	18.69	20.21	19.45a
56 days	24.63	20.64	22.63bc	13.89	13.92	13.90a	18.43	19.67	19.05a
112 days	18.24	20.42	19.33d	13.45	13.05	13.25a	18.69	20.83	19.76a
Means	25.39A	24.90A		13.93A	13.84A		19.51A	20.67A	
CV1 (%)	6.66			14.75			8.27		
CV2 (%)	17.46			7.75			16.70		

* Means followed by the same uppercase letters in row and lowercase letters in column do not differ by Tukey test (5%). CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, MM: mineral, ADIP: acid detergent insoluble protein.

Nutritional value of Tifton 85 hay stored under two conditions

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Introduction Haymaking can be used when there is an extra production of forage, since it allows a balanced livestock management, whose nutrients showed good quality and greater stability throughout the year (Domingues, 2009). However, the changes that occurred during drying, collection and hay storage are crucial for chemical composition of preserved forage. Besides such information, there are few researches concerning changes during haymaking and its storage in Brazil (Jobim et al., 2007). Thus, the trial aimed to study changes on chemical composition of Tifton 85 hay, stored under two conditions.

Material and Methods The experiment was carried out in Marechal Cândido Rondon city, Paraná, Brazil (24° 33 '40"S, 54° 04' 12"W). The Tifton 85 hay was produced on field and baled after 96 hours of dehydration. During harvesting, sampling was performed in 12 randomized points in an experimental area to characterize plants, whose results are: plant height: 60 cm; number of leaves per plant: 6.8; stem base diameter: 1.32 mm; leaf length: 23.5 cm, leaf/stem ratio: 1.71; dry matter (DM): 28.3% DM yield: 6,899 kg ha⁻¹; crude protein (CP): 10.8%; neutral detergent fiber (NDF): 77.0% acid detergent fiber (ADF): 36.4% hemicellulose, 40.6%. The design was completely randomized in a 2x3 subdivided plots, with two storage conditions and three sampling times, with five replications. The storage conditions consisted of two sheds: the first one was an airy room/shed where hay was stored on wooden pallets and the second one was the field where hay was cropped, so the bales were placed on plastic canvas, directly on soil surface then covered with the same material. The samples were collected at the time of baling, as well as after 15 and 30 days of storage. Temperature values of each sample were obtained from bales and samples were collected to determine DM, CP, NDF, ADF and hemicellulose contents.

Results and Discussion Temperature and FDA levels were not affected by the environments or sampling times ($P > 0.05$). On the other hand, there was an interaction effect of DM, CP and NDF, while hemicellulose was changed only by sampling times ($P < 0.05$) (Table 1). The DM contents showed the same levels from the time of baling until 15 days of storage, but there was a decrease in both environments after this period and, at 30 days of storage, hay that was kept in field showed lower DM content when compared to hay stored in the shed. This result is related to the RH of the air, since at 30 days of hay storage, this answer was almost 90% and it should be considered that hay can absorb or lose water to the environment, thus, moisture contents of hay are affected by the environmental RH (Rotz and Abrams, 1988). There was an increase of CP levels when hay was stored in shed up from the moment of baling until 15 days of storage. After that, there was a decrease on those answers. On the other hand, CP contents were kept constant when hay was stored in the field. Due to this increase, at 15 days of storage, the hay stored in the shed showed higher CP levels when compared to hay stored in the field (Table 1). It was registered an initial increase of CP concentration in hay probably due to the loss of non-protein constituents (Rotz and Abrams, 1988), while changes during storage procedure followed the changes in DM contents (Nascimento et al., 2000). The NDF contents decreased with storage for hay stored in the field and these data are in accordance with the findings of Nascimento et al. (2000) and Neres et al. (2010), who observed a decrease in NDF content of alfalfa hay after storage. Hemicellulose also decreased with storage. Nevertheless, decreases for NDF and

hemicellulose levels were observed, but also expected, since according to Rotz and Abrams (1988), most of nutrients are not detected in hay within 30 days storage.

Conclusion The Tifton 85 hay can be stored in an airy shed or field, but protected with plastic canvas for 30 days without sudden changes in nutritional value, however, there will be some decrease in its dry matter content.

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Table 1. Temperature, dry matter content and nutritional value of Tifton 85 hay stored under two conditions at baling and at 15 and 30 days of storage

Environment	Sampling Periods				Sampling Periods			
	Baling	15 days	30 days	Mean	Baling	15 days	30 days	Mean
	Temperature (°C)				Dry Matter (%)			
Shed	32.80	29.70	27.20	29.90a	87.04aAB	90.14aA	83.95aB	87.04
Field	32.80	31.36	33.26	32.47a	88.79aA	92.23aA	73.99bB	85.00
Mean	32.80A	30.53A	30.23A		87.91	91.18	78.97	
CV1 (%)		24.17				6.48		
CV2 (%)		1.28				3.44		
	Crude Protein (%)				NDF(%)			
Shed	9.10aB	10.80aA	8.96aB	9.62	77.97aA	77.23aA	75.79aA	77.00
Field	8.52aA	9.53bA	9.45aA	9.17	79.47aA	76.24aB	74.65aB	76.79
Mean	8.81	10.17	9.20		78.72	76.73	75.22	
CV1 (%)		13.26				0.75		
CV2 (%)		7.87				2.23		
	ADF (%)				Hemicellulose (%)			
Shed	37.63	37.77	37.75	37.71	40.34	39.46	38.04	39.28a
Field	37.99	38.12	37.94	38.02	41.48	38.11	36.72	38.77a
Mean	37.81	37.95	37.84		40.91A	38.79AB	37.38B	
CV1 (%)		2.18				3.59		
CV2 (%)		3.73				4.97		

*Means followed by same uppercase letter on the row and lowercase letter on the column do not differ by Tukey test (5%).

Development of yeasts in Tifton 85 grass silage with different additives

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Introduction Tifton 85 grass takes an important place in the national livestock scenario since it is an alternative tropical forage for silage. At the silo opening, the anaerobic environment becomes aerobic (Amaral et al., 2008). This change allows microorganisms' development as yeasts, which are responsible for aerobic deterioration beginning (Woolford, 1990). Thus, this trial aimed to quantify the population of fungi before Tifton 85 grass storage as well as fungi and yeasts development after silos opening.

Material and Methods The experiment was carried out in Marechal Cândido Rondon city, Paraná, Brazil (24° 33' 40"S, 54° 04' 12"W) under a completely randomized design with four replications in subdivided plots, with four treatments and five sampling time. The treatments were: silage of Tifton 85 grass; silage of Tifton 85 grass plus soybean hulls; silage of Tifton 85 grass plus broken corn, silage of Tifton 85 grass with inoculant and silage of Tifton 85 grass with pre-drying under the sun for 2 hours. PVC silos with Bunsen valves were used. Tifton 85 was collected at 38 days of vegetative growth with a mechanical harvester at 5 cm from soil. It was chopped into pieces with an average length of 3 cm with a forage harvester machine. The proportions between soybean hulls and broken corn added to silage were based on the initial DM content of Tifton 85 to obtain 32% DM for the stored material. Forage was chopped and put under the sun for two hours for dehydration, therefore, 31.99% DM contents were obtained. For the treatment Tifton + inoculants, the used inoculant (Lacto silo Nitral Urbana-Gold ®) showed the following levels of guarantee: 1.0×10^9 colony-forming units (CFU/g) of *Lactobacillus curvatus*, *L. acidophilus*, *L. plantarum*, *L. buchneri*; *Pediococcus acidilactici*, *Enterococcus faecium*, *Lactococcus lactis* and cellulase 85u/g, and a 43g dilution of inoculant was used in 10 liters of water at room temperature, applied at a rate of 200 mL per 100 kg of silage with manual sprayer. Compaction densities were 236 kg of silage per m³ for Tifton 85, pre-dried under the sun and 294 kg of silage per m³ for the other treatments. Silos were opened after 30 days, while the top and bottom portions were eliminated. Samples of 300 g were placed in plastic trays and kept at room temperature for 7 days in the lab. In the 1st, 3rd, 5th and 7th days, samples were collected for yeast counting (inoculated in YEPG medium for 72 h at 28°C).

Results and Discussion Microbiological analyses of Tifton 85 grass and silage mixtures did not detect the presence of yeasts. At silos opening, yeast growth was not registered. Anaerobic conditions and organic acids concentration are two factors that affect yeasts survival during storage of silage (Bravo-Martins et al., 2006), which is provided by oxygen inside the silo (Jonsson and Pahlow, 1984). Yeasts are able to grow at low oxygen concentrations (McDonald et al., 1991) and a wide range of pH (3-8) (Lima et al., 2002). According to Woolford (1990), yeasts are also able to ferment other sugars as well as glucose, with an extra source of energy and put up with adverse effects of low pH and anaerobic conditions in silos. On the third day after silos opening, there were not any yeasts in the treatment with inoculant, however, on the treatment with soybean hulls and broken corn, there was an intense development of these microorganisms (35 and 87×10^3 CFU/g, respectively). After five days of silos opening, associated with the treatments with soybean

hulls and broken corn for Tifton 85 without additives, there was yeast growth. But, one week after opening the silos, it was observed some yeast growth only for the treatments with soybean hulls and broken corn (Figure 1). The results are in accordance with Berger and Bolsen (2006), since they recommend higher rates of silos unloading to prevent silage deterioration with the presence of grains.

Conclusion Silages of Tifton 85 grass with soybean hulls or broken corn allow yeast growth after silos opening.

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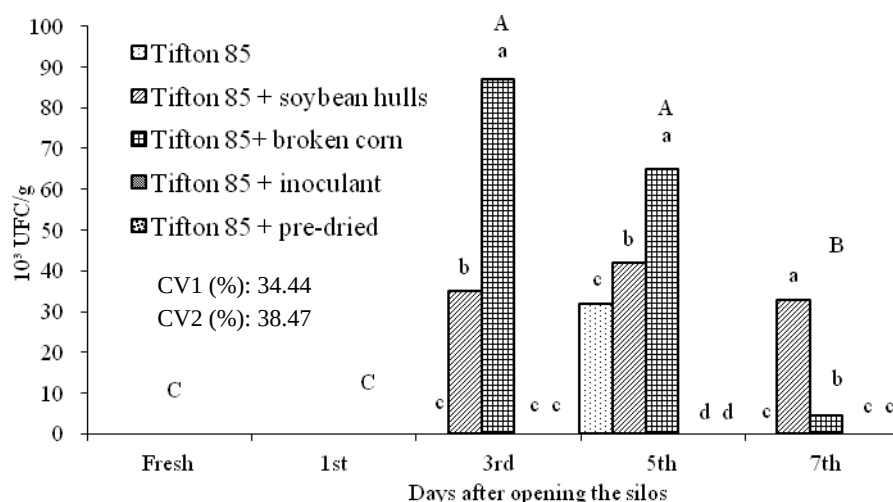


Figure 1. Yeast population in silage of Tifton 85 grass with different additives in fresh material (before ensiling) and at 1, 3, 5 and 7 days after silos opening.

Dehydration curves in the different layers of Tifton 85 in the field

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Introduction The drying curve of forage plants is a function of the material being fenado and climatic conditions of the dehydration period, and typically has an exponential form, so that each additional unit of water loss requires a longer time (Macdonald and Clark, 1987). The changes that occur during the drying of hay, exert a marked influence on the nutritional value of conserved fodder, but in Brazil, few studies that include assessments of the changes that occur during the process of haymaking (Jobim et al., 2007). Thus, work aimed at studying the curves of dehydration of Tifton 85 grass in the different layers of the material provisions of the field.

Materials and Methods The experiment was conducted in Marechal Cândido Rondon, Paraná, Brazil (24° 33 '40"S, 54° 04' 12"W) in a field of Tifton 85 grass implanted for 8 years and designed for the production of hay. The grass Tifton 85 was cut with the help of tractor-implement cutter knives at a height of 5 cm from the ground when he found himself at the age of 38 days regrowth. The experimental design was completely randomized split-plot in 4 x 9, four layers of hay to be sampled (bottom, middle, upper and total) and nine sampling times (0, 8, 32, 47, 56; 71, 80 and 95 h after cutting). The sampling times corresponded to the following times a day (day of the cut): (time 0) 9:00, (8th time) 17:00, Day 2: time (23) 8:00, time (32) 17:00, Day 3 (47th time) 8:00, (56th time) 17:00, Day 4: (time 71) 8:00, (80th time) 17:00, Day 4: (time 95) 17:00. After sampling the hay was packaged in paper bags and dried in an oven with forced air ventilation for the determination of dry matter (DM). From the DM were obtained curves of dehydration. The data were statistically analyzed, and the layers were compared by Tukey test (%), while the accumulation of MS over the sampling times was studied by means of regression analysis.

Results and Discussion The dry matter of Tifton 85 were affected by the layers, drying times and the interaction of factors ($p < 0.01$). In the unfolding of the layers studied at each time of dehydration, at 8, 23 and 32 hours after cutting, the top layer had higher dry matter content of the bottom layer, however, differed from the middle tier and the total (Table 1). At the time of cutting (time zero), and 47, 56, 71 and 80 hours after cutting, the dry matter in all layers were similar. At the time of baling (time 95), the bottom layer had the lowest dry matter content in relation to others. In the unfolding times of dehydration in each layer of Tifton 85, dehydration of the upper layer is adjusted to the cubic model of regression (Figure 1), two sudden drops in the dry matter in the times that corresponded to the dawn of the 4th and 5th day the period of dehydration. This result is due to water coming from the dew, as the hay is hygroscopic and rehydration of the plants with dew occurs when it is absorbed by the internal tissues (Rotz, 1995). Neres et al. (2010) by working with the cultivation of alfalfa observed similar behavior for the material under dehydration, with sharp drop of DM each morning from dew at night. For the other layers, the regression model was significant quadratic and higher dry matter were obtained at 66, 56 and 67 hours after cutting to the middle layers, and lower total (Figure 1).

Conclusion The surface layer of Tifton 85 is more subject to weather conditions, with greater variations in dry matter.

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Table 1. Dry matter of Tifton 85 over the period of dehydration in the different layers studied during the period of 95 hours of dehydration

	0	8	23	32	47	56	71	80	95
Superior	28.42a	47.67a	56.82a	72.16a	75.74a	74.43a	58.90a	85.28a	67.42a
Intermediate	28.52a	41.30ab	46.06ab	65.14ab	69.95a	73.53a	56.41a	84.06a	60.11a
Less	28.37a	35.57b	40.20b	59.59b	68.94a	76.02a	62.43a	82.70a	40.05b
Total	29.51a	45.86ab	47.47ab	69.36ab	72.76a	74.32a	54.95a	87.48a	66.89a
CV1(%)	12.92				CV2(%)				11.12

* Means followed by same letter in columns do not differ statistically by Tukey test (5%).

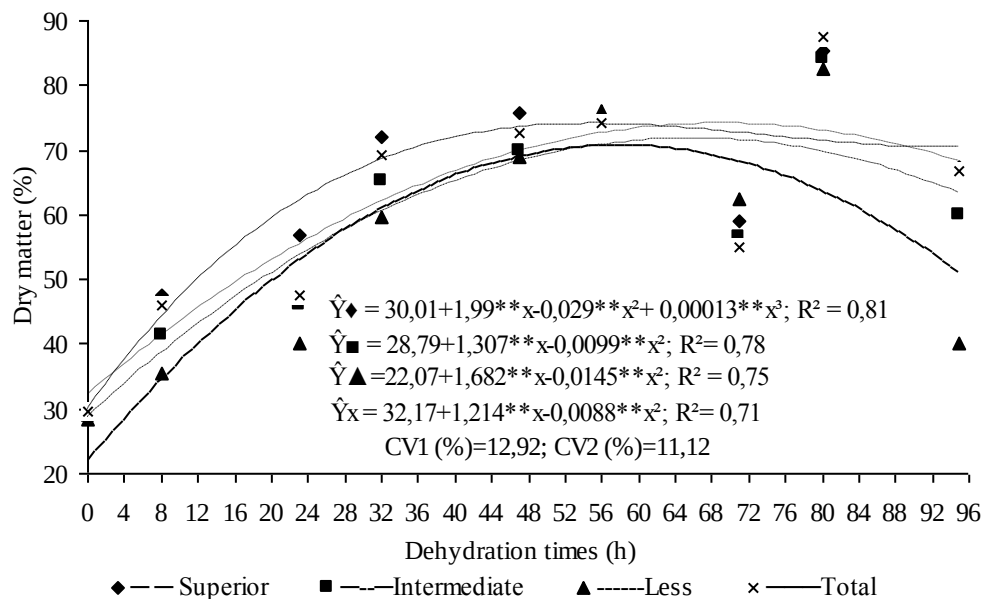


Figure 1. Dehydration curves of Tifton 85 hay in the upper, middle and lower throughout the period of dehydration. (Significant at 1% probability by t test)

Fermentation and aerobic stability of corn silage inoculated with bacterial additives

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Introduction The high nutritional value of corn silage allow the development of opportunist microorganisms responsible by aerobic spoilage. For improve the fermentation and aerobic stability these silages, the utilization of inoculants with obligate and facultative heterofermentatives bacterias has been studied. Thus, the aim this research was to evaluate the effects of bacterial inoculants on fermentation e aerobic stability of corn silage.

Materials and Methods The whole plant corn was harvested with 35% of DM. The treatments were **Control** (forage untreated), **LB** (forage inoculated with 1×10^5 CFU *Lactobacillus buchneri* NCIMB 40788/g), **PA** (forage inoculated with 1×10^5 CFU *Propionibacterium acidipropionici* MA26/4U/g), **BS** (forage inoculated with 1×10^5 CFU *Bacillus subtilis* AY553098/ g), **LP** (forage inoculated with 1×10^5 CFU *Lactobacillus plantarum* MA18/5U/g) and the association **LBLP** (forage inoculated with 1×10^5 CFU *Lactobacillus buchneri*/ g and 1×10^5 CFU *Lactobacillus plantarum*/ g), **BSLP** (inoculated with 1×10^5 CFU *Bacillus subtilis*/ g and 1×10^5 CFU *Lactobacillus plantarum*/ g) and **PALP** (forage inoculated with 1×10^5 CFU *Propionibacterium acidipropionici* /g and 1×10^5 CFU *Lactobacillus plantarum*/ g). The inoculants were diluted in distilled water at the rate of 4 mL kg⁻¹ of fresh forage and uniformly spray on the fresh forage. An amount of chopped corn (5 kg) from each treatment was packed into 7L silos in quadruplicate, sealed with lid and adhesive tape and stored at ambient temperature. After the fermentation period (96 days), the silos were opened, the spoiled forage was discarded, and the remainder was homogenised and sampled to determine the DM content, pH values, ammonia nitrogen(NH₃-TN), lactic acid, acetic acid, yeasts and molds counts. Silage temperature was measured every half hour by a data logger inserted in the center of mass during the aerobic exposure. Room temperature was measured by data logger distributed near of the experimental silos. The data were analysed as a completely randomised design and subjected to an analysis variance with the Statistical Analysis System (1988). The means were separated by the Tukey's test, and the significance level was $P < 0.05$.

Results and Discussion The levels of NH₃/TN and the number of molds did not differ among silages (Table 1). The pH values were lower in control silage and in silage inoculated with *P. acidipropionici* (PA) than silage inoculated with *L. buchneri* and *L. plantarum* (LBLP). The pH values are considered suitable to a desirable fermentation. The control silage showed higher lactic acids levels compared to other silages. The levels of lactate were lower according to Kung e Shaver (2001). The silage added with *L. buchneri* and *L. plantarum* (LBLP) presented higher acid acetic content (2.33% of DM), followed by silage inoculated with *L. buchneri* (1.95% of DM) alone ($P < 0.05$). The silage inoculated with the combination *P. acidipropionici* and *L. plantarum* (PALP) showed lower acetate levels (0.87% of DM) than other silages ($P < 0.05$). The silages inoculated only with *B. subtilis* (BS) or *L. buchneri* (LB) showed lower number of yeasts, followed by silages inoculated with *P. acidipropionici* (PA) or *L. buchneri* and *L. plantarum* (LBLP) ($P < 0.05$). These results are according with the higher production of antifungal compounds by these microorganisms. The silages when inoculated with *L. buchneri* alone or in combination presented more production of acetate, which is an antifungal compound (Ranjit & Kung, 2000). The *P. acidipropionici* (Roughani et al., 2008) and the *B. subtilis* (Todovora & Kozhuharova, 2009) also are known by production of many antifungal compounds. Consequently, the aerobic stability was higher in silages with *L. buchneri* e *B. subtilis* ($P < 0.05$).

All silages treated showed increase of temperature above of room temperature after 36 hours of aerobic exposure (Figure 1).

Conclusions The corn silages inoculated with *L. buchneri* or *B. subtilis* remain stable for more time.

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Table 1. pH values, ammonia N in relation total nitrogen (NH₃/TN), fatty acid levels, number of yeasts and molds in corn silage.

Treatments ¹	pH	NH ₃ /TN (% NT)	Acids		Yeasts CFU/ g of silage	Molds	Aerobic stability (h)
			Latic	Acetic			
			(% of DM)	(% of DM)			
Control	3.81 ^b	5.17 ^a	2.63 ^a	0.93 ^e	3.99 ^a	3.80 ^a	23.3 ^d
LB	3.87 ^{ab}	5.02 ^a	2.44 ^b	1.95 ^b	1.25 ^b	3.85 ^a	100.4 ^a
BS	3.86 ^{ab}	4.85 ^a	1.91 ^d	1.59 ^c	1.00 ^b	4.00 ^a	78.9 ^{abc}
PA	3.81 ^b	4.90 ^a	2.47 ^b	1.57 ^c	2.39 ^{ab}	3.55 ^a	70.3 ^{bc}
LP	3.87 ^{ab}	5.56 ^a	2.44 ^b	1.63 ^c	3.86 ^a	3.80 ^a	54.8 ^c
LBLP	3.89 ^a	5.52 ^a	2.42 ^b	2.33 ^a	2.58 ^{ab}	3.65 ^a	87.1 ^{ab}
BSLP	3.83 ^{ab}	5.03 ^a	2.02 ^c	1.32 ^d	3.34 ^a	3.80 ^a	54.2 ^c
PALP	3.85 ^{ab}	4.64 ^a	2.09 ^c	0.87 ^e	3.89 ^a	3.85 ^a	58.8 ^c
CV (%)²	0.90	14.28	1.47	4.94	24.36	4.00 ^a	14.41

*Means followed by equal letters do not differ by Tukey test (P>0.05).

¹LB: *Lactobacillus buchneri*; PA: *Propionibacterium acidipropionici*; BS: *Bacillus subtilis*; LP: *Lactobacillus plantarum*; LBLP: *L. buchneri* e *L. plantarum*; PALP: *B. subtilis* e *L. plantarum*; P. *acidipropionici* e *L. plantarum*.

² = Coefficient of variation.

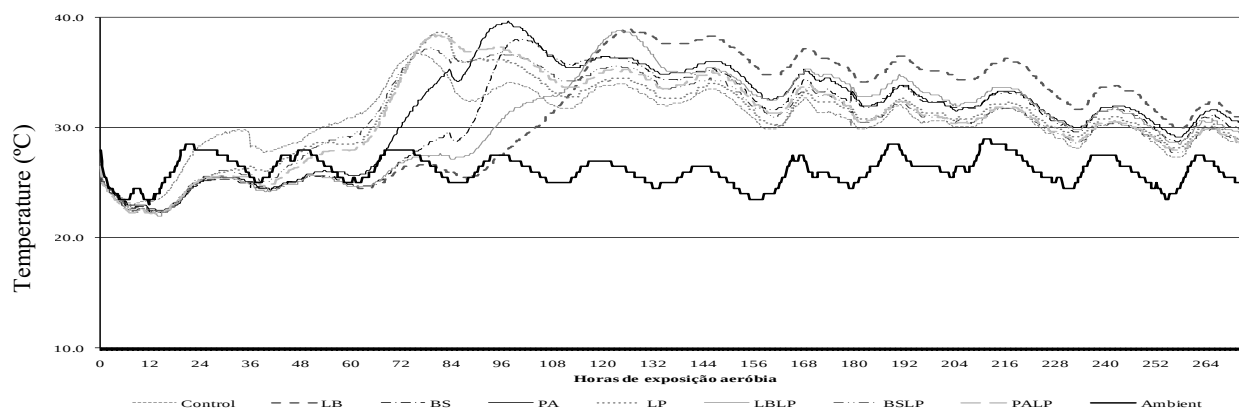


Figure 1. Temperature of silages during aerobic exposure.

Fermentation, microbial occurrence and aerobic stability of corn silages inoculated with *Bacillus subtilis*

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Introduction Corn silage has high concentration of nutrients, which crucially affects its stability after opening the silos under the action of undesirable microorganisms. In this context, the use of microbial inoculants have been proposed in order to increase the aerobic stability of these silages (Kung Jr. et al., 2003). In this sense, many species and strains of the genus *Bacillus* produce a large number of antimicrobial substances including antibiotics. According to Todovora & Kozhuharova (2009), the species *Bacillus subtilis* is one of the most important producers of antifungal and antibacterial compounds from genus *Bacillus*. Strains of *B. subtilis* have been studied in corn silage (Phillip & Fellner, 1992), demonstrating a positive effect on aerobic stability of these bulky. Therefore, the aim of this research was to investigate the effect of *Bacillus subtilis* inoculation on the characteristics and fermentation losses, microbial occurrence and aerobic stability of corn silages.

Materials and Methods The corn was harvested when the grain were in the dough stage. The treatments evaluated were: control (silage without inoculation of *B. subtilis*); inoculation with 5×10^4 colony forming units - CFU of *B. subtilis*/g of forage, with 1×10^5 CFU *B. subtilis*/g of forage and with 5×10^5 CFU *B. subtilis*/g of forage. Silos plastic buckets with a capacity of 7 L were used as experimental. After 60 days of ensiling, the silos were opened, the spoiled forage was discarded, and the remainder was homogenised and sampled to determine the DM content, pH values, ammonia nitrogen ($\text{NH}_3\text{-TN}$), lactic acid, acetic acid, yeasts and molds counts. Silage temperature was measured every half hour by a data logger inserted in the center of mass during the aerobic exposure. Room temperature was measured by data logger distributed near of the experimental silos. The data were analysed as a completely randomised design and subjected to an analysis variance with the Statistical Analysis System (1988). The means were separated by the Tukey's test, and the significance level was $P < 0.05$.

Results and Discussion Inoculation of forage with *B. subtilis* levels did not cause changes in the concentrations of dry matter (DM), $\text{NH}_3\text{/TN}$, acetic and lactic acid and number of molds from silage ($P > 0.05$). However, the pH values, yeasts counts and aerobic stability were affected by inoculation with *B. subtilis* ($P < 0.05$). In control silage was observed lower pH value than that silages in inoculated with *B. subtilis* at doses of 5×10^4 and 1×10^5 CFU/g of forage. However, the pH values are considered suitable to a desirable fermentation (3.5 to 3.7), as described by Borreani et al. (2002). The yeasts counts was lower in silage inoculated with *B. subtilis* at a dose of 5×10^5 CFU/g forage compared to the control treatment, which can be explained by production of antifungal compounds by this microorganism (Todovora & Kozhuharova, 2009). However, the *B. subtilis* levels lowest inoculated at the time of ensiling (5×10^4 and 1×10^5 CFU/g forage) were not enough to reduce the proliferation of yeasts in relation to the control treatment. The aerobic stability was improved in silages inoculated with 1×10^5 and 5×10^5 CFU of *B. subtilis*/g forage. As it is known, the increase of temperature is caused mainly by the action of yeast and bacteria.

Conclusions The inoculation of 5×10^5 CFU of *Bacillus subtilis* /g of forage with controls the growth of yeast and improve the aerobic stability in corn silage.

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Table 1. Characteristics and losses fermentative, microbiological occurrence and aerobic stability of corn silage inoculated with *Bacillus subtilis*¹

Vaiable	Control	5×10^4	1×10^5	5×10^5	VC (%)
DM (%)	37.49 ^A	36.71 ^A	35.49 ^A	36.56 ^A	3.19
pH	3.71 ^B	3.75 ^A	3.76 ^A	3.73 ^{AB}	0.39
NH ₃ /NT (%)	5.27 ^A	6.26 ^A	6.42 ^A	5.56 ^A	19.59
Acetic acid (% of DM)	0.94 ^A	1.18 ^A	1.06 ^A	0.99 ^A	15.13
Lactic acid (% of DM)	4.07 ^A	3.32 ^A	3.52 ^A	3.15 ^A	19.47
Yeasts (Log ₁₀ cfu/g of silage)	4.73 ^A	4.12 ^{AB}	3.82 ^{AB}	3.09 ^B	16.68
Molds (Log ₁₀ cfu/g of silage)	4.90 ^A	5.14 ^A	4.54 ^A	4.47 ^A	21.77
Aerobic stability (hours)	43.6 ^C	93.75 ^B	157.82 ^A	157.87 ^A	9.66

¹Means followed by same letter in the line do not differ by Tukey test at 5% significance.

Nutrients intake and ruminal parameters of heifer fed with corn silages inoculated

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Introduction Corn silage has high concentration of nutrients, which crucially affects its stability after opening the silos under the action of undesirable microorganisms. In this context, the use of microbial inoculants has been proposed in order to increase the aerobic stability of these silages (Kung Jr. et al., 2003). The bacterium most used in this process is *Lactobacillus buchneri*, because it is responsible for increasing acetic fermentation, which has an antifungal effect. However, for increased production of lactic acid has been proposed inoculation with *L. plantarum*, as this BAL is efficient in this process and still has less loss of carbon from CO₂ (McDonald et al., 1991). However, few studies have been conducted with heterofermentative inoculants and their associations in corn silages in Brazil (Zopollatto et al., 2009), as well as their effects on ruminal parameters in cattle. Therefore, the aim of this research was to investigate the effect of bacterial inoculants on the intake and ruminal parameters of cattle fed with inoculated corn silages.

Materials and Methods The corn whole plant was harvested between 30 and 35 % of DM. The treatments evaluated were animals fed with silages: control (silage untreated); LB (corn silage inoculated with 1x10⁵ cfu/g forage of *Lactobacillus buchneri* NCIMB 40788); LBLP (corn silage inoculated with 1x10⁵ cfu/g forage of *L. buchneri* NCIMB 40788 and 1x10⁵ cfu/g forage of *L. plantarum* MA18/5U). Three silos of surface with 10 tons of forage in each were made. After 165 days of fermentation the silos were opened. To evaluate the nutrients intake, three crossbred heifers (average weight of 368 kg) were used. The heifers were cannulated in the rumen. The animals remained in adaptation during 14 days and 3 days to collection of data for 3 periods. The heifers were fed once a day (7:00h) *ad libitum*. The intake was calculated daily. The diet was composed for 80% of corn silage and 20% of concentrates (8% of soybean meal, 87% of corn, 2% of urea and 3% of mineral salt). To determine the apparent digestibility it was carried partial collection of faeces during 3 days in the schedule 8:00; 12:00 and 16:00h, respectively. The total production of faeces was estimated by the internal indicator ADFi (acid detergent fiber indigestible) according to Casali (2006) incubated *in situ* during 264 hours. The ruminal fluid was collected before (time 0) and 3, 6, 9 and 12 hours on the last day of sampling each period. The design used was latin square 3 x 3, with 3 treatments, 3 animals and 3 periods repeated once a time. Data were submitted to analysis of variance comparing the treatment by the test t to 5% significance level.

Results and Discussion The dry matter and nutrients intake were not affected by use of inoculants (P>0.05) (Table 1). According to Kung Jr. et al. (2003) the organic acids produced in inoculated silages arising of fermentation of soluble sugars could decrease the intake, however, this it not observed in this work. The acetic and butyric ruminal concentration were affected significantly by inoculation with BAL obligate and facultative heterofermentative (P<0.05) as well the total concentration of VFA (Table 2). However, the acetate: propionate ratio, pH values and concentration of N-NH³ were not affected (P>0.05). The BAL obligate heterofermentative ferment hexoses to lactic acid, carbon dioxide and ethanol (or acetic acid in the presence of an alternative electron acceptor), and pentoses are converted to lactic and acetic acid (Holzer et al., 2003). Therefore, the ruminal concentration of acetate was higher when the animals were fed corn silages inoculated with *L. buchneri*. To same treatment, there was higher concentration of

butyric acid ruminal. Due to higher concentration of ruminal acetic and butyric acid to animals fed corn silage inoculated with *L. buchneri*, the concentration total volatile fatty acids was higher. The ruminal pH value to heifers fed with silage control was higher than that fed silage inoculated with LPLB. The animals fed with corn silage treated with LP presented intermediate value.

Conclusions The inoculation of *L. buchneri* associate or not to *L. plantarum* in corn silage did not affect the nutrients intake. The ruminal concentration of acetic and butyric acids were affected by inoculation of corn silage with *L. buchneri*.

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Table 1. Nutrients intake of crossbred heifers (kg/day) fed with corn silage inoculated and concentrate.

Itens	Control	LB	LBLP	VC (%)
DMI	7.06	7.14	6.67	7.19
OMI	6.81	6.83	6.38	7.08
CPI	0.74	0.79	0.71	8.53
TCI	5.95	5.9	5.55	7.14
NFCI	2.73	2.72	2.5	7.48
EEI	0.22	0.23	0.22	3.89
NDFI	2.92	2.9	2.77	10.73
ADFI	1.5	1.37	1.4	20.5
TDNI	4.35	4.44	4.1	7.29

*Treatments: Control = silage untreated; LB = inoculated silage with *Lactobacillus buchneri*; LBLP = inoculated silage with *Lactobacillus buchneri* + *Lactobacillus plantarum*.

Table 2. Ruminal parameters of cattle fed with corn silage inoculated and concentrate.

Itens	Control	LB	LBLP	VC (%)
Acetic acid (mMol/mL)	55.75 ^c	63.12 ^a	59.12 ^b	2.50
Propionic acid (mMol/mL)	11.14 ^a	12.88 ^a	13.59 ^a	14.76
Butyric acid (mMol/mL)	4.09 ^b	4.61 ^a	4.15 ^b	5.82
AA:PA	5.07 ^a	5.16 ^a	4.56 ^a	12.03
Total VFA (mMol/mL)	70.18 ^c	80.80 ^a	77.57 ^b	2.95
pH	6.14 ^a	6.09 ^{ab}	6.03 ^b	0.86
N-NH ₃ (mg/dL)	10.23 ^a	10.50 ^a	9.60 ^a	8.48

*Treatments: Control = silage untreated; LB = inoculated silage with *Lactobacillus buchneri*; LBLP = inoculated silage with *Lactobacillus buchneri* + *Lactobacillus plantarum*.

Effect of associated additives in pH and NDF content in sugarcane (*Saccharum officinarum*) silages

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Introduction An alternative to the seasonality of forage production in the grazing-lands is the maintenance of feed supplies such as silages (Carvalho et al., 2007). Sugarcane stands out, mainly, for the high yield per hectare. However, with restrictions to the use in cattle production systems, as the daily need of cutting, the low digestibility of fiber and protein and mineral contents. Sugarcane ensiling can solve, or even reduce the annual forage surplus and the losses by fire or frost, and has been utilized in cattle rations due to these logistic and operational benefits. The fermentative losses of sugarcane silage can turn its utilization impracticable. The predominance of alcoholic fermentations in those silages demands additives to improve the aerobic stability and reduce dry matter losses. The additives act upon the fermentation of silage, alter the ensiled mass and inhibit the development of undesirable microorganisms in the fermentation process (Santos, 2007). In spite of the great volume of studies in the latest years, the use of additives in sugarcane ensiling, whether be bacterial, chemical or organic still represents discrepant results. In this sense, the objectives of this work were to evaluate the effect of associations of additives on pH and on the neutral detergent fiber contents in sugarcane silages.

Material and Methods The experiment was conducted at the Federal Institute of Minas Gerais in Bambuí-MG. The ensiled sugarcane was the variety SP 1049. The cutting and harvest were performed in September 2010 after 12 months of planting (one-year old sugarcane) at 8 cm from soil, followed by defoliation and grinding. Both *Arachis pinto*i and *Leucaena leucocephala* (organic additives) in the sugarcane were ground in a conventional chopper of forages with average particle size from 2 to 3 cm, packed in plastic bags and used at ensiling in artificial silos with the sugarcane. The pH and NDF analyses were performed in the Animal Nutrition Laboratory of the Agricultural Science Department (DCA/IFMG/BAMBUÍ). The experimental design was a completely randomized with five treatments and four replications. The treatments were: sugarcane ensiled with the biological additive *Lactobacillus plantarum* 2.5×10^{10} CFU/g; additive *L. plantarum* associated with 1% CaO, additive *L. plantarum* associated with 1% Urea, additive *L. plantarum* associated with 15% of *L. leucocephala* and additive *L. plantarum* associated with 15% of *A. pinto*i Belmonte cultivar, in percent mixtures of fresh basis. The variance analysis was performed in the statistical package SAS (1996) and the means compared by the Tukey test ($P < 0.05$).

Results and Discussion There was an effect ($P < 0.05$) among treatments in relation to pH and the associations of additives in sugarcane silages presented differences. Sugarcane silage treated with *L. plantarum* associated with 15% *A. Pinto*i presented pH similar to that of the control silage. The sugarcane ensiled with *L. plantarum* associated with *Leucaena* or with urea or CaO were similar and less acidic among the tested silages. Amaral et al. (2009), treated sugarcane silage with 1% CaO, and found increases of up to 5 units in pH, lower than those found in this work. Santos et al. (2008) reported pH values between 8.7 and 4.7 with 1% CaO or 1% limestone, respectively, similar to that of the present work. These results can be related to an increase in lactic acid production. According to Cherney & Cherney (2003), pH is an indicator of the quality of silages

with a low DM content. The increases of pH found in that experiment do not seem to indicate deterioration and reduction in the aerobic stability of silages. The NDF contents showed differences among the treatments. The ensiled sugarcane associating 1% CaO to biological additive presented the lowest NDF content with no differences among the other treatments. According to Balieiro Neto et al. (2007), the addition of CaO in sugarcane ensiling partially solubilizes hemicellulose and the addition of 2% CaO increases *in vitro* digestibility. Cavali et al. (2006), testing doses of CaO in sugarcane silages, found reduction in NDF and hemicellulose content and increase in digestibility. The results of the NDF contents in this experiment corroborate the findings of these authors. The association of biological additive with 1% CaO can have broken down the cell wall of the forage, thus improving its quality as stated by Van Soest (1994). Some bonds which occur in the cell wall formation are susceptible to alkaline agents.

Conclusions Sugarcane silage treated with *L. plantarum* associated with 15% *A. Pintoi* and the control silage showed the lowest pH values and sugarcane ensiled with *L. plantarum* associated with 1% CaO showed the greatest reductions in the NDF contents among the evaluated treatments.

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Table 1. Effect of biological additives, associated with chemical and organic additives in pH and in the Neutral Detergent Fiber content (NDF) of sugarcane silages (*Saccharum officinarum*)

Additives Associated – sugarcane silage	Mean pH	Mean NDF (%)
Biologic + CaO	4.00bc	45.58a
Biologic + Forage peanuts	3.43a	57.22b
Biologic + Leucaena	3.80b	58.41b
Biologic + Urea	3.93bc	60.30b
Biologic (control)	3.60a	65.07b

Means followed by the same letter in the column do not differ by Tukey test (P<0.05)

Potential milk production from silage of different corn genotypes

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Introduction The Brazilian livestock is based on pastures, and the intensification of pasture production may be restricted by climatic factors, limiting the production of quality forage throughout the year. The possibility of simulating scenarios from data regarding the production system contributes in the process of decision making, especially with the use of mathematical models. There is a need for rapid assessment to the results of the maize experiments because genetic breeding is dynamic. The aim of this study was to evaluate different maize genotypes concerning the potential for milk production per ton of forage and per hectare in the Southwest of Paraná, Brazil.

Material and Methods The experiment was conducted at the experimental area of “Universidade Tecnológica Federal do Paraná”, Campus Dois Vizinhos, in the 2009/2010 harvest, the region comprising the third plateau of Paraná, with 520m altitude, latitude 25° 44'South and longitude 53°04'West, the climate is mesothermal humid subtropical (Cfa) according to Köppen classification. To carry out this work, 32 maize genotypes from the Southern super early cycle experiment were evaluated for silage production in the Southwest of Paraná. The genotypes are part of the field trials of maize cultivars that are organized by EMBRAPA and are conducted annually in various regions of Brazil. The experiment was carried out in a simple lattice design. The characters evaluated were the production of dry matter per genotype (DM, t/ha), the number of plants per hectare (NP), green matter (GM, t/ha), and neutral detergent fiber (NDF, % DM). These variables were used as input to the model developed by Undersander et al. (1993). The production of milk per ton of forage (MP, L/t) and milk production per hectare (MP, L/ha) were observed as variable output.

Results and Discussion The NDF content did not differ by analysis of variance (F test), with values between 40.22 and 61.02% DM (2A550 and H2002ALTA genotypes, respectively), and with an average of 49.44%. Therefore, it is clear that the variability of the NDF is high because this variable is directly related to the consumption of forage by animals, which interferes with the milk production. For the milk production of per ton of silage provided (MP/t) there was no statistical difference between genotypes (Table 1). However, milk production ranged from 314.34 liters (H2002ALTA) to 768.61 liters (2A550), with an average of 553.98 liters/t. Milk production per hectare (MP/ha) was different between genotypes, and the genotype with the highest production was the 30A37 with 15,517.90 liters/ha, which according to the number of plants, production of dry matter and low NDF content, provided high milk production. The GNZ2500 genotype with high content of NDF had lower production, 4,572.55 liters/ha. The average observed for all genotypes was 9,188.56 liters of milk per hectare.

Conclusions The use of the deterministic model enabled us to identify which are the best genotypes of corn for silage in the Southwest of Paraná. The genotypes 30A37, AS 1572, 30A77, 20A78, Dx 603, 2A550, 2B433, 2A106 and Dow XBX80822 Test presented characteristics of forage production and biological efficiency in milk production.

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Table 1. Average traits of corn genotypes for the Southern super early cycle.

Genotype	NP*	GM		DM%		NDF	MP/t	MP/ha	
AS1555 YG	61.4	7,783	a*	20.8	ab	49.5	565.4	11,803.7	abcd
AS1572 YG	60.0	6,139	efghij	17.6	abcdefghi	41.7	735.9	13,015.5	ab
AS1578 YG	61.4	5,582	ghijk	14.3	ghijkl	46.7	625.5	8,830.68	bcdef
30A37	62.1	7,564	abcd	21.5	a	42.9	709.6	15,517.9	a
30A77	61.4	6,373	bcdefghi	18.3	abcdefg	47.5	608.9	11,155.8	abcde
20A78	64.2	7,159	abcde	19.1	abcde	43.8	690.3	13,043.3	ab
Dx 908	56.4	6,125	efghij	17.4	abcdefghi	51.8	514.1	9,204.75	bcdef
Dx 603	63.5	6,806	abcdefg	19.8	abcd	46.2	636.1	12,597.5	ab
2A550	58.5	6,288	cdefghij	16.7	bcdefghij	40.2	768.6	12,889.9	ab
2B587	56.4	5,241	ijk	13.7	hijkl	46.6	629.1	8,730.90	bcdef
2B433	62.8	6,609	abcdefgh	16.9	bcdefghij	48.2	592.6	9,865.72	bcdef
AL2007A	61.4	6,648	abcdefgh	18.9	abcdef	49.6	563.3	10,288.7	bcde
H2002ALT	59.2	6,524	abcdefgh	16.4	cdefghijk	61.0	314.3	5,027.80	fg
H25ALTA	61.4	5,252	ijk	12.4	klm	48.7	582.6	7,374.67	cdefg
EMBRAPA	61.4	7,658	abc	19.4	abcde	49.4	566.9	1,1047.9	abcde
EMB.	53.5	3,017	l	8.71	m	48.7	583.3	5,015.67	fg
EMB. Sint.	60.7	5,766	fghijk	15.1	efghijkl	50.6	540.6	8,189.14	bcdef
GNZ 2500	56.4	5,637	ghijk	12.9	jklm	60.2	331.5	4,572.55	g
GNZX 0743	50.7	5,605	ghijk	12.4	klm	50.5	543.1	6,708.60	defg
PRE 12S12	53.5	5,764	fghijk	13.4	ijkl	54.2	461.7	6,238.37	efg
PRE 22S11	58.5	5,593	ghijk	13.7	hijkl	51.2	528.6	7,211.91	cdefg
PRE 22T10	67.1	7,848	a	19.2	abcde	54.9	446.5	8,616.42	bcdef
PRE 22T12	53.5	5,046	jk	14.3	ghijkl	47.8	602.1	8,669.35	bcdef
PREXT010	58.5	7,029	abcdef	17.9	abcdefgh	51.1	530.9	9,698.51	bcdef
PRE 22D11	48.5	5,065	jk	14.5	fghijkl	52.7	493.9	7,130.08	cdefg
SHS-7090	59.2	5,732	fghijk	16.0	defghijk	49.8	559.5	8,961.76	bcdef
SHS-7111	57.8	4,657	k	11.5	lm	51.6	519.5	6,069.87	efg
XBX80822	65.0	6,256	defghij	17.0	bcdefghij	43.3	699.6	11,911.0	abc
AG9040	58.5	5,382	hijk	14.3	ghijkl	52.8	492.6	7,007.67	cdefg
Dow 2A106	62.1	4,694	k	14.5	fghijkl	45.7	647.7	9,462.69	bcdef
BRS3035	60.0	5,338	hijk	14.4	ghijkl	47.8	601.6	8,700.45	bcdef
DKB330	61.4	7,693	ab	20.6	abc	54.3	460.6	9,474.76	bcdef
Average	59.3	6,058		16.0		49.4	567.1	9,188.56	
CV	13.7	11.20		13.3		12.5	23.85	27.27	

* Means followed by different letter differ at 5% probability, DMS-t test.

**Number of plants (NP, 1,000 ha⁻¹), green matter (GM, t/ha), dry matter (DM, t/ha), neutral detergent fiber (NDF,%DM), milk production per feed ton (MP/t, liter) and milk production per hectare (MP/ha, liter).

Hardness influence on corn silage quality

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Introduction Among the forms of forage conservation, silage is the most widely used to reduce the lack of forage. Corn is the forage that has the most important nutritional characteristics. New genotypes are released annually in the market, thus requiring the characterization of these as its purpose (grain or silage). The search for genotypes with better features morphological and productive of interest for the production of silage has been the aim of some research, directing the production system for best results. Thus, the aim of this work was to evaluate the influence of grain hardness (hard, semi-hard and soft) chemical quality and agronomic characteristics of corn genotypes for the production of silage maize genotypes Super Early testing center.

Material and Methods The experiment was conducted at the experimental area of the “Universidade Tecnológica Federal do Paraná, Dois Vizinhos campus”. Genotypes were supplied by Embrapa Maize and Sorghum (Sete Lagoas, MG, Brazil) corresponding to the test center, where 32 genotypes were evaluated Cycle Super-Early (Table 1). The plots were composed of two rows of five meters in length spaced 75 cm between rows and approximately 23 cm between plants. Data were subjected to analysis of variance in lattice design, and the means of groups of corn (hard, semi-hard and soft), were compared between them, by means of contrasts (Scheffé).

Results and Discussion The mean contrast between the type of grain (semi hard, hard and soft) when compared to each other had no significant effect for any of the contrasts (Table 2). Rossi Junior et al. (2006) observed the same in trials evaluating two hybrids of different groups of grains (half-soft and soft), where no significant differences in variables were obtained chemical quality [dry matter (%), crude protein (%), neutral detergent fiber (%) and acid detergent fiber (%)], only digestibility.

Conclusions The characteristic grain hardness does not influence the quality of silage produced.

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Table 1 - Genotype (GEN), trade name, genetic (BG), hardness of the grain (DG), producer (EMP) testing of genotypes belonging to Early Super Center.

Gen.	Comercial Name	BG*	DG**	EMP***	Gen.	Comercial Name	BG	DG	EMP
1	AS1555YG	HS	NI	AGR	17	BRSSint.Super	VAR	SD	BRS
2	AS1572YG	HS	SD	AGR	18	GNZ2500	HS	NI	GNZZ
3	AS1578YG	HS	SD	AGR	19	GNZX0743	HS	SD	GNZZ
4	30A37	HS	SD	AGN	20	PRE12S12	HS	SD	PRE
5	30A77	HS	NI	AGN	21	PRE22S11	HS	SD	PRE
6	20A78	HT	SD	AGN	22	PRE22T10	HT	SD	PRE
7	Dx908	HS	D	DX	23	PRE22T12	HT	SD	PRE
8	Dx603	HT	SD	DX	24	PREXT0109	HT	DM	PRE
9	2A550	HT	SD	DOW	25	PRE22D11	HD	SD	PRE
10	2B587	HS	SD	DOW	26	SHS-7090	HS	SD	SHS
11	2B433	HS	NI	DOW	27	SHS-7111	HS	SD	SHS
12	AL2007A	Var	SD	CATI	28	XBX80822	HS	SD	Semeali
13	H2002ALTA	HI	D	CATI	29	AG9040	HS	SD	BRS
14	H25ALTA	HI	SD	CATI	30	Dow2A106	HS	SD	BRS
15	Embrapa1F640	HS	NI	BRS	31	BRS3035	HT	SD	BRS
16	BRSGorutuba	Var	NI	BRS	32	DKB330YG	HS	D	BRS

* Genetic Basis: HS – simple hibrid, HT – triple hibrid, Var – open polinization, HD – double hibrid, HI – intervartietal hibrid; ** grain hardness: NI – don't informed, SD – half-hard, D - hard, DM - half-soft; *** Business: 1: Agroeste, 2: Coodetec; 3: Delta; 4: DI SOLO; 5: Syngenta Seeds; 6: Embrapa; 7: Prezzotto; 8: Santa Helena Sementes; 9: Pioneer Sementes; 10: Instituto Agronômico de Campinas; 11: Agromen Tecnologia; 12: Biomatrix; 13: Gêneze Sementes; 14: Dow AgroSciences; 15: DSMM/CATI e 16: Semeali.

Table 2 - Contrasts average testing super center early in the type of grain.

Var	SD	D	DMS	CV	SD	DM	DMS	CV	D	DM	DMS	CV
EP	2.28	2.37	0.32	1.26	2.38	2.37	0.53	1.26	2.37	2.37	0.60	1.260
NP	58701	61905	14759	2.35	58701	62143	24520	2.35	61905	62143	27691	2.35
MV	59625	65909	22155	3.36	59625	75641	36806	3.36	65909	75641	41566	3.36
MSE	0.50	0.47	0.10	2.02	0.50	0.49	0.17	2.02	0.47	0.49	0.19	2.02
EF	474.00	452.00	296.00	6.16	474.00	536.00	492.00	6.16	452.00	536.00	556.00	6.16
MSP	0.32	0.30	0.07	0.00	0.32	0.31	0.11	0.00	0.30	0.31	0.13	0
MSH	18.84	19.67	8.69	4.15	18.84	23.23	14.43	4.15	19.67	23.23	16.30	4.15
PB	7.23	7.38	2.65	3.50	7.23	8.18	4.40	3.50	7.38	8.18	4.97	3.5
ADF	27.38	26.15	17.32	6.29	27.38	21.18	28.77	6.29	26.15	21.18	32.49	6.29
NFF	50.10	47.56	20.19	3.97	50.10	42.92	33.55	3.97	47.56	42.92	37.89	3.97
TDN	68.81	69.62	11.33	1.56	68.81	72.92	18.83	1.56	69.62	72.92	21.26	1.56
ELL	1.55	1.59	0.47	2.90	1.55	1.72	0.79	2.90	1.59	1.72	0.89	2.9

*Var: characteristics; D: hard; SD: semi-hard; DM: soft, least significant difference (DMS) e coefficient of variation (CV) for the characteristics plant height (EP, m), number of plants per acre (NP), dry cob (MSE, %), effluent release (EF, gramas) e total plant dry matter (MSP, %), dry matter per acre (MSH, t/ha), crude protein (PB, %), acid detergent fiber (ADF, %), neutral detergent fiber (NDF, %), digestible nutrients total (TDN, %), net energy of lactation (ELL).

In vitro gas production of corn silage with different levels of enzymatic extract

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Introduction The use of filamentous fungi to produce enzymes of commercial interest is advantageous due to the fact that these microorganisms excrete these enzymes to the culture medium, which facilitates the process of obtaining and purifying these molecules (Reis, 2007). The addition of these enzymes in the diets of ruminants aims to increase the digestibility of fibrous feed. However, few studies that have tested their addition to diets are available, and found a great variability of results, making comparison between studies and validation of the efficacy of the product. A significant factor is great variability and level of enzyme to be applied. Nsereko et al. (2002) speculated that the application of a moderate level of enzymes in ruminant diets caused a beneficial break on the surface structure of the food before or after eating. Thus, it is noted that the optimal level of enzyme used depends on the diet, and high amounts reduce microbial attachment and limited digestion of food.

Materials and methods The enzymatic extract of *Aspergillus niger* was obtained by submerged fermentation (FSbm) SR in a liquid medium containing 1% wheat bran as substrate. The vials were kept for 72 hours at 30°C under 100 rpm agitation. The xylanolytic and cellulolytic activities were assayed using 3',5'-dinitrosalicylic acid (DNS) (Miller, 1959), using 1% (v/w) birchwood xylan and CMcellulose as substrates, at 39°C, respectively. One unit (U) was defined as the amount of enzyme that releases 1 µmol of reducing sugar per min. Gas production was determined by the technique of gas production, according to the methodology described by Mauricio et al. (1999). The rumen fluid was taken from crossbred sheep Dorper x Santa Inês fitted with rumen cannula and maintained with diet based on corn silage. The enzyme doses were 0.0 (control), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mL, added at the time of incubation. The experimental design was completely randomized design with nine treatments and four replications. Means were analyzed by regression test at 5% probability for the effect of time.

Results and discussion The enzymatic extract added to the silage at the time of incubation contained 25.78 U / mL xylanase and 1.54 U / mL cellulase at 39 ° C and pH 6.0. Table 1 shows the adjusted equations of cumulative gas production along with the optimal time of fermentation and the maximum gas production for each dose. Curves estimated cumulative gas production are shown in Figure 1. The curves of gas production showed quadratic trend, in which it was observed little difference between the equations. It was observed that the addition of fibrolytic enzymes increased the gas production when compared with treatment without enzymes. In general, it was found that the maximum gas production occurred between 42 and 43 hours of fermentation, noting that the dose was 4.0 mL with the highest gas production (331.34 mL / g DM). In another experiment of gas production, Jalilvand et al. (2008) found that the addition of enzymes (commercial) affected ruminal fermentation of forages differently, according to the fiber content and composition of the polysaccharides present and also that the concentration of enzyme addition is an important factor in the effectiveness them.

Conclusion The addition of enzyme extract containing fibrolytic enzymes increased the gas production of corn silage.

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Table 1. Equations and coefficient of determination adjusted for cumulative gas production.

Enzyme doses (mL)	Regression Equations	Time ¹	Gas production ²	R ²
0.0	$y = -0.1103x^2 + 9.4574x + 104.88$	42.87	307.61	0.93
0.5	$y = -0.1128x^2 + 9.6318x + 109.95$	42.69	315.56	0.92
1.0	$y = -0.1150x^2 + 9.7826x + 104.24$	42.53	312.28	0.92
1.5	$y = -0.1203x^2 + 10.234x + 111.46$	42.54	329.11	0.92
2.0	$y = -0.1120x^2 + 9.7182x + 108.91$	43.38	319.72	0.91
2.5	$y = -0.1173x^2 + 9.9904x + 106.16$	42.58	318.88	0.92
3.0	$y = -0.1249x^2 + 10.527x + 109.36$	42.14	331.17	0.92
3.5	$y = -0.1226x^2 + 10.405x + 106.47$	42.43	327.24	0.92
4.0	$y = -0.1238x^2 + 10.473x + 109.85$	42.30	331.34	0.92

¹ Optimal time of fermentation. ² Maximum cumulative gas production.

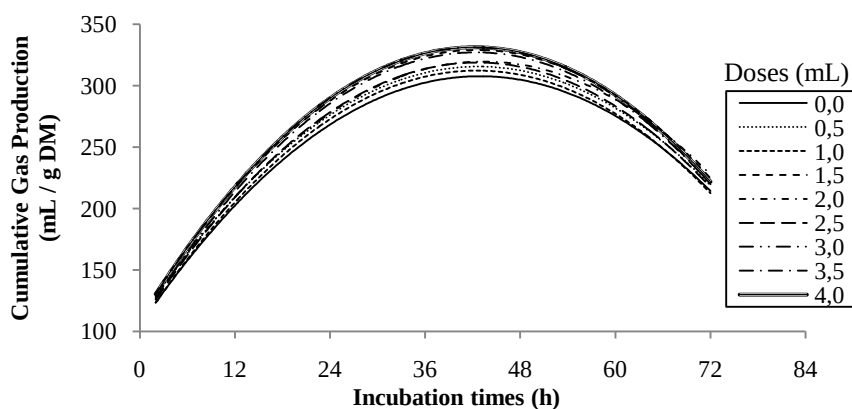


Figure 1. Cumulative gas production of corn silage with different enzyme levels.

Total digestible nutrients of corn silage with different levels of enzymatic extract

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Introduction Fungi of the genus *Aspergillus niger* are considered good producers of fibrolytic enzymes involved in the process of degradation of fiber, thus increasing the digestibility of food. Physiologically, there are a number of possible modes of action of exogenous enzymes and, as described, these effects can be as simple as the release of soluble carbohydrates or as complex as the removal of structural barriers ions. The effect depends on the type of food and type of enzyme used (Krueger & Adesogan, 2008). Another factor that should be studied is the level of enzyme to be applied. Some authors demonstrated that the addition of high levels of enzymes may be less effective than lower levels (Beauchemin et al., 2003). The optimal level of enzyme used depends on the diet, and high amounts reduce microbial attachment and limited digestion of food. The objective of this study was to evaluate the effect of adding the enzymatic extract of *Aspergillus niger* on the levels of total digestible nutrients (TDN) of corn silage.

Materials and methods The enzymatic extract of *Aspergillus niger* was obtained by submerged fermentation (FSbm) SR in a liquid medium containing 1% wheat bran as substrate. The vials were kept for 72 hours at 30°C under 100 rpm agitation. The xylanolytic and cellulolytic activities were assayed using 3',5'-dinitrosalicylic acid (DNS) (Miller, 1959), using 1% (v/w) birchwood xylan and CMcellulose as substrates, at 39°C, respectively. One unit (U) was defined as the amount of enzyme that releases 1 µmol of reducing sugar per min. The values of total digestible nutrients (TDN) were determined by the technique of gas production, according to the methodology described by Mauricio et al. (1999). The rumen fluid was taken from crossbred sheep Dorper x Santa Inês fitted with rumen cannula and maintained with diet based on corn silage. The enzyme doses were 0.0 (control), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mL, added at the time of incubation. We used the following equation: $EM (MJ / kg DM) = 2.20 + (0.136 * gas_{24}) + (0.0057 * CP) + (0.00029 * EE)$, where: EM is the metabolizable energy; gas₂₄ is the in vitro gas production in 24 hours, in mL / 0.2 g DM, and CP, mineral and EE are expressed in g / kg DM. Subsequently, the results were converted to TND, according to the equations proposed by NRC (2001). The experimental design was completely randomized design with nine treatments and four replications. Means were analyzed by regression test at 5% probability for the effect of time.

Results e discussion The enzymatic extract added to the silage at the time of incubation contained 25.78 U / mL xylanase and 1.54 U / mL cellulase at 39 ° C and pH 6.0. Table 1 shows the regression equations of TND on 24 and 48 hours of fermentation. The estimated curves for the same fermentation time, depending on the dose, are shown in Figure 1. There was a quadratic effect for the TDN 24 hours, and found the optimal dose of 3.8 mL. We observed a 6.1% increase in the TDN level when using the optimal dose (3.8 mL) compared with the control (0.0 mL). This increase in the TDN level is considered relevant since similar studies have shown a maximum increase of 4.0% (Queiroz et al., 2004). For the TDN in 48 hours fermentation it was observed linear effect, ie, an increase in the TDN level as the addition of enzyme extract. The use of

fibrolytic enzymes is an attractive way to improve energy utilization of tropical forages. Thus, more studies should be performed in order to reduce the variability of results and validate the effectiveness of the product.

Conclusion The addition of an enzymatic extract containing fibrolytic enzymes increased the total digestible nutrient content of corn silage.

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Table 1. Regression equations of TDN on 24 and 48 hours of fermentation.

TDN	Effect	R ²	F (Pr)	Optimal dose
24 hours	TDN = 64.70 + 2.21Dose - 0.291(Dose) ²	0.6761	6.26 (0.034)	3.80
48 hours	TDN = 62.93 + 0.52 Dose	0.4438	5.59 (0.051)	-

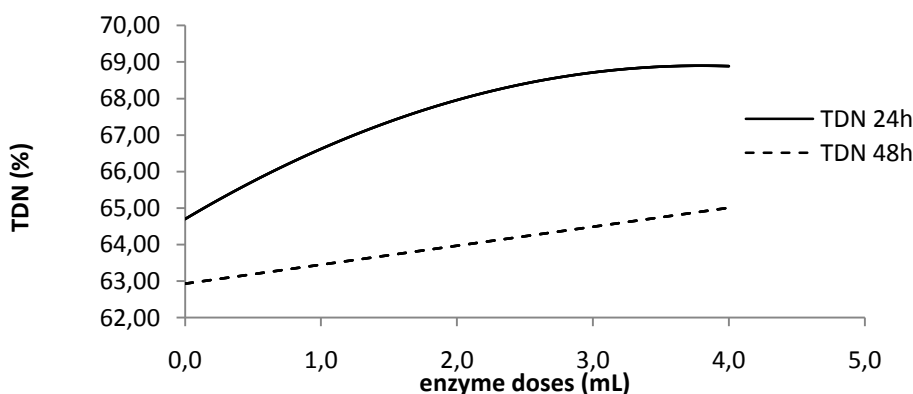


Figure 1. TDN estimated for 24 and 48 hours of fermentation depending on the doses of enzymatic extract used.

Aerobic stability of rations containing cassava fodder silage and mesquite pod meal

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Introduction Silage making is an excellent alternative for times of drought in semiarid regions mainly for small producers. Cassava (*Manihot esculenta* Crantz) is adapted to these regions and its fodder portion can be stored as silage. Aerobic stability of silage can be described as the resistance of the deterioration in forage mass after silo opening and the speed which the mass deteriorates after exposed to air and how it is affected by weather, where the daytime increases temperature and decreases in nocturnal activity and control the growth of microorganisms (Jobim et al. 2007). This study aimed to determine the aerobic stability of cassava fodder silage (CFS) and the addition of mesquite pod meal (MPM).

Materials and Methods Cassava fodder silage (CFS) was stored in plastic buckets and mesquite pod meal (MPM) was obtained from Riocon Company. The rations, calculated according to NRC (2007) presented forage:concentrate ratios of 100:0, 80:20, 60:40 and 40:60. The aerobic stability was determined by simulating the environment trough plastic buckets without lids with digital thermometers placed on the mass. Measures were recorded daily for environment temperature and mass temperature in the buckets at 10:00am and 4:00pm during ten days. Samples were collected every three days (1, 4, 7 and 10-day) to determine the chemical composition, according to Silva and Queiroz (2002). The variables of aerobic stability were evaluated according to Jobim et al. (2007) that are expressed as: number of days to rise 2°C in temperature, number of days to reach the maximum temperature, the sum of daily mean temperature accumulated from day-0 to day-5 and from day-0 to day-10 days and maximum temperature reached by the mass. The determination of total dry matter losses (DML) during the aerobic stability were calculated according to Schmidt (2006). This assay was distributed with four treatments and four replications in a completely randomized design, and means compared by Tukey test at 5% probability.

Results and Discussion The forage:concentrate ratio 60:40 and 80:20 presented 1.9°C above environment temperature on the fourth day and sixth day, and forage:concentrate ratio 100:0 and 40:60 revealed 1.6°C difference in the sixth and eighth days, respectively. The maximum temperatures were recorded on the tenth day except for the ratio 60:40, which occurred on the ninth day (Table 1). The presence of cyanogenic compounds in cassava shoots may indicate maintenance of the likely silage stability during use. Despite of the lower stability of CFS plus MPM, the time to rise (four days) maximum temperature (10 days) was higher regarding research using grasses as found by Loures (2004) and Schmidt (2006) who reported 2 to 4 days to maximum elevation and temperature, respectively.

Conclusion Cassava fodder silage showed greater stability in relation to the association with meal mesquite pods. The DM losses are directly proportional to stability loss of the evaluated diets. The feed can be evaluated for longer feeding than grass silages, without losing its nutritional value, allowing use of suitable forage. Diets based on CFS and MPM diets are viable and have satisfactory term degradation.

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Table 1. Temperature and total dry matter losses in Cassava fodder silage (CFS) and mesquite pod meal (MPM) at different forage:concentrate ratio.

Treatment	Mean temperature				Days to increase		Accumulated Temperature		T. Max.	DML (%)
	D-1	D-4	D-7	D-10	Start	Max	0–5 days	0–10 days		
100:0 CFS/MPM	23.0	20.8	22.5	24.5	8	10	- 2.5	1.5	24.5	1.92 ^D
80:20 CFS/MPM	23.0	21.1	23.5	24.4	6	9	- 2.0	1.4	24.6	10.28 ^B
60:40 CFS/MPM	23.0	21.4	23.3	25.3	4	10	- 1.6	2.3	25.3	17.74 ^A
40:60 CFS/MPM	23.0	21.1	22.8	24.6	6	10	- 2.3	1.6	24.6	3.24 ^C

* Means followed by same letter in columns do not differ by Tukey test at 5% probability

Organic acids production in pre-wilted Tifton 85 silages¹

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Introduction The ensiling process is the technique of converting soluble carbohydrates into lactic acid, causing decrease of pH silage mass to levels that inhibit the microbial activity, but retains its characteristics (Ferrari Junior et al., 2009). Tropical forages stand out for high production however, the excess moisture at the ensiling time increases the risk of undesirable fermentation. In order to reduce the moisture excess, the addition of citrus pulp is a viable alternative due its high capacity to retain moisture. In this context, microbial inoculants promote the digestion of structural and nonstructural carbohydrates as starch, yielding soluble sugars used as substrates for lactic fermentation. The aim of this study was to quantify organic acids in the pre-wilted Tifton 85 silage made with the addition of enzymatic bacterial inoculants and citrus pulp.

Materials and Methods This experiment was conducted at EMBRAPA – Gado de Leite, located in the city of Juiz de Fora, Minas Gerais state, Brazil (21°33'22"S 43°06'15"W). The treatments were: pre-wilted Tifton 85 silage with the addition of microbial inoculants; pre-wilted Tifton 85 silage with the addition of microbial inoculants and citrus pulp; pre-wilted Tifton 85 silage without microbial inoculants and with the addition of citrus pulp; pre-wilted Tifton 85 silage with no added microbial inoculants and with the addition of citrus pulp. The material was chopped and exposed to sunlight during 6 hours. The enzymatic bacterial inoculant containing *Streptococcus faecium*, *Lactobacillus plantarum*, *Lactobacillus salivarius*, cellulases and hemicellulases enzymes, was used following the manufacturer's recommendations. The citrus pulp was added at rate of 8 % fresh basis. The experimental silos were made in tubes of 10 cm diameter and 50 cm length, and stored for 60 days. The analysis of organic acids were made in a high-performance liquid chromatography (HPLC), a SHIMADZU, SPD-10A VP, coupled to an ultraviolet detector (UV), using 210 nm wavelength. The experimental design was a completely randomized in a factorial arrangement 2 (with or without microbial inoculant) x 2 (with or without citrus pulp), using 5 replications. The results were analyzed using the ANOVA procedure and means compared by the SNK test at 5% probability.

Results and discussion In pre-wilted Tifton 85 silages the use of citrus pulp reduced ($P < 0.05$) the levels of lactic acid, while the microbial inoculants increased it. Among evaluated silages, only silages without microbial inoculants and with citrus pulp had lower levels than those indicated by Catchpoole and Henzell (1971) ranging from 3 to 13 % of lactic acid. In pre-wilted Tifton 85 silage made with microbial inoculants, the addition of citrus pulp reduced ($P < 0.05$) the levels of acetic acid. However in silage made without citrus pulp and with the use of microbial inoculants acetic acid levels increased ($P < 0.05$). The addition of citrus pulp in silages without microbial inoculant reduced ($P < 0.05$) concentration of propionic acid. The use of microbial inoculants in silage without citrus pulp reduced ($P < 0.05$) the levels of propionic acid, however, silages made with citrus pulp and microbial inoculant increased the levels of propionic acid. The addition of citrus pulp had no effect ($P > 0.05$) on the concentration of butyric acid in pre-wilted Tifton 85 silage made with or without inoculant. The use of microbial inoculants in pre-wilted

Tifton 85 silage reduced ($P < 0.05$) levels of butyric acid in silages, but in all silages exceeded the levels indicated by Ferreira (2001), thus indicating the occurrence of undesirable fermentations.

Conclusions The addition of citrus pulp reduced the levels of lactic acid, while the use of microbial inoculant provided an increase in levels of lactic acid and reduced levels of butyric acid in pre-wilted Tifton 85 silages.

Acknowledgments To FAPEMIG for funding the Project and support for the participation in the event and CAPES for scholarships granted.

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Table 1. Lactic, acetic, propionic and butyric acids content in pre-wilted Tifton 85 silages.

	Lactic Acid (% DM)			Acetic Acid (% DM)		
	Inoculant			Inoculant		
	Without	With	Mean	Without	With	Mean
Citrus pulp						
Without	4.24bA	5.88aA	5.06	0.84bA	1.70aA	1.27
With	1.46bB	3.76aB	2.61	1.10aA	0.79bB	0.95
Mean	2.85	4.82		0.97	1.25	
	Propionic Acid (% DM)			Butyric Acid (% DM)		
	Inoculant			Inoculant		
	Without	With	Mean	Without	With	Mean
Citrus pulp						
Without	0.27aA	0.21bA	0.24	3.88aA	2.28bA	3.08
With	0.09bB	0.17aA	0.13	3.16aA	2.51bA	2.83
Mean	0.18	0.19		3.52	2.39	

Means followed by the same letter, lowercase in line and uppercase in column, did not differ statistically $*(P > 0.05)$.

Losses in wilted Tifton 85 grass silage with different additives¹

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Introduction Because the seasonality in forage production it is necessary to preserve roughage in the summer in order to use it during the dry season. Silage of wilted Tifton 85 grass may be used for this purpose, but its conservation is difficult. Secondary fermentation, high effluent production and aerobic deterioration can increase the dry matter loss from 7 to 40% (McDonald et al., 1991). The use of additives in tropical grass silages can overcome part of these limitations caused by the high moisture content, low soluble carbohydrate and the great buffering capacity. According to Woolford (1984), these factors inhibit the occurrence of an adequate fermentative process and impair a better quality silage production. In order to decrease these losses, several practices have been used, but they have to be evaluated. The use of citrus pulp and bacterial inoculants increases the lactic acid production, accelerate the pH drop, improve preservation and reduce losses (Pitt, 1990). The objective of this work was to determine the losses in wilted Tifton 85 silage with the addition of enzymatic bacterial inoculants and of citrus pulp.

Material and Methods The experiment was carried out at the EMBRAPA Dairy Cattle, located in Coronel Pacheco, MG, Brazil. The four treatments of wilted Tifton 85 silage were: with inoculant, with inoculant and citrus pulp; with no inoculant or citrus pulp; with citrus pulp. The grass was harvested, chopped and exposed to sunlight during 6 hours. After this time a sample was collected for chemical analyses and the forage was submitted to the experimental treatments. The enzymatic bacterial inoculant containing *Streptococcus faecium*, *Lactobacillus plantarum*, *Lactobacillus salivarius* and the enzymes cellulases and hemicellulases, was used following the manufacturer's recommendations. The citrus pulp was added as 8% of fresh basis. Experimental silos, 10 cm diameter and 50 cm length, were made from PVC tubes and had rubber caps in both ends, one of them fitted with a Bunsen valve to allow gas escape. There was a dry sand bag, of known weight, at the bottom of each silo in order to absorb the effluent produced. Silos were weighed before and after forage filling. After 60 days of fermentation silos were weighed and opened. The sand bag as well the forage were removed and also weighed. Differences between initial and final weights of silo, sand bag and forage were used to determine the gas, effluent and dry matter loss, respectively. A completely randomized experimental design was used, with a factorial arrangement 2 (with or without inoculant) x 2 (with or without citrus pulp). The results were analyzed using the ANOVA procedure. Data were compared by the SNK test at 5% probability.

Results and Discussion: The results are showed in Table 1. Gas loss decreased only when citrus pulp and microbial inoculants were used together ($P < 0.05$). The treatments did not affect effluent loss ($P > 0.05$), probably due to the dry matter content of ensiled material (30.95%). Following the gas loss pattern, total loss was reduced only when citrus pulp and bacterial inoculants were used together. It can be explained by the greater participation of gas loss in total losses.

Conclusions: The use of microbial inoculants was effective on reducing gas loss, as well as total losses of wilted Tifton 85 grass silage only when associated to citrus pulp.

Acknowledgement To FAPEMIG, for funding the Project and supporting the participation in the event. To CAPES for scholarships granted.

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Table 1. Gas, effluent and total losses of wilted Tifton 85 grass silage with citrus pulp and enzymatic bacterial inoculant.

	Gas losses (%)			Effluent losses (%)			Total losses (%)		
	Inoculant			Inoculant			Inoculant		
Citrus Pulp	Without	With	Mean	Without	With	Mean	Without	With	Mean
Without	2.0aA	1.38aA	1.69	0.29aA	0.21aA	0.25	2.28aA	1.59aA	1.94
With	2.25aA	1.42bA	1.83	0.13aA	0.16aA	0.14	2.37aA	1.58bA	1.98
Mean	2.12	1.4		0.22	0.17		2.33	1.59	
CV		27.72			65.74			22.67	

Means followed by the same letter, uppercase in the same column and lowercase in the same line, do not differ *(P>0.05).

Development of fungi in hays of white and black oats under two cutting heights

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Introduction The oat is a winter forage with high nutritional value, which can be used as pasture or conserved forage, in the hay or silage making. The management adopted for the production of hay, associated with the storage conditions interferes in the quality and sanity of hay, as well as losses in the process (Domingues, 2009). During storage, the population of fungi such as *Fusarium* and *Cladosporium* is reduced. On the other hand, the typical epiphytic genres, such as *Aspergillus* and *Penicillium*, increases (Hlodversson and Kaspersson (1986). The objective of this research was to evaluate the occurrence of fungi in white and black oat before cutting and in the hay obtained under two cutting heights.

Materials and Methods The experiment was conducted in Marechal Cândido Rondon, PR (24°33'40"S, 54° 04 '12"W). The seeding rates were 70 and 60 kg/ha for black oat (*Avena strigosa*) and white (*Avena sativa*) oat, respectively, using precision seeder attached to the tractor in lines spaced 0.175 m. The experimental design was a randomized block in a 2x2x3 factorial design, with two oats (*Avena sativa* and *Avena sativa*), two cutting heights (10 and 20 cm) and three assessment periods (before cutting, at the haymaking and after 30 days storage), with six replications. After sampling, the material was processed with the aid of scissors for particle size reduction. Fungi were isolated by induction of growth in BDA culture media (200 g potato, 20 g dextrose, 15 g agar and 1000 mL of distilled water), by induced sporulation. Identification of fungi was performed using specific identification keys (Samson et al., 1995). The data were transformed in logarithm (Log) and subjected to analysis of variance and means were compared by Tukey test at 5% probability.

Results and Discussion For *Aspergillus*, *Penicillium* and total counts of fungi there was no significant effect of the evaluation period, cutting height and of the interaction evaluation period x time. Higher counts of *Aspergillus* were observed after storage, in which the 10 cm cutting height resulted in higher development of this fungus (Figure 1). This result may be related to the higher proximity to the ground, resulting in higher soil contact with hay. The *Aspergillus* can serve as biological indicators of storage conditions, and their quantification in conserved forage is of fundamental importance because it represents a potential mycotoxins producer (Moser, 1995), occurring more frequently in hot and humid environments. The population of *Cladosporium* was not influenced by treatments, with an average of 1×10^2 CFU/g. According to Nascimento et al. (2000), fungi *Cladosporium* tend to disappear during the storage period. For *Fusarium* and *Rizophus* population, it was observed significant effects ($P < 0.05$) of evaluation period, of oats species and the interaction (oats x assessment periods). Higher counts of these fungi were observed after hay storage. However the white oat favored population increase of *Rizophus* at the time of baling. The same occurred for oats after storage (Figure 2).

Conclusion The storage of white and black oat hay increased *Aspergillus* and *Penicillium* population.

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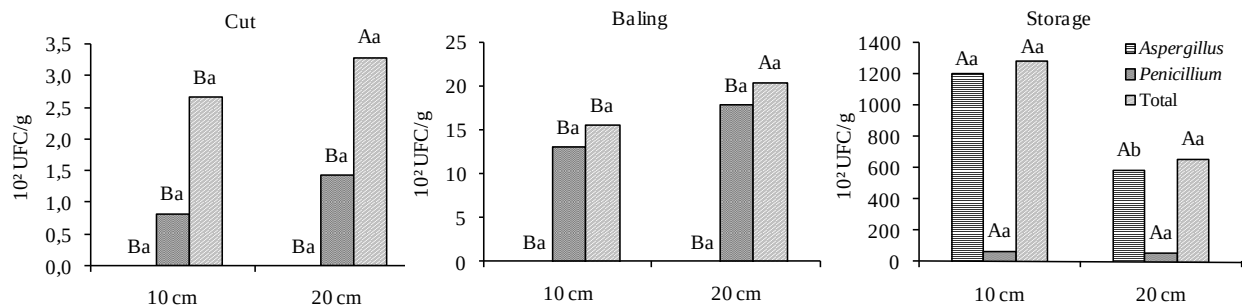


Figure 1. Occurrence of fungi *Aspergillus*, *Penicillium* and total counts, in the black and white oat hay under two cutting heights, at the time of cutting, baling and after the 30 days of storage. (Bars followed by the same uppercase letter for each fungi, and lowercase for each evaluation period do not differ by Tukey test at 5% probability).

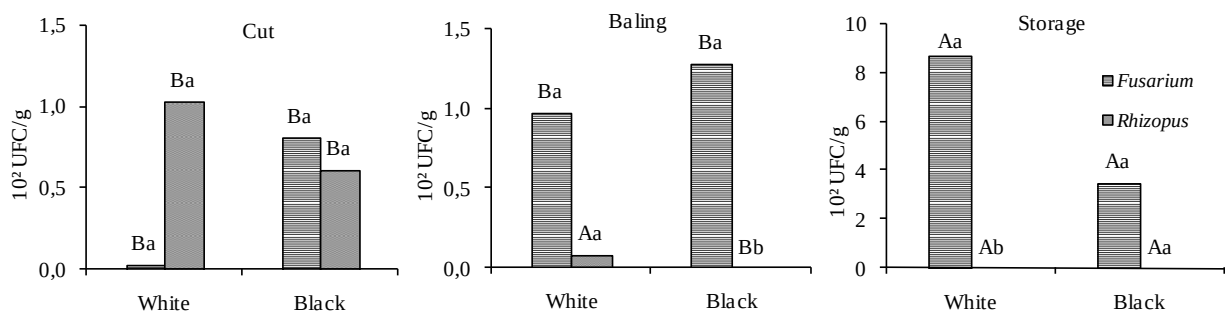


Figure 2. Occurrence of fungi *Fusarium* and *Rizophus*, in the black and white oat hay at the time of cutting, baling and after the 30 days of storage. (For each fungi, bars followed by the same uppercase letter between the periods of assessment and lowercase between the oats specie did not differ by Tukey test at 5% probability).

Aerobic stability of wet brewers waste ensiled with different nutritional additives

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Introduction The use of agroindustrial residues in animal feed allows minimizing production costs, improve productivity and minimize environmental impacts caused by their improper destination. The use of silage wet brewery waste (WBW) has emerged for its nutritional quality and availability during the year. The high water content of the WBW is limiting for ensiling, being necessary the drying process or the inclusion of absorbent additives. After opening the silos, exposure to oxygen contributes to the growth of microorganisms that deteriorate the silage. According to Oude Elferink et al. (2000), the aerobic stability can be defined as resistance to temperature increase of silage in panel of the silo and during supply to the animal at the trough. Thus, the objective was to evaluate the aerobic stability of wet brewers waste, ensiled with different nutritional additives.

Materials and Methods The experiment was conducted in Marechal Cândido Rondon, Paraná, Brazil (24°33'40"S 54°04'12"W). The wet brewery waste (BWB) was ensiled in PVC silos (100mm), 50 cm height and 10 cm diameter, with a capacity of approximately 3.00 kg of waste. The silage was compacted and the silos were sealed with PVC cover adapted to allow free escape of gases through Bunsen valves. The experiment design was a subdivided plot, where the treatments were: ensiled wet brewery waste (EWBW), WBW with wheat bran (EWBW + WB), EWBW with corn grits (EWBW + CG) and EWBW with soybean hulls (EWBW + SH). The silos opening was after 52 days of fermentation, and there were taken temperature and pH (time 0) values. After opening the silo, the material was placed in plastic trays, about 1.5 kg, staying for 10 days for aerobic stability evaluation. Trays with the samples were kept in a closed room, monitoring the temperature with digital thermometer in each sample twice daily (8a.m. and 8 p.m.). The pH of samples was monitored according to the methodology proposed by Cherney and Cherney (2003). The aerobic stability was calculated as the time, in hours, to raise the temperature 2°C higher than the room temperature (Kung Jr. et al., 2000). Data were subjected to analysis of variance and means compared by Tukey test at 5% probability.

Results and Discussion The aerobic stability was not affected ($P > 0.05$) by the treatments tested. There was no heating until about 40 hours after opening the silos, indicating that evaluated silages have good stability. However, according to Siqueira et al. (2010), the interpretation of the results of aerobic stability considering additives should be undertaken with caution. The variable days to achieve the maximum temperature of the ensiled mass was also unaffected by the different nutritional additives. The maximum temperature reached, discounting the environment temperature, was higher ($P < 0.05$) for silage EWBW + SH, and the lowest temperature of the silage was presented by EWBW without additional additives. The temperature measurements taken twice a day can be a critical factor in the accuracy of the information. The maximum value of pH (pHmax) was observed in silage EWBW + SH. However, the number of days to achieve the maximum pH (DpHmax) on this silage was higher than the others. The values of pH in the silo opening, although different among silages, showed to be next, within the range considered standard (Rotz and Muck, 1994). According to the model of aerobic deterioration of Muck et al.

(1991), several factors affect silage stability, including pH, acids, residual soluble carbohydrates and initial population of yeasts and filamentous fungi. The temperature alone is not the most appropriate parameter for characterizing aerobic stability.

Conclusion The aerobic stability of wet brewery waste silage was not affected by adding nutritional additives at the time of ensiling.

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Table 1. H 2°C values (time in hours to raise temperatures by 2°C); DTmax (number of days to reach the maximum temperature), Tmax (maximum temperature reached by the silage mass); pH 0 (pH measured at the time of silo opening); pHmax (maximum pH); DpHmax (number of days to reach the maximum pH)

Variables	Treatments				CV %
	EWBW	EWBW + WB	EWBW + CG	EWBW + SH	
H 2°C, hours ^{ns}	39.00	48.00	48.00	48.00	19.67
DTmax, days ^{ns}	6.00	3.75	4.25	5.25	30.73
Tmax, °C	26.10c	29.30ab	27.20bc	30.15a	4.13
pH 0	3.52c	3.81a	3.65b	3.82a	0.23
pHmax, pH	6.19c	7.44b	6.12c	8.54a	3.92
DpHmax, days	9.50ab	9.50ab	8.50b	10.00a	5.33

^{ns} P>0.05;

Means followed by same letter in row did not differ (P> 0.05) by Tukey test.

Sensorial traits, pH, losses, and dry matter recovery of elephantgrass silage with different proportions of cotton processing residue

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Introduction Elephantgrass is used on many cattle raising farms to be a strategic source of forage in the dry season of the year. In general, it is left to grow freely for just one cut, resulting in low forage quality, negatively affecting animal performance. Making silage seems to be the best way to conserve it. However its high moisture content and low amount of soluble carbohydrates limits the fermentation process. Dry additives have been added in elephantgrass ensiling to improve the pattern of fermentation (Quadros et al., 2001). The state of Bahia is the second largest producer of cotton in Brazil, releasing huge amounts of residue. The objectives of this work was to evaluate the sensorial traits, pH, gas and effluent losses, and dry matter (DM) recovery of elephantgrass silage with different proportions of cotton processing residue.

Materials and Methods The experiment was conducted at the Bahia State University, in Barreiras, BA, Brazil (12° 09' 10" S 44° 59' 24" W). Elephantgrass with 60 days of regrowth, with 20 % DM, was cut into average particle sizes of 3.0 mm, then it was stored exclusively or with 5, 10, 15, and 20% of cotton processing residue (fresh basis). There were used 25 plastic buckets, sized of 0.02 m³, as experimental silos containing 4 kg of sand at the bottom, having 5 of them each treatment, following a completely random design. The grass and the residue were mixed conforming to each treatment and compacted using feet, reaching the density of 340 kg/m³. After 50 days, the silos were weighted and opened, measuring gas and effluent losses by gravimetric evaluation. After that, pH was measured using Silva and Queiroz (2002) methodology. Samples of 200g were dried in forced air circulation ovens, with temperature regulated at 140°F for 72 hours to determine DM content. Another three samples of 200 g were evaluated for sensorial traits, by 3 person, using the Meyer et al. (1989) scale. Data was analyzed using ASSISTAT, assuming that significant differences among average values when P values were fewer than 5%. Furthermore, data was analyzed by orthogonal polynomials, verifying the linear, quadratic, or cubic effects, and the significance degree.

Results and discussion The DM content increased linearly ($P<0.01$) with the increasing of the proportions of cotton residue, from 18.4 % in the exclusive elephantgrass silage to 31.6 % with 20 % residue. The pH was higher ($P<0.01$) in the silages with 10, 15, and 20 % of cotton residue in comparison to 0 and 5 %, instead of what was the observed by Quadros et al. (2003) when adding from 5 to 20 % coffee hulls to elephantgrass silage. The ideal pH for good silage starts at 3.8 to 4.2 (McDonald et al., 1991). According to these authors, grass silage dry matter contents of 20 % are normally accepted as a lower limit to decrease pH close to 4.0 and then preserve ensiled herbage satisfactorily. However, when herbage is too wet, even lowering its pH to 4.0 would not be enough to inhibit clostridial growth. The interaction between pH and the DM should also be considered to control undesirable silage fermentation (Quadros et al, 2003). The effluent losses dropped with the inclusion of cotton residue ($P<0.01$) (Table 1). On the other hand, gas losses did not follow a defined pattern with the increasing of cotton residue, affecting the results of total losses and DM recovery. The worth result was obtained with 20 % of residue, compared with 0, 5, and 15 %, while with 10% did not differ among them ($P>0.05$). The sensorial traits, divided in

characteristics and sanity, was an easy way to evaluate silages in the field. The characteristics of silage were better using 20% cotton residue ($P < 0.05$) than the exclusive elephantgrass, while with 5, 10, and 15% of residue did not differ among them ($P > 0.05$) (Table 1). The score, according to Meyer et al. (1989), below or equal to 15 indicates non satisfying silage, 16 to 19 regular, 20 to 25 satisfying, 26 to 30 good to very good.

Conclusion The inclusion of cotton residue can be recommended to increase both the sensorial traits and DM content, and reduce effluent losses. The higher pH seems to not have affected the silage fermentation pattern. The extension of gas losses needs to be studied further.

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Table 1. Sensorial traits, DM content, pH and losses in the elephantgrass silage with different proportions of cotton residue

Variables	Cotton residue proportion					SEM ¹	Effect
	0%	5%	10%	15%	20%		
DM (%)	18.4 ^d	22.9 ^{cd}	26.5 ^{bc}	30.5 ^{ab}	31.6 ^a	4.71	Linear**
pH	4.01 ^b	4.10 ^b	4.37 ^a	4.49 ^a	4.45 ^a	0.18	Quadratic*
Losses by effluent (kg/ton)	51.6 ^a	4.49 ^b	2.67 ^b	3.50 ^b	1.76 ^b	4.45	Linear**
Losses by gas (%)	1.09 ^b	1.01 ^b	1.83 ^{ab}	1.52 ^b	2.90 ^a	1.10	NS
DM recovery (%)	89.1 ^a	89.9 ^a	81.7 ^{ab}	84.8 ^a	71.0 ^b	11.0	NS
Total DM losses (%)	15.6 ^b	10.5 ^b	18.5 ^{ab}	15.5 ^b	29.2 ^a	10.9	NS
Sensorial traits							
Characteristic	15.0 ^b	21.5 ^{ab}	21.4 ^{ab}	21.0 ^{ab}	25.3 ^a	6.90	Cubic*
Sanity	-0.53 ^a	-0.53 ^a	-2.93 ^a	-1.80 ^a	-1.60 ^a	3.84	NS

^{a-d}Means within a row with different superscripts differ ($P < 0.05$).

²SEM = standard error of the mean. NS $P > 0.05$; * $P < 0.05$; ** $P < 0.01$.

Aerobic stability of elephantgrass silage with different proportions of cotton processing residue

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Introduction At the silo opening, the anaerobic environment, one with the responsibility of forage conservation, became aerobic. In this event, microorganisms, dormant in the oxygen absence grow faster, promoting intense metabolic activity, emitting heat and consuming nutrients, increasing losses of dry matter (DM) and decreasing the nutritive value of forage (Siqueira et al., 2005). Losses after opening depend on silo management, however the extension of deterioration of forage is linked to the aerobic stability of silage (Nussio et al., 2002). Bahia is the second largest producer of cotton in Brazil, resulting in the availability of huge amount of residue. The objective of this work was to evaluate the aerobic stability of elephantgrass silage with different proportions of cotton processing residue.

Materials and Methods The experiment was conducted at the Bahia State University, Barreiras, BA, Brazil (12° 09' 10" S 44° 59' 24" W). Elephantgrass with 60 days of regrowth, with 20 % of DM, was cut in average particle sizes of 3 mm, and then it was stored exclusively or with 5, 10, 15, and 20% of cotton processing residue (fresh basis). There were 25 plastic buckets used as experimental silos sized of 0.02 m³ containing 4 kg of sand at the bottom, 4 of them for each treatment following a completely random design. The grass and the residue were mixed according to the treatment and compacted using feet, reaching the density of 340 kg/m³. After 50 days the silos were opened and a sample of 500g was placed in a polypropylene box. Temperatures were measured using a digital thermometer at different times post-opening (0, 3rd, 15th, 24th, 48th, 72th, 96th, 120th hour). The pH was determined by the Silva and Queiroz (2002) methodology at 0, 48th, and 96th hour post-opening. Data was analyzed by orthogonal polynomials.

Results and discussion The aerobic stability was affected by increasing cotton residue. The exclusive elephantgrass silage increased the temperature linearly through the time reaching 93.2 °F, almost the same with 5 % cotton residue (Figure 1). On the other hand, storing elephantgrass with 10, 15, and 20 % of residue resulted in better stability post-opening. The results obtained in this work agree with the Andrade et al. (2010), when adding 5 and 10 % of soybean hulls to elephantgrass silage. The break of stability occurred just in the treatment control, at the 96th post-opening. The low levels of temperature at the 16th hour post-opening in all silages (Figure 1) probably in relation to lower environment temperatures during the first night. The pH of exclusive elephantgrass silage and with 5 % cotton residue began with lower pH until the 48th hour post-opening, however after that they had a great increase, proportionally higher when compared to 10, 15, and 20 % residue, which showed more stability through the same time post-opening (Figure 1). As a consequence of undesirable microorganisms growth, it had soluble carbohydrates utilization, nitrogen compounds, and vitamins of silages, resulting in the decreasing of the nutritive value (Siqueira et al., 2005).

Conclusion The inclusion of 10, 15 and 20 % cotton residue increased aerobic stability of elephantgrass silage.

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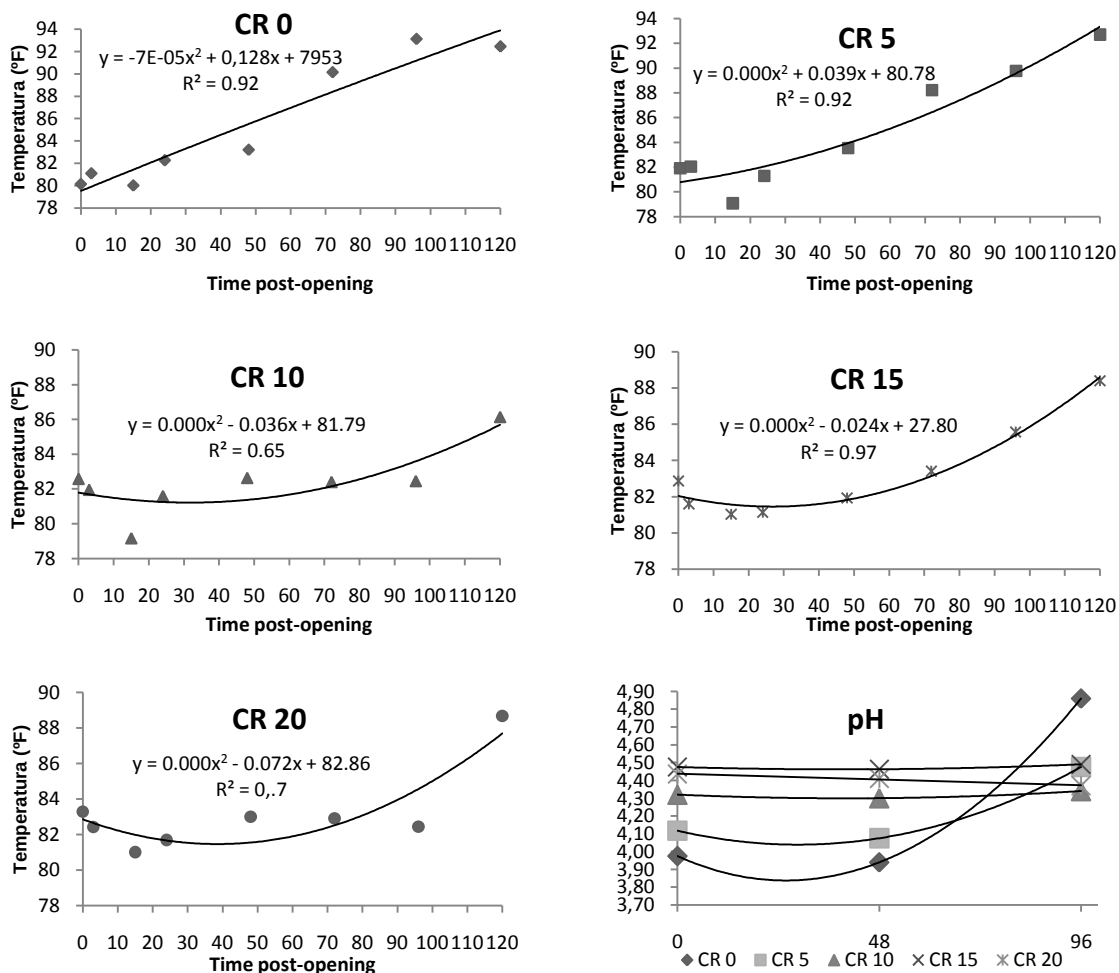


Figure 1. Aerobic stability of elephantgrass silage with different proportion (0. 5. 10. 15. and 20 %) of cotton residue (CR).

Nitrogen fertilization and inclusion of inoculants in maize silage production

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Introduction The maize silage is one of the most used products for ruminants feeding. However, productive and qualitative characteristics are dependent on the handling used in its confection. The expression of plant potential is related to nutrients availability, as nitrogen (N). This nutrient is important for tissue growth and proteins composition (Brouwer and Flood, 1995). However, the technology used in maize production can be impracticable if the fermentation process after ensiling is not adequate. Therefore, the use of microbial inoculants in the ensiling becomes an interesting characteristic, because according to Henderson (1993), these additives contribute to the reduction of enzymatic proteolysis, due to the fast pH drop inside the silo, what favors the production of great amounts of lactic acid, and represents, therefore, the possibility of higher dry matter recovery. This paper was conducted with the aim to evaluate chemical and fermentation characteristics of maize silage managed with different levels of nitrogen fertilization and ensiled with or without the inclusion of inoculant.

Materials and Methods The trial was carried out in Palmeira das Missões (27°53'58"S, 53°18'49"W), RS, Brazil. The treatments evaluated consisted of two factors: nitrogen fertilization as urea (N = 45%) (0, 100, 200 and 300 kg urea/ha) and inoculant (mini-silos with or without inclusion of inoculant). We used a 2 x 4 factorial arrangement (four levels of nitrogen fertilization x silage with or without inoculant), in a complete randomized design, with three replications. Twelve plots were made with 15 m² each. When the grains were at half milky half dough kernel stage, the plants were harvested manually (discarding 50 cm borders) at 20 cm height, chopped in a stationary chopper (adjusted to 2 cm mean particle size) and weighed. Mini-silos were composed of 20 L buckets, coated by black plastic bags, with 2 mini-silos per plot (one with and one without inoculante addition). The inoculant consisted of the following components: *Lactobacillus plantarum* (8 x 10⁹ cfu/g), *Lactobacillus buchneri* (10¹¹ cfu/g) and *Enterococcus faecium* (2 x 10⁹ cfu/g). The mini-silos had been weighed soon after its confection and later in the opening day, being the differences between the weights multiplied by its respective dry matter content, and transformed into percentage of the initial mass. Silage samples were collected for the determination of DM, CP, NDF, ADF and TDN content, metabolizable energy (ME), net energy for lactation (NEL), net energy for gain (NEG), net energy for maintenance (NEM) digestible energy (DE, %DM), according to procedures described by Silva and Queiroz (2002). Two kilograms of silage of each silo were removed and conditioned in styrofoam boxes with 2 L capacity. Temperatures were recorded at 0 (immediately after the allocation of the material in the styrofoam boxes), 12, 24, 48, 72, 96 and 120 hours for aerobic stability determination. The results of the analyzed temperatures were calculated as the rate of temperature rise, using the maximum temperature observed divided by the time necessary to reach the maximum temperature (Ruppel et al., 1995). The results were submitted to the analysis of variance and the means compared by the Tukey test (5% probability). The statistical program Assistat (2011) was used for the statistical analyses.

Results and Discussion With exception for the CP, for the other studied variables it was not observed effect (P>0.05) of inoculant and fertilization. The nitrogen fertilization increased CP, TDN, ME, NEL, NEM, NEG, and decreased the ADF content (Table 1). The inclusion of

inoculant did not affect the chemical quality of silage. The highest CP content for the groups that had received fertilization with urea were due to protein deposition in the grain, related to the N content in the plant mainly at the beginning of the flowering. Therefore, the urea inclusion increased the available N in the soil for plant absorption (Brouwer and Flood, 1995). In relation to the percentage of dry matter loss and the aerobic stability differences had not been verified ($P>0.05$) between the treatments with nitrogen fertilization or the use or not of inoculant. This fact possibly occurred due to the handling adopted in the silage confection. Probably there was an ideal condition and the storage time was enough to provide good fermentation.

Conclusion The nitrogen fertilization affects positively the silage quality. The correct procedures in the ensiling process do not require the use of inoculant to improve quality.

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Table 1. Chemical composition of maize silage treated with different levels of urea, and ensiled with (WI) or without (WHI) inclusion of inoculant

Variable	Urea (kg/ha)								CV (%)
	0		100		200		300		
	WI	WHI	WI	WHI	WI	WHI	WI	WHI	
DM, %	30.43aA	29.97aA	30.53aA	32.53aA	29.13aA	32.87aA	30.49aA	28.67aA	6.64
CP, %	6.32aA	6.38Ca	7.02bA	7.18bA	7.99aA	8.04aA	7.72aA	7.82abA	3.94
NDF, %	60.08aA	58.18aA	59.43aA	60.63aA	59.28aA	61.07aA	57.31aA	58.02aA	4.87
ADF, %	28.37aA	28.68aA	27.63bA	27.12bA	26.44bA	26.78bA	26.33bA	27.03bA	3.92
TDN, %	66.98bA	66.76bA	67.5abA	67.86abA	68.33aA	68.10aA	68.41aA	67.91aA	1.10
ME, Mcal/kg	2.42bA	2.41bA	2.44abA	2.45abA	2.47aA	2.46aA	2.47aA	2.45aA	1.10
NEL, Mcal/kg	1.52bA	1.52bA	1.54abA	1.55abA	1.56aA	1.55aA	1.56aA	1.55aA	1.15
NEM, Mcal/kg	1.65bA	1.65bA	1.67abA	1.68abA	1.70aA	1.70aA	1.69abA	1.68abA	1.32
NEG, Mcal/kg	0.93bA	0.93bA	0.95abA	0.96abA	0.98aA	0.97aA	0.97abA	0.96abA	2.3
DE, %	66.8bA	66.56bA	67.37abA	67.78abA	68.30aA	68.05aA	68.39aA	67.84aA	1.23

Means followed by the same capital letters (inoculant effect) and lowercase letters (nitrogen fertilization effect) do not differ ($P>0.05$) by Tukey test.

Maize plants managed with different levels of nitrogen fertilization

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Introduction Maize silage productive and qualitative characteristics are influenced by the techniques used at the ensiling process. Improvements in soil quality brings economic benefits to the production, therefore the expression of income potentials of the plant is related to nutrients availability, as the nitrogen (N). The nitrogen fertilization is the one who brings more increments to the dry matter (DM) production. Therefore, most tropical soils are deficient in organic residues (France et al., 2007). The search for the ideal dosage of nitrogen aims to optimum plant performance, mainly in relation to the amount of produced silage mass (SM). This paper was conducted with the aim to evaluate productive parameters and the chemical composition of maize plants to ensiling managed with different levels of nitrogen fertilization.

Materials and Methods The trial was carried out in Palmeira das Missões (27°53'58"S, 53°18'49"W), RS, Brazil. The treatments evaluated consisted of different levels of nitrogen fertilization as urea (N = 45 %): 0, 100, 200 and 300 kg urea/ha, in a complete randomized design, with three replications. Twelve plots were made with 15 m² each one. The urea was divided in two applications, being half applied when the culture presented four leaves and the remaining when it presented eight leaves. When the grains were at half milky half dough kernel stage, five plants and five ears randomly chosen were evaluated in each plot (discarding 50 cm of the borders) and analyzed for: ear diameter and length with straw (DWS and LWS, respectively) and without straw (DWOS and LWOS, respectively), cob diameter (CD) and plant height (PH). At the selected ears there were also evaluated the number of grain rows (GR) and average grains per row (AG). Ears number per plot (ENP) and plants number per plot (PNP) were also evaluated. The plants were manually harvested (discarding 50 cm of the borders) to a 20 cm height, chopped (adjusted for 2 cm mean particle) and weighed. Samples were taken for the determination of DM, CP, NDF, ADF, TDN, metabolizable energy (ME), net energy for lactation (NEL), net energy for gain (NEG), net energy for maintenance (NEM), digestible energy (DE, %DM), according to Silva and Queiroz (2002). The results were submitted to the analysis of variance and means compared by the Tukey test (5% probability). The statistical program Assistat (2011) was used for statistical analyses.

Results and Discussion Dry matter and silage mass productions (kg/ha) were higher in the treatment that received 300 kg urea/ha with no difference ($P>0.05$) from treatments with 100 and 200 kg urea/ha (Table 1). Similar pattern was observed for the morphological parameters (ear length without straw, plant height and average grains per line per ear) and chemical (crude protein) (Table 2). The gains in production (SM and DM) with the nitrogen fertilization occurred due to the increment in plant height, ear length, and grains production. Grain production also contributed for the higher protein content. In addition, nitrogen fertilization also provides greater protein deposition in grains, because there is a relation with N content in the plant (Brouwer and Flood, 1995).

Conclusion The nitrogen fertilization brings benefits to maize plants in the productive and chemical parameters.

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Table 1. Productive and morphologic parameters of maize plants managed with different levels of nitrogen fertilization (urea kg/ha).

	Treatments (urea kg /ha)				CV (%)
	0	100	200	300	
SM (kg/ha)	32.142.86 b	36.507.93 ab	39.285.71 ab	44.444.44 a	12.29
DM (kg/ha)	9.170.89 b	11.563.18 ab	12.571.43 ab	13.146.67 a	11.67
DWS, mm	56.20 a	57.13 a	58.47 a	59.8 a	2.73
LWS, cm	24.13 a	25.97 a	24.98 a	25.79 a	3.33
DWOS, mm	44.73 a	46.13 a	48.80 a	48.17 a	3.56
LWOS, cm	19.06 b	24.27 a	20.49 ab	20.24 ab	7.59
CD, mm	26.13 a	26.60 a	28.33 a	28.40 a	4.47
GR	17.40 a	17.73 a	17.87 a	17.80 a	3.33
AG	32.73 b	37.91 a	36.57 a	35.38 a	2.79
PH, m	2.28 b	2.36 ab	2.5 a	2.41 ab	3.32
ENP	108.67 a	102.00 a	117.00 a	120.00 a	9.58
PNP	89.67 a	81.00 a	85.67 a	90.67 a	5.79

Different letters in the same line differ (P<0.05) by Tukey test.

Table 2. Chemical parameters of maize plants managed with different levels of nitrogen fertilization (urea kg/ha)

Components	Treatments (urea kg /ha)				CV (%)
	0	100	200	300	
DM, %	30.21 a	31.53 a	31.00 a	29.58 a	4.47
CP, %	7.30 c	7.93 b	9.01 a	8.80 a	2.86
NDF, %	64.88 a	65.29 a	63.34 a	62.36 a	4.88
ADF, %	27.73 a	29.58 a	26.69 a	28.76 a	4.30
TDN, %	67.43 a	66.14 a	68.16 a	66.71 a	1.27
ME, Mcal/kg	2.43 a	2.39 a	2.46 a	2.41 a	1.20
NEL, Mcal/kg	1.54 a	1.50 a	1.55 a	1.52 a	1.32
NEM, Mcal/kg	1.67 a	1.63 a	1.69 a	1.65 a	1.42
NEG, Mcal/kg	0.95 a	0.91 a	0.97 a	0.93 a	2.51
DE, %	67.30 a	65.86 a	68.11 a	66.5 a	1.41

Different letters in the same line differ (P<0.05) by Tukey test.

Effect of inclusion of perennial soybean (*Neonotonia wightii*) on the chemical composition and *in vitro* dry matter digestibility of elephant grass silage⁴.

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Introduction The elephant grass is a forage specie of great importance in ruminant feeding, ease of deployment, adaptation and management, as well as high productivity, a disadvantage of the elephant grass is their seasonality of production (Lopes, 2011). Thus, the use of forage in the process of silage is increasing, however despite the high elephant grass production has a high dry matter content and low content of soluble carbohydrates. Pre wilting is a technique that aims to increase the dry matter since the inclusion of legumes is to improve the nutritional value. The objective of this study was to evaluate the effect of adding soy bean perennial on the *in vitro* dry matter and chemical composition of elephant grass silages

Materials and Methods The experiment was conducted in an area formed with elephant grass cv. Taiwan A-146 belonging to the Institute of Animal Science, UFRJ in Seropédica – RJ. There was a uniformity cut followed by a top dressing with 100 kg / ha of N and K₂O. The elephant grass and perennial soybean (*Neonotonia wightii*) were collected manually with 70 days of growth, plants were cut and subjected to a period of wilting in the sun for 6 hours and then chopped. The treatments were: Treatment 1 - grass wilted and without addition of perennial soybean; Treatment 2 - grass wilted whit 10% perennial soybean; Treatment 3 - grass wilted whit 20% perennial soybean; Treatment 4 - grass wilted whit 30% perennial soybean. We used PVC experimental silos with 10 cm in diameter and 30 cm high. The ensiled material was compacted manually in order to provide density of about 600 kg / m³ of silage. After 60 days, the silos were opened and sampled for chemical analysis. Chemical analysis of silages were performed at the Laboratory of Embrapa Dairy Cattle. We evaluated the levels of dry matter, crude protein, neutral detergent fiber, acid detergent fiber, cellulose, lignin using techniques described by Silva and Queiroz (2002). The *in vitro* dry matter digestibility by the method of Tilley and Terry (1963).

The experimental design was a completely randomized design with five replications, and the results interpreted through analysis of variance and regression. Choosing the best model was based on the coefficient of determination and significance of regression coefficients, and SNK test at 5% probability.

Results and Discussion: There was a positive linear effect for crude protein, *in vitro* dry matter digestibility, lignin, and pH values ($P < 0.05$) (Table 1), demonstrating that the inclusion the perennial soybean in elephant grass silage was beneficial to increase the levels of protein and *in vitro* dry matter digestibility. The pH values behaved the same way but this rise may indicate the occurrence of secondary fermentation in silage, and the inclusion until 25% of perennial soybean can provide appropriate values of pH (McDonald, 1991), as rising levels of lignin can reduce consumption, but no detectable decline on *in vitro* dry matter despite the increase in lignin. In variables NDF, ADF, cellulose and hemicellulose effect was observed ($P < 0.05$) negative (Table 1), showing that there was a decrease of the values of these variables as the levels of inclusion of perennial soybean increased, probably due to the dilution effect provided by the inclusion of perennial soybean.

Conclusions Including up to 25% perennial soy bean (*Neonotonia wightii*) in the elephant grass silages had a positive effect on their chemical composition and in vitro dry matter digestibility.

Thankfulness To FAPEMIG for funding the Project and support for the participation in the event and CAPES for scholarships granted.

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Table 1. Regression equations for the variables pH, in vitro dry matter digestibility (IVDMD), neutral detergent fiber (NDF), hemicellulose, lignin and crude protein in silage of elephant with inclusion of perennial soybean.

Variable	Inclusion Levels of Perennial Soybean (%)				Regression equations
	0	10	20	30	
pH	3.87	4.19	4.16	4.25	$Y = 3.95 + 0.01X$ ($R^2 = 71.36\%$)
IVDMD	48.41	50.89	54.72	57.97	$Y = 48.12 + 0.32X$ ($R^2 = 99.37\%$)
NDF	68.95	65.37	63.01	60.16	$Y = 68.68 - 0.29X$ ($R^2 = 99.33\%$)
ADF	42.12	41.51	39.35	38.82	$Y = 42.26 - 0.12X$ ($R^2 = 93.47\%$)
Cellulose	35.98	34.50	32.23	30.07	$Y = 36.20 - 0.20X$ ($R^2 = 99.23\%$)
Hemicellulose	26.83	23.86	23.66	21.34	$Y = 26.42 - 0.17X$ ($R^2 = 91.46\%$)
Lignin	5.15	6.31	6.26	7.62	$Y = 5.23 + 0.07X$ ($R^2 = 88.41\%$)
Crude Protein	9.11	11.04	12.89	14.53	$Y = 9.17 + 0.18X$ ($R^2 = 99.87\%$)

Meta-analysis of sorghum silages maturity stage produced in Brazil

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Introduction The maturity stage in which forage plants are harvested for silage is one of the factors that interfere on the fermentation and, therefore on the nutritional value of silage. Concerning the importance of this factor on the sorghum silage production, several researchers have been studying it using different hybrids in different edaphoclimatic conditions in order to set the appropriate time for harvesting to maximize the production of silages in terms of quantity and quality. Thus, the chemical composition of sorghum silages produced in different stages of maturity in experiments carried out in Brazil were evaluated in a meta-analytical research.

Materials and Methods To compose the database (DB), we have looked for public domain scientific papers published between January, 1989 and December, 2010, covering the last 22 years of researches about sorghum silage in Brazil. So, the database was built of 82 works with 604 treatments, which totalized 797 silos. The list of selected papers can be requested for the authors of this paper. In this meta-analytical study, it have been selected experiments which presented parameters relating to the chemical composition of sorghum silage (SS), concerning the maturity stage of the grain at the harvesting time. The considered stages of maturity were MI:Milky; MI-PA:Milky-Pasty; PA:Pasty; PA-DO: Pasty-Dough and DO:Dough, so the DB was composed by 191 treatments coming from 28 works, comprising 581 silos. The development of the work was based on the methodologies idealized by Normand (1999) and Lovatto et al. (2007). Statistical analyses were performed through mixed models according to Littell et al. (2006), considering the work as a random effect and the maturity stages as a fixed effect. The dry matter (DM) content was assumed as a co-variable item, considering the difference between each maturity stage (Table 1). It was used Tukey-Kramer test to compare means when necessary.

Results and Discussions When sorghum plants are harvested with the grain completely at the milky stage, they generate low dry matter silage content (Table 1), which is different ($P<0.01$) from all other maturity stages. Probably, in the milky stage every plant remains green and as soon as it evolves its maturity stage, it begins to get senescent, contributing to the DM increase. When the photoassimilates of the grain start to get pasty consistency (milky-pasty), there are no differences ($P>0.05$) between the other maturity stages. During the maturity grain evolution occurs deposition of the backup nutrients (starch) in the panicle. However, this action is not enough to dilute the cell wall contents of produced silages. Silage stored with low DM levels (21.86%) may increase effluent losses, causing deleterious effects on the environment. There was no change on crude protein (CP) content ($P>0.05$) and ammonia nitrogen content ($N-NH_3$) since the nitrogen content is reduced in sorghum and, as we were dealing with silage experiments, we have concluded that silage first steps (chopping, compaction and sealing) have probably been well applied. The $N-NH_3$ content showed that proteolysis occurred in acceptable levels. Despite the technological evolution that has been happening in Brazil, nowadays the determination of soluble sugars and organic acids is done in few experiments. So, it was not possible to consider

the results of soluble carbohydrates in this study. However, the results of lactic and acetic acids allowed us to assume that the bacteria proliferation was optimized by soluble sugars, which have probably limited proteolysis. During the stages in which the grain is partial or totally milky, there was more lactic acid production ($P<0.02$), but it did not cause differences in pH. The fibrous carbohydrate and lignin contents did not change ($P>0.05$) in all maturity stages. Considering that sorghum silage is primarily an energy source, especially for rumen microorganisms, it is essential that researches look for ways to reduce fiber content, since it decreases the concentration of energy and may limit voluntary intake.

Conclusions The different stages of sorghum grain maturity modify the dry matter content of silage, as well as the production of lactic acid, but they do not modify the proportions of fibrous carbohydrates, lignin and pH value.

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Table 1. Dry matter content of silage sorghum produced under experimental conditions in Brazil between 1989 and 2010 and average chemical values according to dry matter as co-variable

Parameter	N	Maturity Stages ¹					P=
		MI	MI-PA	PA	PA-DO	DO	
DM (%)	172	21.86a	26.73b	30.62b	29.82b	34.49b	<0.001
CP (%DM)	191	7.31	8.72	7.23	7.32	6.96	0.5355
N-NH ₃ (%TN)	121	5.84	9.33	6.44	1.92	5.29	0.4590
NDF (%DM)	182	59.72	57.07	57.28	57.82	58.78	0.6490
ADF (%DM)	175	35.35	34.25	33.71	35.57	33.82	0.6771
Hemicellulose (%DM)	144	22.42	22.94	21.84	21.12	22.53	0.9641
Cellulose (%DM)	136	24.27	30.82	23.82	24.48	24.55	0.1530
ADL (%DM)	159	5.29	5.18	5.20	5.76	5.46	0.9217
pH	127	3.74	3.89	3.84	3.88	3.78	0.6204
Lactic acid (%DM)	72	9.93a	10.46a	6.83b	--	6.91b	0.0172
Acetic acid (%DM)	64	1.71	1.77	1.35	--	1.17	0.0523
Propionic acid (%DM)	18	0.35	--	0.42	--	0.38	0.7304
Butiric acid (%DM)	40	0.07	0.06	0.12	--	0.07	0.5631

¹ Evaluated Maturity stages: MI = Milky; MI-PA= Milky-Pasty; PA= Pasty; PA-DO= Pasty-Dough; DO= Dough;

Meta-analysis of sorghum silages produced in experiments in Brazil: Types of silo

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Introduction The hybrid tendency, its maturity stage, the chop length and the additives inclusion, for example, are factors that can interfere on the nutritional value of sorghum silages in an isolate, antagonist and/or in a synergic way. Considering the amplitude of the possible interactions between the factors mentioned above, several researchers have been conducting experiments that evaluate, simultaneously, multiple treatments to hybrids, which precludes the possibility of using field silos in these conditions. Then, laboratory silos are generally used to simulate field conditions. However, the silo size, its covering and its sealing may influence silage quality. So, through a meta-analytical comparison, we aimed to answer if laboratory silos represent the field silos conditions concerning the chemical composition of sorghum silage produced in Brazil.

Materials and Methods To compose the database (DB), we have looked for public domain scientific papers published between January, 1989 and December, 2010, covering the last 22 years of researches about sorghum silage in Brazil. The database was built of 82 works with 604 treatments, which totalized 797 silos. The list of selected papers can be requested for the authors of this paper. In this meta-analytical study, there were selected experiments which presented parameters relating to the chemical composition of sorghum silage (SS), concerning the type of silo, classified as: field silo (FIE) and laboratory silo (LAB). The database was composed by 312 treatments from 58 studies, totalizing 786 silos. The development of this work was based on methodologies idealized by Normand (1999) and Lovatto et al. (2007). Statistical analyses were performed through mixed models according to Littell et al. (2006), considering the work as a random effect and the types of silos as a fixed effect.

Results and Discussion There was no difference ($P > 0.05$) between silo types on analyzed parameters (Table 1). It is important to mention that all silos studied in this work are from scientific papers developed in Brazil. In other words, probably, the field silos were made and managed according to the silage and ensiling premises. Furthermore, in both laboratory silos and field silos there was not aerobic instability that could severely influence the silage nutritional value in rural properties. Thus, it is essential to conduct experiments that compare the research conditions to the real field condition. In a great part of Brazilian rural properties that use silage at the production system, the producers are quite concerned at the ensiling moment, but they are not much concerned about silo management during the silage feedout which can cause biological and economic losses. In this study, the neutral detergent fiber (NDF) content was 57.71% for laboratory silos and 60.65% for field silos, showing that even under experimental conditions, these percentages are pretty high, requiring changes to preserve high energy feed. According to Van Soest (1994), there is a high negative correlation between the content of NDF and ruminant's dry matter intake. So, silage with similar levels as those ones we have mentioned

above may limit animals' voluntary feed intake which have standard genetic compatible with the forage and also with the production cost of semi or totally confined systems.

Conclusions The laboratory silos are used to simulate the silage process in real conditions.

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Table 1. Chemical composition of Brazilian sorghum silages, according to silo type

Parameter	Number of silos according to the type				P=
	n	LAB	N	FIE	
DM (%)	177	32.81	89	29.91	0.2963
CP (%DM)	197	7.69	94	6.72	0.1401
N-NH ₃ (%TN)	146	7.13	27	6.05	0.6333
NDIN (%TN)	12	13.85	28	20.21	0.4979
ADIN (%TN)	41	6.99	32	11.61	0.2596
Soluble carbohydrates (%DM)	78	1.73	6	1.80	0.9841
NDF (%DM)	186	57.71	77	60.65	0.1388
ADF (%DM)	186	34.21	73	36.85	0.1733
Hemicellulose (%DM)	148	22.83	26	19.35	0.1151
Cellulose (%DM)	148	27.71	17	28.39	0.8290
ADL (%DM)	164	5.14	49	6.32	0.0611
EE (%DM)	17	3.04	57	2.33	0.1594
Lactic acid (%DM)	90	8.74	14	9.26	0.8669
pH	155	3.83	31	3.96	0.1382

LAB= Laboratory; FIE= field

Corn silages of plants cultivated under different arrangements - Costs of production

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Introduction In a great part of rural properties in the Northwest of Rio Grande do Sul, silage production have fallen short of the genetic potential of the hybrid corn currently commercialized, owing to edaphoclimatic changes that most of the times are not minimized or completely corrected, such as water deficit and the correction of soil fertility for plants that will be ensiled. However, when there is the use of inputs and pesticides in order to improve the plant phenotype, the cost of production increases. Therefore, it is necessary to know discriminately the investments that have been performed, aiming to the profit from the livestock production (milk and meat), which may ensure the permanence of producers in the rural activity. An alternative for reducing production costs per unit produced can be the management of available resources. According to Argenta et al. (2001), the arrangement of plants in different row spacings can provide better utilization of soil nutrients, resulting in higher production and besides it is possible to dilute the costs. Then, the aim of this paper was to evaluate the production costs of silage corn plants grown in different row spacings.

Materials and Methods The experiment was performed in Palmeira das Missões – RS. According to Streck et al. (2002), the soil is classified as a typical dystrophic red latosol. On November 11th, 2010, it was carried out a desiccation with herbicide and then the seeds were sowed under direct planting system on straw. A hybrid of an early-maturing corn with grain tendency and also with orange semi-hard grain type was planted, totalizing 65,000 seeds per hectare. The evaluated treatments were 30, 45, 60, 75, and 90 centimeters between each row spacing, distributed in plots (25m²), according to the randomized block design with four replications. The fertilization for maintenance corresponded to 300 kg of monoammonium phosphate and 100 kg of potassium chloride. The 400 kg of urea per hectare nitrogen top dressing fertilization were divided into three applications. It was also accomplished two insecticide applications to control the fall armyworm (*Spodoptera sp.*) occurrence. At the ensilage process, plants were weighed separately according to the treatments and then chopped in a harvester. After that, mini-silos were confectioned in two-liter buckets; they were sealed right after the silage was over and sent to the laboratory. An economic analysis was carried out based on the results of production per hectare, on the inputs price quotation and on the implements sold in Palmeira das Missões. To determine the silage production operating costs, the following expenditures were considered: financial value of the inputs needed (seeds, fertilizers and pesticides), cost of tractor conservation and refueling (lubricant and diesel oil), harvesting and transportation of fresh material to the silo, compaction, sealing, implements depreciation, labor costs and social taxes.

Results and Discussion The most costly operations are sowing and fertilization (Table 1), which were accounted in a single cost in this paper, because the producer works with compatible technological levels. Considering that to reach genetic potential expression of the seed it is

necessary to conduct steps such as fertilization and soil management to optimize the phenotype characteristics of the seed. The total operating costs concerning the corn cultivation and ensiling in one hectare were R\$ 2,573.53 in May 2011. From this amount, the seed and the fertilizer corresponded to 52.33% of the production cost and it was necessary to manage properly the other available resources considering the high initial investment. The present trial was carried out in plots and the sowing and harvesting were performed manually. So, it is necessary to evaluate treatments under field conditions to determine the losses of silage during the harvest from each small row spacing. Thus, the smallest spacings produced greater amount of silage per hectare (Table 2), which has diluted the costs. Comparing the spacings 45 and 90 cm there was a variation of 9.28% and 8.67% in the costs per hectare and per kilogram, respectively.

Conclusions Each row spacing has led to different production costs.

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Table 1. Total operating cost of crop production and corn silage per hectare

Operation	Costs (R\$/ha)	Percentage
Desiccation	113.545	4.41
Sowing + fertilization	1,346.976	52.33
Pulverization	94.450	3.67
Harvesting	296.196	11.50
Transport	272.134	10.57
Distribution and Compaction	340.298	13.22
Sealing	109.928	4.30
Total	2,573.527	100

Table 2. Production and cost of dry matter produced per hectare under different row spacing (cm)

Row spacing (cm)	DM production (kg/ha)	Cost/ton of DM (R\$)	Cost/kg of DM (R\$)
30	18,666	137.872	0.137
45	17,147	150.077	0.150
60	17,205	149.580	0.150
47	16,688	154.208	0.154
90	15,692	163.999	0.163

The effects of commercial microbial inoculants on the fermentation and aerobic stability of barley silage

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Introduction When used as inoculants, some species of heterolactic and propionic acid bacteria may extend the aerobic stability of the silages by the production of acetic acid or propionic acid, respectively (Kung et al., 2003). The objectives of this study were to determine the effectiveness of commercial microbial inoculants on the fermentation and aerobic stability of barley silage.

Materials and Methods Chopped whole-plant barley (41 %DM) was taken to a shed, divided into small piles and the following treatments were applied to each pile: (1) Water, negative control (**C**); (2) Easy Sile Advance (Micron Bio-Systems Inc., Buena Vista, CA) (**M**): 150,000 CFU of *Pediococcus acidilactici*, *P. pentosaceus*, *Lactobacillus plantarum* and *L. brevis* per g of forage; (3) Biotol Plus II (Lallemand Animal Nutrition, Milwaukee, WI) (**P**): 100,000 CFU of *P. pentosaceus* 12455 + 20,000 CFU of *Propionibacterium freudenreichii* R2453 per g of forage; and (4) Biotol Buchneri 500 (Lallemand Animal Nutrition) (**B**): 400,000 CFU of *L. buchneri* 40788 + 100,000 CFU of *P. pentosaceus* 12455 per g of forage. Approximately 6.5 kg of forage was packed into 9-L pails (quadruplicates per treatment) and allowed to ensile for 2 and 60 days in a closed barn at room temperature. Upon silo opening, fermentation products and nutrient profile of the silages were determined, and silages stored for 60 days were also tested for aerobic stability, according to Kung and Ranjit (2001). Data was analyzed by ANOVA according to the GLM procedure of the SPSS 19.0 with treatments as fixed effects and a non-parametric Kruskal-Wallis test for the not normally distributed variable per sampling time.

Results and Discussion The nutrient and fermentation profiles are shown on Table 1. Compared to the other treatments, B treated silages had the lowest level of lactic acid and highest level of acetic acid, which has been normally observed. Treatment B also led to silages with higher contents of starch and lower contents of ADICP; high levels of ADICP can be indicative of heating events in the silage. The aerobic stability of barley silages is shown in Figure 1. Silages treated with B were the most stable when exposed to air, followed by silages treated with P; treatment with M resulted in no improvement in aerobic stability compared to untreated silages.

Conclusion Inoculants containing *Lactobacillus buchneri* 40788 or *Propionibacterium freudenreichii* R2453 were effective at improving the aerobic stability of the resulting barley silages compared to untreated ones.

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Table 1. Fermentation and nutrient profile of barley silages treated with different inoculants after 60 days of ensiling.

Item	Treatment ¹				SEM
	C	M	P	B	
DM, %	34.89	32.77	33.35	33.81	1.30
pH	3.97 ^b	4.14 ^{ab}	4.11 ^b	4.46 ^a	0.08*
Lactic acid, %DM	4.14 ^a	4.76 ^a	4.04 ^a	1.77 ^b	0.38
Acetic acid, %DM	0.44 ^c	0.36 ^b	1.60 ^b	1.96 ^a	0.10
Sugar, %DM	2.21 ^{ab}	1.83 ^b	1.60 ^b	2.80 ^a	0.22
Starch, %DM	15.85 ^b	14.86 ^b	13.86 ^b	19.52 ^a	1.00
Ammonia-N, CP	0.35	0.42	0.36	0.42	0.10*
ADICP ² , CP	0.87 ^a	0.88 ^a	0.83 ^{ab}	0.79 ^b	0.03

¹C = Control; M = Easy Sile Advance; P = Biotol Plus II; B = Biotol Buchneri 500;

²ADICP = Acid detergent insoluble crude protein; ^{a,b}Means in the same row with different different superscripts differ ($P < 0.05$); *non parametric Kruskal-Wallis test.

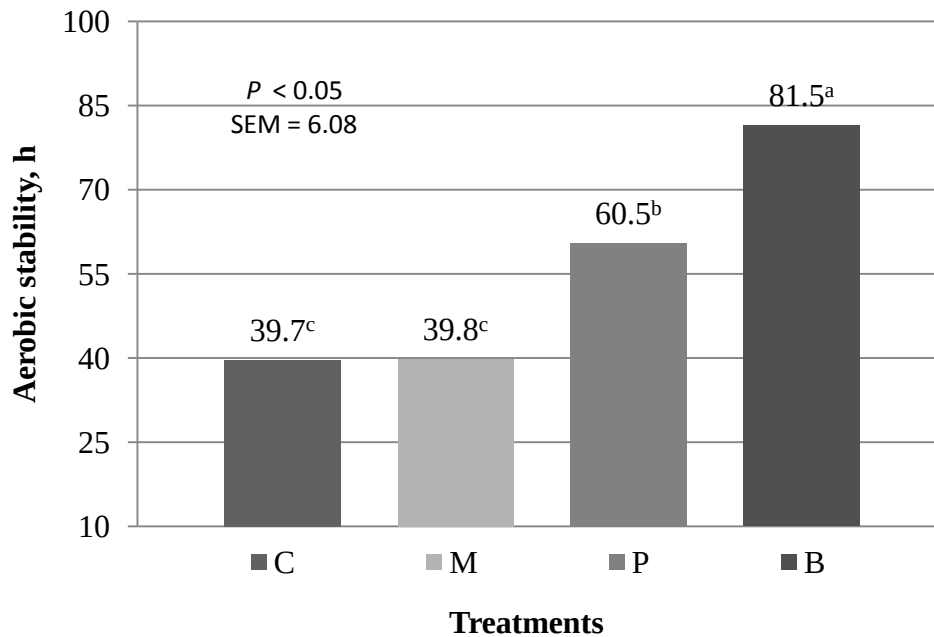


Figure 1. Aerobic stability of barley silages treated with different inoculants after 60 days of ensiling.

Effect of sealing strategies on the panel temperature of corn silage

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Introduction Aerobic deterioration is the mainly factor that contributes to losses during ensiling process (Ashbell and Liska, 1988). Because the deterioration is essentially triggered by microorganisms' growth, an essential prerequisite is to minimize the presence of oxygen after sealing the silo. Anaerobic environment inhibit the presence of fungi which is responsible for the silage heating and, in turn, losses of nutrients. Thus, the plastic film is important in the set of strategies associated with sealing and anaerobiosis maintenance. The experiment aimed to measure the panel temperature in corn silage sealed with different plastic films.

Materials and Methods The experiment was carried out at the Department of Animal Science of USP/ESALQ. The treatments consisted of four sealing strategies: 1) silo covering with 200 µm v, 2) silo covering with 200 µm black-on-white polyethylene film overlaid with sugarcane bagasse (10 cm thickness), 3) silo covering with 45 µm polyamide film combined with a protective mesh tarp; 4) silo covering with 125 µm black-on-white coextruded oxygen barrier film. Plants were harvested with 35 % DM and ensiled in four bunker silos, with 75 t fresh forage capacity. Evaluations of silages were made in the panel immediately after silage feedout in the morning. Four regions of the silo panel (210 x 150 cm) were sampled: a) top side (TS), b) top center (TC), c) bottom side (BS) and d) center (C). Four samples were collected over time, with intervals of 21 days from July to September 2011. After silage removal, thermometers were placed on the panels, arranged in the width of 0, 60, 120, 180 and 240 cm from the side of the silos and the height of 0, 10, 20, 30, 70, 110 and 150 cm from the top of the silos, totaling 35 temperature collection points in each silo. From these points there were generated means for the four mentioned regions. The experimental design was randomized complete blocks, in a 4x4 factorial scheme (four sealing strategies and four sampling sites). Data were analyzed as repeated measurements by using the MIXED procedure of SAS. Means of plastic films were compared by Tukey's test ($\alpha = 5\%$). Orthogonal contrasts were used to evaluate silo zones.

Results and Discussion The higher panel temperatures were observed on the top zones, closer to the plastic film (TS and TC). As expected, these areas are more susceptible to aerobic deterioration due to the oxygen permeation and lower density. In the other hand, C and BS zones were well preserved and showed temperatures closer to the environment. The temperature difference between the warmest and coldest zones exceeded 7°C (Table 1). Overlaying the plastic film with sugarcane bagasse was effective to prevent heating (Table 2). This strategy weight down the cover and avoid directly solar radiation incidence, decreasing gas exchange between silage and environment. Polyethylene coextruded with polyamide film (treatment 4) was as effective as overlaying sugarcane bagasse in a silo sealed with polyethylene film. Black-on-white polyethylene film without any protection (treatment 1) and the polyamide film combined with a protective mesh tarp (treatment 3) showed the highest panel temperatures. Polyamide film is characterized by low oxygen permeability and in this way it was expected lower panel temperature, contrary to observed. More trials should be carried out to check this found.

Conclusion The adoption of polyethylene film overlaid with sugarcane bagasse was effective to reduce the panel temperature in corn silage stored in bunker silos.

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Table 1. Panel temperature of silage corn under different sampling points

Zone ¹	Temperature (°C)
TS	33.47
TC	33.09
BS	27.03
C	26.28
SEM ²	0.90
Contrasts	
(TS+TC) x (BS+C) ³	<0.01
TS x BS	<0.01
TC x C	<0.01

¹TS – top side; TC – top center; BS – bottom side; C – center;

²SEM: standard error of the mean;

³Top vs. bottom contrast;

Table 2. Panel temperature of corn silage under different sealing strategies in the moment of silos opening

Treatment	Temperature (°C)
1) black-on-white polyethylene film	30.66 ^{ab}
2) black-on-white polyethylene film overlaid with sugarcane bagasse	27.80 ^b
3) polyamide film combined with a protective mesh tarp	32.33 ^a
4) black-on-white coextruded oxygen barrier film	29.09 ^b
SEM	0.93
<i>P</i>	<0.01

SEM: standard error of the mean

Heterolactic bacteria effects on sugarcane ensiling

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Introduction Sugarcane ensiling without additives shows DM (dry matter) losses due to the alcoholic fermentation resulting of the high sugar content and epiphytic yeast population. Although, it triggered an increase interest among scientists and farmers, especially because of the logistics and operation benefits. One way to decrease the DM losses related to the fermentation process and improve the aerobic stability is the use of microbial inoculants containing heterolactic bacteria, which have shown the capacity to reduce yeast population in corn and temperate grass silages (Ranjit, 2000; Taylor, 2002). The objective of this study was to evaluate the DM losses during the fermentation and aerobic exposition of sugarcane silage inoculated with *L. buchneri*.

Material and Methods This study was carried out at the Department of Animal Science of USP/ESALQ. The sugarcane was harvested with 29.7% DM and 21.3° brix. The treatments were: 1) sugarcane ensiled without inoculant; 2) sugarcane inoculated with *L. buchneri* (1×10^6 ufc.g⁻¹ fresh forage). Four replications of each treatment were done. The experimental silos were 20L plastic buckets with lids adapted with Bunsen valves and an effluent collecting structure (sand + screen + cotton cloth). After a 77-d storage period, the silos were opened and samples (4kg) placed, without packing, in 20 L buckets without lids and allocated in a temperature controlled room through 10 days. Temperature sensors (data loggers) were placed in the geometric center of the silage mass and the temperature was recorded every 15 minutes. Samples were collected for pH and DM determination. DM recovery (DMR) was calculated according to Jobim et al. (2007). Data were analyzed by the MIXED procedure of SAS. Treatment means were compared by Tukey test with 5% significance.

Results and Discussion Sugarcane silage inoculated with *L. buchneri* improved the fermentative process, reducing ($P < 0.05$) DM losses in 58.9% and, consequently, increasing ($P < 0.05$) dry matter recovery (Table 1), due to a 68.9% reduction ($P < 0.05$) in gas production of. Significant effects of these inoculants, during the aerobic exposition, were not ($P > 0.05$) observed (Figure 1). Usually, *L. buchneri* additivated silages, show lower DM losses and higher stability during silage feedout (Driehuis et al. 1999; Ranjit, 2000; Mendes et al. 2008). However, both silages showed high aerobic stability (Table 2), probably due to the high acetic acid content.

Conclusion Inoculating *L. buchneri* in sugarcane silage was effective to decrease fermentation losses.

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Table 1. Effects of *L. buchneri* on fermentation of sugarcane silages

Variables	Control	Additive	SEM	P
Opening DM, %	27.18	29.62	0.81	0.08
Opening pH	3.74	3.64	0.03	0.07
DM losses (%)	19.65	8.15	1.39	<0.01
DM recovery (%)	80.35	91.85	1.39	<0.01
Gas losses (% DM)	17.26	5.37	1.49	<0.01
Effluent production (kg/t fresh forage)	27.25	28.41	3.28	0.82

Table 2. Effects of *L. buchneri* on aerobic stability of sugarcane silages

Aerobic stability	Control	Additive	SEM	P
pH after five days	3.41	3.44	0.04	0.75
pH after 10 days	4.57	4.84	0.16	0.45
Aerobic stability (h)	94.1	78.0	9.08	0.26
DM losses, 5 days of aerobic exposition (%)	10.37	7.17	1.57	0.20

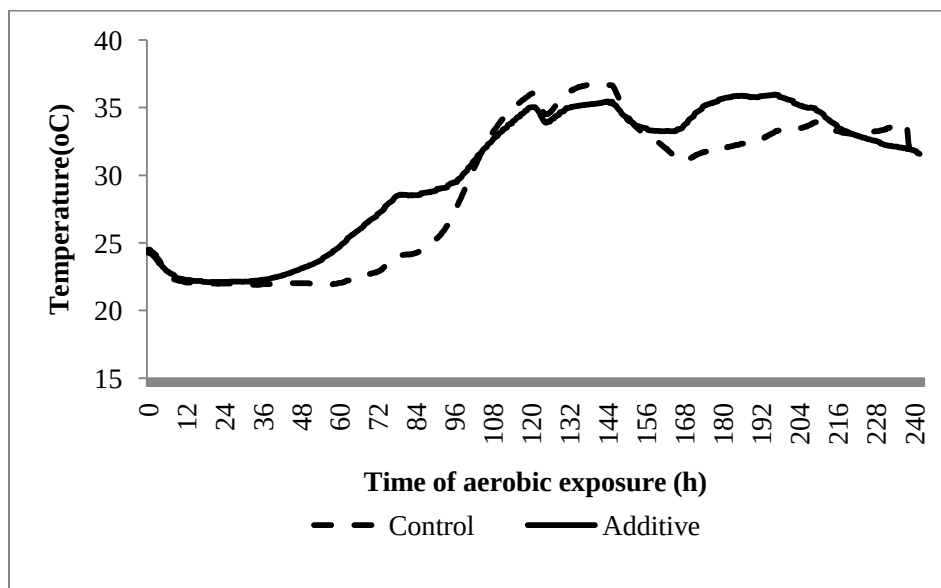


Figure 1. Effect of *L. buchneri* on sugarcane silages temperature during aerobic exposure

Genetically modified maize for silage production

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Introduction To prepare maize silage, seeks to identify cultivars with higher productivity and nutritive value, by increasing the participation of grains and higher digestibility of the forage portion. Results show that genetically modified foods have the composition (laboratory tests) and nutritional value (animal studies) equivalent to traditional foods and according to regulatory agencies (WHO), there is no risk of consumption of these food sources (Phipps and Beever, 2000; Calsamiglia et al. 2007). Up to the present, transgenic DNA and protein were not found in milk, meat and eggs, emphasizing the benefits to producers, consumers and the environment, both in developed and developing countries, with particular emphasis on the ability of transgenic technology to increase productivity, improves food quality and reduces environmental impacts in agriculture (Phipps and Beever, 2000; Calsamiglia et al., 2007). The objective of this study was to determine productivity and nutritive value of transgenic and conventional maize cultivars, using different technologies available in Brazil.

Materials and Methods Five experiments were installed in the State of São Paulo (cities: Votuporanga, Pindorama, Andradina, Mococa and Tatuí) on 2009/10 and 2010/11 harvest, to evaluate ten maize cultivars, five genetically modified (ImpactoTL, DKB390YG, 30S40Y, 2B688Hx and 30F90Y) and five their conventional pairs. The plots were distributed in a completely randomized block design with four replicates. Each plot consisted of eight lines of 10 meters. Plants were harvested when the dry matter (DM) content was between 32 to 36%. Five plants per line (10 plants/plot) were randomly harvested, fractionated and the fractions weighed: ear (removed with stalk), leaves and stem+tassel. After this phase, cob+grain and bracts were separated, weighed and air dried, and after dried at 60°C for about 24 hours. Later on, the grains were separated from the cob and quantified. The leaves and stems were chopped in a mechanical chopper, sampled (minimum 500 g) and dried at 60°C, for 72 hours. Other 10 plants per plot (whole plant, including ears), were chopped, sampled (500 g) and dried at 60°C, for 72 hours. Samples were grounded in a Wiley mill and analyzed by NIRS (Shenk and Westerhaus, 1991) to obtain the following chemical composition: crude protein (CP), *in vitro* organic matter digestibility (IVOMD), digestible dry matter production (DDMP) was estimated by using productivity and digestibility traits, neutral detergent fiber (NDF), acid detergent fiber (ADF), nitrogen in NDF (NDF-N), cellulose (CEL), hemicellulose (HEMI) and starch. Data were analyzed by using the GLM procedure of SAS, including effects of city, block and type of plant (conventional vs. transgenic).

Results and Discussion Maize plants showed no differences for agronomic and chemical traits (Tables 1 and 2). Pereira (2009) also found no difference ($P > 0.10$) in chemical composition between Bt and non Bt hybrids and its silages. The authors concluded that Bt event did not interfere in the nutritional quality of produced silage. In another study, Pereira (2009) evaluated the performance and blood parameters of cows fed conventional ("non Bt") or transgenic maize silage ("Bt maize" TC-1507 Herculex). In that trial, Bt maize did not show any negative change in productivity or even in milk composition when compared to conventional maize. Although

transgenic maize can be resistant to pests and diseases and, in turn have lower field losses, the transgenic maize did not stand out in relation to conventional hybrid for nutritional variables.

Conclusions Cultivars of conventional and transgenic maize do not differ in agronomic or chemical variables at the time of ensiling.

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Table 1. Productivity and fractionation on the conventional and transgenic maize plant

Variable	Conventional	Transgenic	SEM	P
DM, %	37.49	38.19	0.47	0.31
DMP, t.ha ⁻¹	19.95	20.50	0.23	0.09
Leaves, %	20.05	19.98	0.20	0.82
Stem, %	27.78	28.33	0.35	0.29
Ear, %	52.16	51.73	0.38	0.44
Grain, %	33.33	33.34	0.36	0.98
Grain/ear, %	63.16	63.68	0.36	0.32

DM = dry matter; DMP = dry matter production.

Table 2. Chemical composition of conventional and transgenic maize plant

Variable	Conventional	Transgenic	SEM	P
CP, % DM	7.41	7.41	0.05	0.98
NDF-N, % DM	6.97	7.06	0.07	0.39
NDF, % DM	54.17	54.19	0.30	0.97
ADF, % DM	30.08	30.24	0.30	0.71
CEL, % DM	25.58	25.56	0.16	0.93
HEMI, % DM	26.86	26.60	0.24	0.45
STARCH, % DM	22.89	22.94	0.25	0.89
IVOMD, % DM	61.87	61.88	0.19	0.98
DDMP, t.ha ⁻¹	12.38	12.70	0.14	0.12

DM = dry matter; CP = crude protein; NDF-N = nitrogen in NDF, NDF = neutral detergent fiber, ADF = acid detergent fiber; CEL = cellulose, HEMI = hemicellulose; IVOMD = in vitro organic matter digestibility; DDMP = digestible dry matter production.

Soluble carbohydrates content according to maize drying method

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Introduction Dry matter (DM) determination is the start point of feeds analysis, enabling to express the nutrients content in the same basis. The DM determination of forages, with more than 40% moisture, is predominantly performed by conventional oven drying. However, during heating, sample chemical composition can be altered (Deinum and Maassen, 1994). In order to prevent it, several methods have been proposed to cease enzymatic and microbial activities as soon as possible in the samples. The objective of this study was to compare drying methods in whole corn plant (fresh or frozen) in order to evaluate DM and alcohol soluble carbohydrates contents.

Material and methods Whole corn plants were harvested at 2/3 milk line and chopped in a stationary machine and conditioned. Fresh (day zero) or frozen (120 d storage period) samples. Were considered the following methods:

- a) Conventional oven-drying at 55°C for 48h;
- b) High-temperature pretreatment at 100°C for 1h followed by conventional oven-drying;
- c) Microwave pretreatment for 1 minute (1100W to achieve 70°C) followed by conventional oven-drying;
- d) Microwave-drying, until a constant sample weight.

Four replications of each treatment were done. The dried samples were grounded in a Wiley mill through a 1 mm sieve. Soluble carbohydrates (SC) were obtained in alcohol extracts (ethanol 80%) and determined by the phenol-sulfuric acid method, according to Hall (2000). Statistical analysis was performed by using the MIXED procedure of SAS considering a completely randomized design. The means were compared by Tukey test ($\alpha = 0.05$).

Results and Discussion The microwave treatment lead to the lowest DM content across the procedures in both fresh and frozen samples ($P < 0.05$) opposed to showed by Oetzel et al. (1993) in corn silage samples. More extensive DM oxidation during sample heating might be a possible explanation. Soluble carbohydrates content was similar ($P > 0.05$) across treatments. There was no interaction ($P > 0.05$) between drying methods and storage (fresh or frozen) samples. Although pretreatment have been recommended in forage samples to prevent soluble carbohydrates losses (Pelletier et al., 2010), in the current study it was not efficient.

Conclusion Drying methods did not result in significant changes of soluble carbohydrates fraction.

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Table 1. Effect of drying methods on dry matter (DM) and soluble carbohydrates (SC) contents

Treatment	% DM	% SC
Conventional-Oven Drying (55°C)	38.78 ^a	5.16 ^a
High temperature pretreatment	38.53 ^a	5.31 ^a
Microwave pretreatment	39.42 ^a	5.67 ^a
Microwave-drying	35.88 ^b	4.96 ^a
SEM	0.58	0.19
Effect	<0.01	0.19

Means in the same line with different superscript differ (P<0.05)

Nutritive value prediction of maize hybrids for silage production

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Introduction Maize crop has a high production of digestible dry matter per hectare and has traditionally been regarded as standard forage for silage production (McDonald, 1991). However, its nutritive value depends on the interactions between genotype, environmental factors and management practices. Thus, both chemical (e.g. NDF %) and agronomic (e.g. plant height) variables should be considered in cultivar selection programs. Therefore, the study of the relations between plant characteristics would provide better target selection, favoring productivity and quality characteristics of most interest (Gomes et al., 2004). The objective of this study was to establish which variable would have greater importance in predicting: a) *in vitro* organic matter digestibility (IVOMD) and b) digestible dry matter production (DDMP) using stepwise regression. The best equations were selected based on the criterion of simplicity (no more than three independent variables), coefficient of determination (R^2) and root mean square residue (RMSR).

Materials and Methods A database from the Evaluation Program of Maize Silage Cultivars for Silage Production of IAC-APTA, obtained during 2010/2011 harvest, was used in this trial. There were evaluated 15 cultivars (12 conventional and 3 transgenic) in four experiments in the state of São Paulo (cities: Votuporanga, Mococa, Andradina, and Tatuí). Beside these, 10 cultivars (five transgenic cultivars and their conventional pairs) were tested in Pindorama city. The plots were distributed in a randomized block design with four replications. Each experimental unit consisted of six lines of 5 meters each spaced 80 or 90 cm. The side lines were considered edge, and the four central lines of 5 meters were the working lines, where the assessments were done. Two lines were harvested at the silage point (DM ~ 35%) and two lines were harvested at the grain maturity stage. After counting plants total number, 10 were harvested, weighed and fractionated to determine the fractions: stem+tassel, leaves and ear. Later, the grains were removed and weighed. Samples of grounded whole plant were dried in a forced air oven at 60°C and ground in a Wiley mill with 1 mm sieve. The chemical composition was determined by reflectance infrared spectroscopy proximal (NIRS) as described by Mello et al. (2006). The associations between chemical and agronomic variables for the cultivars, aiming to predict the nutritive value was established through stepwise multiple regressions using the REG procedure of SAS.

Results and Discussion The prediction equations of nutritive value based on agronomic and chemical variables selection are presented in Table 1. In general, the models for DDMP had better adjustment in relation to the IVOMD variable evidenced by the higher R^2 and lower RMSR values. As expected, as a new variable was included in the stepwise regression, the model adjusted better to the observed data. The DDMP is directly related to the dry matter production (DMP), being the only agronomic variable considered of major importance in these models ($R^2 = 0.94$, RMSR = 2.63), with the other variables considered in the chemical regressions. This result confirms the previously described by Paziani et al. (2009), in a simple correlation (Pearson) study where it was evident that the dry matter production is one of the main factors that determine the production of digestible dry matter in corn plants ($r = 0.85$). The NDF was crucial in predicting IVOMD. However, it was not as significant ($R^2 = 0.54$, RMSR = 2.43) as the DMP in the

equations for DDMP. The nitrogen linked to NDF (N-NDF) was useful to determination of IVOMD ($R^2 = 0.64$, RMSR = 2.17).

Conclusion Variables associated with the fiber fraction of forage, NDF and N-NDF are important in determining the nutritional value in corn plants for silage production.

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Table 1. Stepwise regressions of chemical and agronomic variables to predict digestibility and digestible dry matter production in corn plants

Variables	Regression model	R^2	RMSR†
1	IVOMD = 88.77 - 0.51 (NDF)	0.54	2.43
2	IVOMD = 85.33 - 1.07 (N-NDF) - 0.30 (NDF)	0.64	2.17
3	IVOMD = 79.60 + 0.78 (CP) - 1.13 (N-NDF) - 0.29 (NDF)	0.66	2.10
1	DDMP = 751.81 + 0.58 (DMP)	0.94	2.63
2	DDMP = 6923.99 + 0.58 (DMP) - 115.77 (NDF)	0.97	1.82
3	DDMP = 6031.90 + 0.59 (DMP) - 197.29 (N-NDF) - 78.93 (NDF)	0.97	1.72

† Root mean square residue; IVOMD: *in vitro* organic matter digestibility; DDMP: digestible dry matter production, NDF: neutral detergent fiber; N-NDF: nitrogen linked to NDF, CP: crude protein, DMP: dry matter production.

Acid lactic bacteria inoculation in corn silage and fermentative losses evaluation at different opening times

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Introduction During the corn silage fermentation process, losses can overcome 20% of ensiled mass. The use of additives aims to guarantee that lactic acid bacteria dominates the fermentation process, resulting in a well preserved forage, with high nutritional value (McDonald et al., 1991). According to Kung Jr. (2009) inoculating microbial additives in corn silage results in variable effects. In some studies, a higher acid lactic production and better recovery of dry matter after fermentation were observed, while consistent results were not detected in other ones. The objective of this study was to verify the effects of an additive containing lactic acid bacteria strains in the dry matter recovery, effluent losses and gas losses in corn silages at different silo opening periods.

Materials and Methods This study was carried out at East Santa Catarina, Lajeado city. The samples were analyzed at the University of Santa Catarina State, in the Animal Nutrition Laboratory (UDESC). The corn was harvested with 42.14% dry matter (DM) content, with a harvester coupled to a tractor and chopped to a 20 mm mean particle size. Two treatments were applied: 1) control (no additive) and 2) microbial inoculant containing the following acid lactic bacteria: *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Enterococcus faecium*, *Lactobacillus casei*, *Treptococcus lactis*, *Aspergillus oryzae* and *Bacillus subtilis*, in a dose to reach 2.5×10^9 cfu/g of fresh forage. There were used four replications and three silo opening times (days 30, 60 and 90). The inoculant was diluted in distilled water and homogeneously applied to the fresh forage. The experimental silos consisted of 20L plastic buckets with Bunsen valves for gas losses evaluations. Forage compaction was done expecting to reach 580 kg/m³ density. The gas losses (% DM) were calculated by the difference of the initial and final DM weight of the experimental silos, and effluent production by the difference of initial and final weights of the silos containing sand, plastic screen and cotton cloth, as proposed Jobim et al. (2007). Total DM losses were calculated by the difference between initial forage DM weight, placed in each experimental silo, and the forage DM weight at the opening day, deducting the produced effluent. Statistical analyses were done using the GLM procedure of SAS, and means compared by the Student “t” test, with 5% significance.

Results and Discussion The effluent losses did not differ ($P>0.05$) between opening times, indicating that the effluent losses occur with more intensity at the beginning of the fermentation process. However it was observed a lower ($P<0.05$) effluent production per ton of fresh forage for the additive treatment (18.8 kg/t) in comparison to control treatment (21.3 kg/t), 90 days after ensiling (Table 1). According to Balieiro Neto et al. (2005), reductions in dry matter losses, during the storage process, are observed when there is a lower leaching by the additive effect or when significant changes happen at the fermentation process. Nevertheless, this effect was not observed in this study because there was no difference ($P>0.05$) in the dry matter recovery between treatments or opening periods. Dry matter recovery means, considering all the opening periods, were 86.9 and 86.6 for additive and control, respectively. The gas production was affected ($P<0.05$) by the additives on days 30 and 60, with the lowest values observed for the

control treatment. Higher losses, in elevated storing times, can be related to secondary fermentation, usually mediated by the heterolactic bacteria, who presents slower fermentative metabolism. These microorganisms are featured by the CO₂ production during the lactic acid and carbohydrates conversion to acetic and propionic acids, resulting in dry matter losses (McDonald et al., 1991).

Conclusion According to the study conditions, the microbial additive was efficient only to reduce corn silage effluent production on day 90, with no significant effects on the fermentation process.

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Table 1. Corn silage effluent production (kg of effluent/t fresh forage) on different opening periods, using a microbial additive

Period	Day 30	Day 60	Day 90
Additive	17.5 ^B	16.6 ^B	18.8 ^B
Control	19.2 ^B	18.3 ^B	21.3 ^A

Means followed by same capital letters in columns, do not differ Student “t” test, 5% significance.

Table 2. Corn silage gas losses (% DM) on different opening periods, using a microbial additive

Period	Day 30	Day 60	Day 90
Additive	1.3 ^{bC}	1.7 ^{bB}	2.4 ^{aA}
Control	1.0 ^{aC}	1.4 ^{aB}	1.9 ^{aB}

Means followed by same capital letters in columns and lowercase in lines, do not differ Student “t” test, 5% significance.

Fitting linear vs. non linear regression to sucrose accumulation in sugarcane

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Introduction The lack of forage availability during dry season is a constant challenge to maintain livestock production in tropical grassland areas. In Brazil, sugarcane has been employed mainly to sugar and ethanol industry purposes. Currently, this forage source has triggering an increase interest among dairy and beef farmers due to the high productivity (over 100 t ha⁻¹) in the same period as forage shortage in pastures. Soluble carbohydrate fraction, especially stalk sucrose contents is the most important nutrient associated with sugarcane feed value because of the poor quality of fibrous fraction. Therefore, knowledge about how sugar accumulation dynamics occurs is crucial. The objective of this research was to compare mathematical models in order to predict crop harvesting start point.

Materials and Methods Whole sugarcane plants (IAC 86 2480 variety) were collected from field plots in Ribeirão Preto, SP, Brazil (22°12'S, 47°52'W) during crop growing phase (2nd cut): 150, 180, 210, 240, 270, 300, 330 and 360 days after harvesting (DAH). Two sucrose measures were considered: a) apparent sucrose content (POL), which is commonly used in sugar industry and b) ethanol soluble carbohydrates (SC) according to the methodology proposed by Hall (2000). Three regression models were considered for sucrose accumulation: a) quadratic, b) broken-line, and c) logistic. Quadratic model is the most widely spread model used in sugar industry to describe sugar curves, especially at ripening phase. Broken-line is a linear regression procedure that is simple, fast and reliable and is commonly employed in others animal nutrition research fields, ex. prediction of amino acid requirements. Logistic model (non linear) fits better when phenomena describes a sigmoid growth (non linear) and the inflection point of variable in study is closer to 50% of the maximum pool predicted (Winsor, 1932). Harvesting criteria in the mathematical models were defined as: 1) quadratic: as the point of maximum accumulation, 2) broken-line: when asymptote was matched and 3) logistic: at 95% arises of maximum predicted sucrose accumulation. GLM (quadratic) and NLIN (broken-line and logistic) procedures of SAS were performed to establish the sucrose accumulation curve. Models adjustments were compared by R² and RMSE (root of mean square error).

Results and Discussion As expected, POL was a more reliable measure comparing with SC due to the highest R² and lowest RMSE values (Table 1), probably because other soluble compounds were present in SC fraction (Hall, 2000). In addition, more variability was observed in SC database. Pattern of predicted maximum sucrose pool was maintained across measures: broken-line (earliest) followed by logistic and quadratic models (latest) (Figure 1). Although quadratic harvesting point predictions were closer between POL and SC, but these values did not reflect plant biological significance in terms of growth and ripening crop phases. Based on the present data set, sugarcane harvesting could start around 9th month of regrowth. Therefore, based on POL and SC respectively, broken-line and logistic regressions are suitable tools to determine physiological maturity of sugarcane.

Conclusion Broken-line and logistic regressions are suitable tools to determine harvesting start point of sugarcane for animal nutrition.

References

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Table 1. Mathematical models to predict sucrose accumulation in sugarcane

Model	Measure	Parameter			R ²	RMSE
Quadratic $Y = a + bx + cx^2$	POL	<i>a</i>	<i>b</i>	<i>c</i>	0.68	1.68
	SC	-11.094	0.163	-0.0003	0.61	5.93
Broken-Line [†] $Y = l + u(r - x_{lr})$	POL	<i>l</i>	<i>u</i>	<i>r</i>	0.69	1.36
	SC	14.387	-0.069	248.2	0.57	4.65
Logistic $Y = a / [1 + \exp(c - kx)]$	POL	<i>a</i>	<i>c</i>	<i>k</i>	0.67	1.39
	SC	14.815	-3.586	0.024	0.61	4.56
		46.448	-3.848	0.027		

[†] By definition $r - x_{lr}$ is zero when $x > r$

POL: apparent sucrose content; SC: ethanol soluble carbohydrates

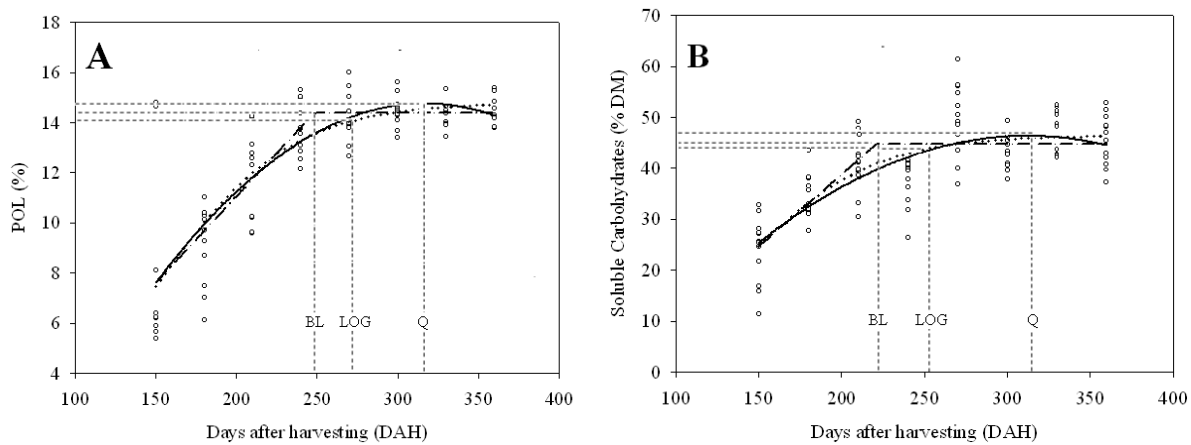


Figure 1. Sucrose accumulation curves according to quadratic (Q), broken-line (BL), logistic (LOG) models. (A) POL. (B) Soluble Carbohydrates.