Effects of a Propionic Acid-Based Additive on Short-Term Ensiling Characteristics of Corn and on Dairy Cows Performance

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December 2006

A thesis submitted to McGill University
in partial fulfillment of the requirements
of the degree of Master of Science

Your file Votre référence
ISBN: 978-0-494-32742-5
Our file Notre référence
ISBN: 978-0-494-32742-5

DEDICATION

This document is dedicated to Amir Brendel, the one and only!

ACKNOWLEDGMENTS

First and foremost, I am grateful to my supervisor, Dr. Arif Mustafa, for his guidance throughout the project. I have learnt a lot from his expertise in the area of dairy nutrition, and from his impressive analytic ability.

I am also most thankful to my co-supervisor, Dr. Philippe Seguin from the department of Plant Science, for his useful advice and many instructive discussions. In particular, I acknowledge his help with the statistical analysis included in this work.

On the personal side, I would like to thank both Dr. Mustafa and Dr. Seguin for their kindness and patience. I took great pleasure in working under their supervision. I was lucky to have two supervisors who were available and helpful whenever I needed them, and at the same time allowed me to work independently and freely.

I am thankful to Dr. Leroy E. Phillip, who was a member of my supervisory committee, for his help and advice throughout the project; and also to Dr. Suha Jabaji-Hare from the Plant Science department, for helping me to identify the mold species.

I would like to thank the fall 2004 crew of the Macdonald Campus Farm for their help in carrying out the experiment described in this work. I am especially grateful to Paul Meldrum for his extreme kindness and for his willingness to provide help with any issue, small as large, at any time, during the last two years.

My warm gratitude is given to the members of our lab, Fadi Hassanat, Marsha Wysote and Einar Vargas, for their help and friendship, and mainly for providing music, fun and relaxed work environment. In particular, I thank Fadi Hassanat, for his help with operating the GC system and with handling several troubleshoots in the lab.

Many thanks to Marie Claude Ferland for providing the French translation of the abstract, and for many enjoyable lunches and ice cream socials.

Finally, I thank Naama Gal whose knowledge of programming saved me several hours of work, and for being there for me always.

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ABSTRACT

Forage shortage may force producers to feed unfermented or partially fermented forages, which are more susceptible to aerobic deterioration. Propionic acid-based additives can be added to ensiled forages to inhibit yeast and mold growth, and improve the aerobic stability of silages. The objectives of this study were to determine the effects of a propionic acid-based silage additive (Solution Foin) on ensiling characteristics, aerobic stability and feeding value of short-term ensiled corn forage. Chopped whole corn was left untreated or treated with Solution Foin (contains 700 g kg^{-1} propionic acid and 300 g kg^{-1} NH_4OH). The additive was added to forage prior ensiling at a rate of 5 L ton^{-1} (wet basis). Treated and untreated forages were placed in six plastic silo bags (three each). Silo bags were opened one day after ensiling and daily samples were collected for 30 consecutive days. Effects on animal performance of feeding treated or untreated forage were determined in a completely randomized design, using 30 lactating cows (178±55 days in milk) fed total mixed ration (50:50 forage:concentrate) with the major forage portion consisting of untreated or treated forage. The feeding study started one day post-ensiling. Results showed that Solution Foin reduced ($P < 0.05$) yeast and mold populations between d 5 and 14 post-ensiling. The highest differences ($P < 0.002$) were observed on d 10, at which point yeast and mold populations for untreated silage were 7.86 and 2.51 log cfu g^{-1}, respectively. The corresponding values for treated silage were 4.35 and 0.00 log cfu g^{-1}, respectively. Aerobic stability of treated ensiled forage was also improved ($P < 0.01$) from day 0 (by 159 h) to day 10 (by 33 h) post-ensiling. Solution Foin also increased ($P < 0.05$) the overall lactic acid and WSC content, and lowered the temperature of the treated ensiled forage in the field. No treatment differences were detected in the forage pH and acetic acid concentration. Dairy cows fed treated forage had similar feed intake (average 23 kg d^{-1}) and milk yield (average 29 kg d^{-1}) to cows fed the untreated forage. It was concluded that Solution Foin can be used to improve the aerobic stability and ensiled forage characteristics of short-term corn forage, likely by reducing yeast and mold populations.

ABRÉGÉ

Les effets d'un préservatif à base d'acide proprionique sur les qualités d'ensilage du mais et la performance des vaches laitières à court terme

Les manques de fourrages au moment de la récolte peuvent forcer les producteurs à servir des ensilages non ou partialement fermentés, qui sont plus susceptibles à la dégradation aérobie. Les préservatifs à base d'acide proprionique sont ajoutés aux ensilages afin de prévenir la propagation des moisissures et des champignons et du même coup améliorer la stabilité aérobie des ensilages. Le but de ce projet est de déterminer les effets à court terme d'un préservatif à base d'acide proprionique (Solution Foin) sur les qualités d'ensilage, la stabilité aérobie et la valeur nutritive des ensilages de mais. L'ensilage de mais a été traité ou non avec Solution Foin (700 g kg^{-1} d'acide proprionique et 300 g kg^{-1} NH_4OH). L'ajout du préservatif c'est fait avant la mise en sac à un niveau de 5 L $tonne^{-1}$ (base humide). Les ensilages traités et non traités on été entreposés dans des six silos couloirs de plastiques (trois de chaque). Les silos ont été ouverts le jour suivant la mise en sace et des échantillons quotidiens ont été collectés pour les 30 jours suivants. Les performances animales de la consommation d'ensilages traités ou non traités ont été évaluées à l'aide d'un modèle aléatoire complet. Trente vaches en lactation (178±55 JEL) consommant une ration totale mélangée (50:50 Fourrage:Concentré) où la majorité des fourrages provient des ensilages traités ou non traités. L'étude débuta le jour suivant la récolte des ensilages et les résultats démontrent que Solution Foin réduit ($P < 0.05$) les populations de moisissures entre les jours 5 et 14 suivant la mise en sac. Les différences les plus marquantes ($P < 0.002$) ont été observées au jour 10, les populations de champignons et de moisissures pour les ensilages non traités étaient respectivement de 7.86 et 2.51 log cfu g^{-1}. Alors que les valeurs correspondantes pour les ensilages traités étaient respectivement de 4.35 et 0.00 log cfu g^{-1}. La stabilité aérobie des ensilages traités a aussi été améliorée ($P < 0.01$) entre les jours 0 (par 159 h.) et 10 (par 33 h.). Solution Foin a aussi permis d'augmenter ($P < 0.05$) la teneur en acide lactique et HCS et de diminuer la température des ensilages traités. Aucune différence significative n'a été notée au niveau du pH et de la teneur en acide

acétique. La consommation volontaire en matière sèche (23 kg d^{-1} en moyenne) et la production de lait (29 kg d^{-1} en moyenne) des vaches consommant les ensilages traités a été similaire à celles consommant les ensilages non traités. L'utilisation de Solution Foin sur les ensilages de mais permet de réduire les populations de champignons et de moisissures à court terme, ce qui améliore la stabilité aérobie et les qualités de fourragères de ces ensilages.

1. General Introduction

Corn silage is a major forage source for ruminants because of its high energy content. Silage making is a process in which chopped plant material is ensiled under anaerobic conditions. After the silo is filled and sealed, plant material and microbes continue to respire until O_2 is depleted. The fermentation phase starts under anaerobic conditions where lactic acid bacteria (LAB) predominate the bacterial population. These bacteria ferment sugars to lactic acid and the silage pH drops significantly. The resulting acidic environment is hostile to most microbes and thus silage will become stable as long as the silo is kept airtight, until it is opened for feeding. During the feed-out phase, O_2 can penetrate into the silo and silage deterioration could occur. Deterioration of silage is usually associated with a rapid growth of aerobic microbes, such as yeasts and molds, followed by: DM losses, increase in pH and temperatures, decreased silage palatability and feed intake, and in some cases production of mycotoxins.

In order to improve the fermentation process and aerobic stability of silage, many additives can be applied, during harvest or when the forage is ensiled. The most common ones are bacterial inoculants and short-chain organic acids. Undissociated organic acids can penetrate fungi cells, release H^+ and thus inhibit their growth. Compared with other organic acids, propionic acid has the strongest fungi-static effect, and therefore can be used to improve silage aerobic stability. However, propionic acid may be unpleasant to handle as it is volatile and corrosive, and consequently buffered propionic acid-based additives are more commonly used.

Producers occasionally face situations where it is necessary to open the silos before the completion of the ensiling process due to feed shortage. Unfermented or partially fermented ensiled forages are less stable once exposed to air. Inadequate fermentation frequently leads to accelerated silage deterioration, since the aerobic microbes are still relatively active. To our knowledge, no study has been conducted to evaluate the effects of propionic acid-based additives on aerobic stability and feeding value of unfermented or partially fermented silages.

Hence, the main objectives of this research were to evaluate the effects of a buffered propionic acid-based additive (Solution Foin) on: microbial population, aerobic stability, and feeding value of short-term ensiled corn forage.

The hypotheses of the study were:

1) Treated short-term ensiled corn forage with Solution Foin will have fewer yeast and mold populations, and therefore
2) Will improve stability upon aerobic exposure;
3) Improved aerobic stability of partially fermented corn forage will be reflected in superior animal performance.

2. Literature Review

2.1. Silage background

Silage can be defined as fermented, high-moisture, forage usually made from the entire above ground part of preserved plants. The main objective of the ensiling process is to preserve forages for periods where feed is unavailable (McDonald et al., 1991).

Silage is preserved and stored in silos, sealed structures in which the crops are fermented and stored until used (Weinberg and Ashbell, 2003). During ensilage, the forage undergoes anaerobic fermentation in which bacteria produce organic acids such lactic, acetic and butyric acids, from sugars present in the raw material. The net result is a reduction in pH, which prevents the growth of spoilage micro-organisms; the majority of which are intolerant to acidic conditions. A successful preservation of silage depends on two factors: 1) anaerobic conditions, which restrain plant respiration, aerobic microbial growth and stimulate fermentation by lactic acid bacteria (LAB), and 2) a rapid drop in pH, which inhibits plant proteolytic enzymes and anaerobic undesirable micro-organisms (Muck and Pitt, 1994; Driehuis et al., 1999; Oude Elferink et al., 2001).

Although the ensiling process is simple in principle, silages are in fact complicated ecological systems, which involve multiple interactions of different chemical and microbiological (Figure 2-1) processes (Jonsson, 1991; Weinberg and Muck, 1996).

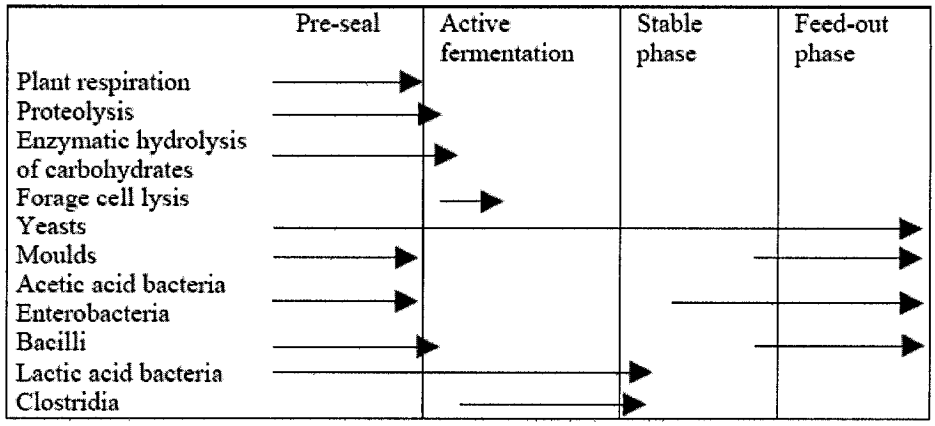


Figure 2-1: Major contributors during the ensiling process (Adapted from Muck, 1993).

2.1.1. Historical development of silage

Silage making is probably more than 3000 years old. The ancient Egyptians and Greeks stored grains and whole plants in silos. Silos were also found in the ruins of Carthage, indicating that forage was ensiled there around 1200 B.C. The development of silage-making technology is traced back 300 years ago (Schukking, 1976; Jonsson, 1991). Despite of the early knowledge of ensilage as a conservation technique in many parts of the world, it was not until the latter part of the 19th century that interest in this process became more widespread (McDonald et al., 1991). According to Siefers (2000) the first silo in the USA was built in 1873 in Illinois and eight years later, the first one was constructed in Ontario, Canada.

The principles of ensilage are now more fully understood and the condition necessary for obtaining a good silage are well defined (McDonald et al., 1991). Improvements in harvesting, wilting, transportation, ensiling techniques, and more effective preservatives have contributed significantly to the production of good quality silages.

2.1.2. Types of silos

According to McDonald et al. (1991) silos can be classified into seven main categories: tower, surface-walled bunker (consist of three solid walls), stack – without retaining walls, flexible-walled, vacuum, plastic silo bags and big bale.

Tower or upright silos are the most popular type in Canada and the northern parts of the Unites States. Horizontal silos are used predominantly in low-rain-fall areas and are characterized by their low cost and large storage capacity (Smith et al., 1986; Savoie and Jofreit, 2003). The walled bunker silos are becoming more popular as they are easier to fill and empty, and compared with other types of silos, there is usually less waste (Woolford, 1984b). The plastic silo bags are a modification of the bunker silos. The flexible properties of the silo bags enable ensiling in various sites and wide range of forage quantities.

2.1.3. Feeding silages

The nutritional value of silage is different from that of fresh or dried forages. This is mainly due to the significant changes in carbohydrate and protein fractions that take place during the ensiling process (Table 2-1). Silage generally provides better consistency of nutrient content compared with other forms of forage (Rotz et al., 2003).

In well-preserved silage, LAB, which dominate the fermentation process, will consume most of the available water-soluble carbohydrates (WSC) for organic acid production. Specific enzymes present in the plant material, will convert polysaccharides or complex oligosaccharides (mostly cell wall) to fermentable sugars and thus increase the content of digestible carbohydrates (McDonald et al., 1991; Rooke and Hatfield, 2003). Proteolysis, which occurs more in wetter than drier silages (Thomas, 1978), increases the proportion of soluble intake protein and ruminally degraded protein, and lowers the proportion of ruminally undegraded protein (Albrecht and Beauchemin, 2003).

Table 2-1: Chemical composition of fresh alfalfa and alfalfa silage

	Fresh alfalfa	Alfalfa silage	P-value
Dry matter (%)	23.7	50.2	0.003
Soluble protein (% Crude Protein)	39.1	72.2	0.02
Ruminal undegraded protein (% CP)	30.5	13.9	0.02
Acid detergent fibre (% Dry Matter)	28.2	33.9	0.05
Neutral detergent fibre (% DM)	33.1	39.8	0.07
Digestible energy (Mcal kg^{-1} DM)	3.8	4.1	0.19

(Adapted from Whiting et al., 2004)

Silage is generally superior to hay in terms of its energy, crude fibre and crude protein contents and often superior to the original crop in terms of energy content (Woolford, 1984b). Silage quality is affected by weather and thus better adapted to harvesting the crop at the optimum stage of maturity for high nutritional value (Pahlow et al., 2003).

It is estimated that 200 million tonnes of DM are ensiled worldwide annually at a production cost between 100 and 150 US dollars per ton of DM. This cost comprises of land and cultivation (~50%), harvesting and covering (30%), silo (13%), and additives (9%) (Wilkins et al., 1999). In Western Europe approximately 60% of the forage conserved for winter feed is in the form of silage (Wilkinson and Stark, 1992). About 150 million tons of silages, mostly corn and legume silages (mainly alfalfa) are produced in the US. As a result, a small reduction in losses or improvement in feed value could easily be worth $100 million annually to farmers (Muck, 1996).

2.2. Microbiology of ensiling

Epiphytic micro-flora (the micro-organisms naturally present on forage crops) are responsible for spontaneous silage fermentation (Lin et al., 1992). The microbial population of standing or freshly harvested forage crops is considerably different from that found during the ensiling process or in the final product in

terms of numbers as well as taxonomical makeup (Pahlow et al., 2003). The total number of bacteria on freshly harvested forages varies between 10^6 to 10^8 g^{-1} DM (Moon and Henk, 1980). The majority of these bacteria are strictly aerobic with minimal contribution to the ensiling process (McDonald et al., 1991).

2.2.1. The fermentation process

There are two main fermentations, which can occur during ensiling process namely the lactic acid and the clostridial (butyric acid) fermentation. These are often referred to as primary and secondary fermentation, respectively (Woolford, 1984b).

Because silage is preserved by lactic acid fermentation, homo-fermentative bacteria are more beneficial. Other micro-organisms either cause inefficient fermentation or lead to silage deterioration. Following ensiling, the anaerobic micro-organisms begin to grow and compete for available nutrients. The changes in the first few days are critical for the success or failure of the subsequent fermentation (McDonald et al., 1991).

Anaerobic environment, adequate substrate, and sufficient number of LAB are required for rapid fermentation (Muck, 1988). If conditions are suitable, LAB will rapidly acidify the environment to such an extent that the competing micro-organisms will not be able to survive and the end result will be stable, low pH silage with higher lactic acid concentrations (Figure 2-2). If the pH does not decline quickly enough, the undesirable micro-organisms (mainly yeasts, clostridia and enterobacteria) will be able to compete for nutrients, and consequently lead to silage deterioration (McDonald et al., 1991; Muck, 1996). A well preserved silage is defined as one that has a high concentration of lactic acid and low concentrations of butyric acid and NH_3 (Weiss et al., 2003).

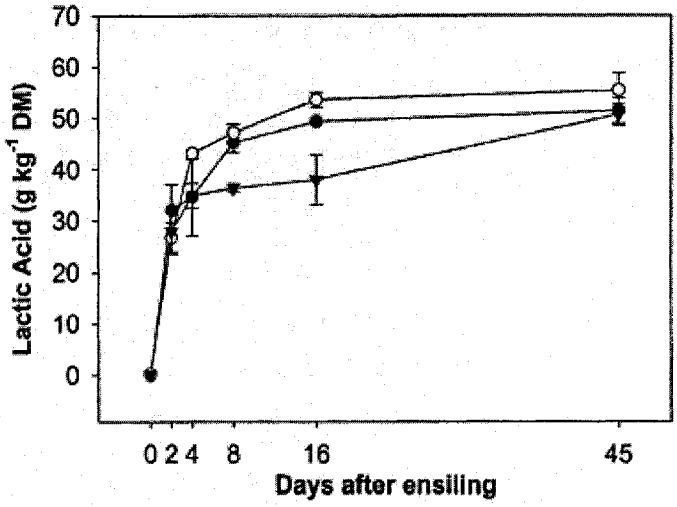


Figure 2-2: Changes in lactic acid of ensiled whole-crop forage faba bean (●), pea (○), and soybean (▼) (Mustafa and Seguin, 2003).

2.2.2. Phases of ensiling

The ensiling process can be divided into four distinct phases: aerobic, anaerobic, stable and feed-out phase.

2.2.2.1. Aerobic phase

At the beginning of the ensiling process, the freshly chopped forages and more importantly the aerobic bacteria, continue to respire within the silo structure (Holland and Kezar, 1990). Plant proteolytic enzymes (which are most significant in the first day in the silo) and aerobic bacteria, metabolize plant sugars and O_2 trapped in the packed forage, producing CO_2, water and heat (Muck, 1988; Kunkle et al., 2006). This process eliminates O_2 from the silo and thus creating an anaerobic environment for fermentation (Muck, 1988). In well-sealed silo, O_2 is rapidly depleted from the atmosphere, with 90% being removed within 15 min and less than 0.5% remaining after 30 min (Sprague, as cited in Woolford, 1990). Plant proteases initiate the decomposition of proteins to amino acids and non-protein nitrogen, and carbohydrases increase the amount of soluble carbohydrates available for LAB fermentation (Pahlow et al., 2003).

Under ideal forage and storage conditions, this phase will last for only few hours (Holland and Kezar, 1990). No major changes in the initial pH of the

chopped forages (i.e. 6.0 to 6.5) will take place during this phase (Weinberg and Muck, 1996).

2.2.2.2. Anaerobic phase

During the early stages of this phase, facultative and obligate anaerobic micro-organisms such as enterobacteria, clostridia, certain bacilli and yeasts can theoretically compete with the LAB for soluble carbohydrates, which are increasingly released from the collapsing plant cells and tissues (Pahlow et al., 2003). The decline in pH starts when there are approximately 10^8 colony-forming units (cfu) of LAB g^{-1} forage (Muck, 1988). Britt et al. (1975) showed that pH decline rapidly between the first and the third day of ensiling, followed by a gradual decline at the later stages. The anaerobic bacteria ferment plant sugars and producing organic acids, such as acetic and lactic acids (Muck, 1988; Kunkle et al., 2006), which will lead to a rapid and significant drop in the pH of the ensiled forages (Weinberg and Muck, 1996), associated with rise in silage temperature, generated from lactic acid fermentation (Kung and Ranjit, 2001).

In grass forages, lactic acid may comprise as much as 150 g kg^{-1} of the resultant silage DM (Gill et al., 1986). As the pH of the ensiled forage falls below 5.0, acetic bacteria decline in numbers as their growth is inhibited at low pH (Holland and Kezar, 1990). Lactic acid bacteria multiply and become the predominant bacteria (Kroulik et al., 1955; Weinberg and Muck, 1996). Once the pH drops below 4, plant proteolytic activity, and protein breakdown, decrease significantly (Muck, 1988; Henderson, 1993).

The duration of this phase varies between several days to several weeks (after the silage becomes anaerobic) depending on the characteristics of the ensiled forage and the ensiling conditions (Weinberg and Muck, 1996; Pahlow et al., 2003).

2.2.2.3. Stable phase

During this phase, silage becomes stable and can remain stable for long periods if air does not penetrate (Kunkle et al., 2006). Only acid tolerant enzymes

continue to be active, causing a slow acid hydrolysis of structural carbohydrates (Pahlow et al., 2003). Once the pH is below 4.5, the LAB predominate, causing a further reduction in pH. Lactic-acid fermentation slows or stops when the fermentable sugars are depleted or the low pH inhibits bacterial growth. The LAB population typically undergoes a 3+ logarithmic reduction below their peak number (about 10^{10} cfu g^{-1} fresh matter) (Kunkle et al., 2006). Fermentation products such as acetic, propionic and butyric acids are inhibitory to yeasts and molds (Muck, 1996). Several acid tolerant yeasts species survive this period in an inactive state, along with bacilli and clostridia, which become dormant (Pahlow et al., 2003).

In the event of insufficient acid production, clostridia, which ferment lactic acid to butyric acid and amino acids to NH_3, might become active, in a process called 'secondary' or 'clostridial fermentation' (Weinberg and Muck, 1996). Clostridial bacteria grow without O_2, degrade sugars, and covert lactic acid to butyric acid, thus causing the pH to rise. They also break down protein to amines and other undesirable end products. Insufficient concentration of lactic acid leads to increased decomposition, DM loss, and reduced palatability of the silage (Thomas, 1978; Kunkle et al., 2006). Once silage pH is increased, other aerobic micro-organisms can grow rapidly on the other remaining substrates (Muck, 1996).

2.2.2.4. Feed-out phase

Aerobic exposure of silage re-activates aerobic micro-organisms, mostly yeasts and molds, bacilli and acetic acid bacteria (Weinberg and Muck, 1996). These micro-organisms start to multiply in the presence of air and cause major chemical changes which lead to reduction in lactic acid concentration, rise in pH, and a substantial reduction in nutritional value (Pahlow et al., 2003). Aerobic deterioration is usually characterized by rise in temperature and the appearance of molds (McDonald et al., 1991). High temperatures during the summer increase aerobic spoilage and reduce bunk life of silages (Kunkle et al., 2006). It has been

indicated that up to 50% of the silage DM losses occur from secondary aerobic decomposition (Woolford, 1990).

2.2.3. Bacterial growth during ensiling

Epiphytic micro-flora, the micro-organisms naturally present on forage crops, are responsible for silage fermentation and also influence the effectiveness of silage bacterial inoculation. According to Woolford (1984b) there are four significant groups of micro-organisms that are involved in silage fermentation: a) LAB, b) endospore-forming bacteria, c) coliform bacteria, and d) fungi (yeast and filamentous fungi); in addition, propionic acid bacteria are occasionally found.

The distribution of epiphytic micro-organisms is variable and affects several factors including forage species, stage of maturity, weather, wilting, and chopping process (Muck, 1988; Lin et al., 1992). The total number of bacteria on fresh grass has been shown to vary between 10^6 and 10^9 g^{-1} DM (Moon and Henk, 1980; Stirling, as cited in McDonald et al., 1991). Dellaglio (1985) found that LAB are usually present on grass in numbers 1000 times lower than their main competitors – fungi and enterobacteria. After ensiling, the micro-organisms capable of anaerobic growth begin to grow and compete for available nutrients (McDonald et al., 1991).

2.2.3.1. Lactic acid bacteria (LAB)

The epiphytic LAB (i.e. lactobacilli, pediococci, leuconostocs) are essential for desirable fermentation during the ensiling process. The population of epiphytic bacteria is variable and ranges from the limit of detection (i.e. 10^1) to 10^7 cfu g^{-1} fresh matter on corn and sorghums. In corn, the lowest counts typically occur during cool weather while higher counts can be found in early maturing varieties (Pahlow et al., 2003).

Lactobacilli numbers increase rapidly during early phases of ensiling (Thomas, 1978). Weise (1969) reported a million fold increase in LAB within 48 h of ensiling. Lactic acid bacteria are facultative anaerobes, but grow better under

reduced O_2 tension with optimum growth temperature of 30 to 40°C; at least half of the fermentation end-product carbon of LAB is lactate (Holt et al., 1994).

There are two main types of LAB, homo- and hetero-fermentative. Both groups can be found during ensiling. Homo-fermentative bacteria ferment WSC almost exclusively to lactic acid, while hetero-fermentative LAB produce other organic acids in addition to lactic acid (McGechan, 1990). The LAB can grow under aerobic and anaerobic conditions but, unlike other micro-organisms, which can change from fermentative to respiratory pathways in the presence of O_2, their metabolism remains fermentative (Gill et al., 1986; McDonald et al., 1991).

A distinctive feature of the LAB is their high tolerance to acidic conditions. The optimum pH for growth ranges between 4.0 and 6.8, though some species can grow under pH as low as 3.5. Growth temperature is very variable and ranges between 5 and 50°C, but the optimum for most strains is about 30°C. Under anaerobic conditions the LAB can ferment a wide range of substrates, mainly sugars, using a variety of pathways (McDonald et al., 1991)

The concentration of lactic acid in silages varying from 50 to 160 g kg^{-1} DM; lower concentration may occur when acid additives are used (McDonald et al., 1991). Owing to the lower buffer capacity of cereal, relative to crop forages, the concentration of lactic acid in cereal silages is normally less than those in extensively fermented crop silages (Weiss et al., 2003).

2.2.3.2. Acetic acid bacteria

Acetic acid bacteria are obligate aerobic, acid tolerant bacteria, which are able to oxidize ethanol to acetic acid. The optimum pH for these bacteria ranges from 5.4 to 6.3 and the most favourable growth temperature is 25 to 30°C (Holt et al., 1994). The concentration of acetic acid in silages varies between 10 and 100 g kg^{-1} DM, with most well-preserved silages containing 20 to 50 g kg^{-1} DM (McDonald et al., 1991).

Acetic acid bacteria can utilize lactic acid, acetic acid, glucose and ethanol at low pH, and thus cause aerobic deterioration. Yeasts and acetic acid bacteria

often proliferate together in corn silage, usually with the yeast being predominant (Muck and Pitt, 1994).

Ethanol is the preferred substrate for acetic acid bacteria and is oxidized in preference to acetic and lactic acid (Spoelstra et al., 1988). When ethanol is depleted, the metabolism is switched to the oxidation of lactic and acetic acids. Thus, acetic acid bacteria can be responsible for the onset of aerobic deterioration of corn silage, with or without the presence of yeasts. These findings were confirmed in later study (Muck and Pitt, 1994).

However, Moon (as cited in Spoelstra et al., 1988) showed that acetic acid in combination with lactic acid can inhibit yeast and mold growth. The competition between yeast and acetic acid bacteria depends on the DM content of the silage and the concentration of the fermentation acids. Both yeast and acetic acid bacteria play a role in silage deterioration, when the silage contains a relatively low concentration of acetic acid (i.e. < 6 g kg^{-1}) and the initial population of yeast is above 10^3 g^{-1} (Courtin and Spoelstra, 1990).

2.2.3.3. Clostridial (butyric acid) bacteria

Clostridium cells are obligatory anaerobes (although tolerance to O_2 varies widely) with optimum growth temperature of 10 to 65°C. They produce mixtures of organic acids and alcohols from WSC with many species producing potent exotoxins (Holt et al., 1994; Pahlow et al., 2003).

Although considered mainly anaerobic, several studies reported clostridial growth during aerobic deterioration of silages (Jonsson, 1991; Kwella and Weissbach as cited in Pahlow et al., 2003). A possible explanation for this occurrence could be the coexistence of aerobic and anaerobic niches in the silo, in which clostridia profit from the oxidation of the preserving acids by aerobic organisms such as yeast (McDonald et al., 1991; Pahlow et al., 2003).

Clostridial growth is inhibited in well-preserved silages. This is primarily due to low pH (i.e. <5), low moisture content (i.e. <700 g kg^{-1}) and high concentrations of organic acids (Thomas, 1978; Jonsson, 1991; Muck, 1996) The

butyric acid fermentation, as carried out by Clostridium species (e.g. *C. tryobutyricum*) can be described as:

$$2 \text{ lactic acid} \longrightarrow 1 \text{ butyric acid} + 2H_2 + 2CO_2$$

Such fermentation leads to an increase in the silage pH because two moles of lactic acid are converted to one mole of (weaker) butyric acid (Pahlow et al., 2003). The fermentation of lactic acid to butyric acid is the principle cause of spoilage during ensilage, and this activity renders conditions more conductive to the proliferation of proteolytic clostridia (Woolford, 1984b). Clostridia usually derive their energy from the fermentation of organic compounds such as WSC and protein (Pahlow et al., 2003). Depletion of the protein by clostridia, can lead to a significant reduction in the nutritive value; protein nitrogen is converted to NH_3 which may reduce silage palatability (McGechan, 1990). Extensive growth of clostridia generally takes place during the later stages of the ensilage process.

Proteolytic clostridia primarily ferment amino acids, which in turn results in both DM and energy losses. However, the main cause of concern is the production of butyric acid (Muck, 1988). Butyric acid has an offensive smell, which can drastically reduce silage palatability (Woolford, 1975b; McGechan, 1990). Clostridial silages typically have a high pH (i.e. > 5 in low DM silage), contain little or no lactic acid, and high concentrations of butyric acid (i.e. > 5 g kg^{-1} DM) and NH_3 (Jonsson, 1991; Voss, as cited in Pahlow et al., 2003). Because of the magnitude of energy and DM losses along with subsequent effects on animal performance from clostridial fermentation, many silage evaluation protocols that assess fermentation quality rely on the concentration of butyric acid and NH_3 (McDonald et al., 1991; Zimmer, as cited in Pahlow et al., 2003).

2.2.3.4. Propionic acid bacteria

Propionic acid bacteria (propionibacteria) are facultative anaerobes but have variable O_2-tolerance; their optimum growth temperature is from 30 to 37°C (Holt et al., 1994). These bacteria utilize carbohydrates and lactic acid to produce propionic and acetic acids, CO_2 and H_2 (Holt et al., 1994; Driehuis et al., 1999).

All strains of propionic acid bacteria can ferment glucose, fructose, and glycerol, and many can use substrates like lactate, and some sugars. Butyric acid can be produced by propionibacteria as a result of lactic acid fermentation (Pahlow et al., 2003).

The role and importance of propionibacteria in silage is not clear since no comprehensive research has been conducted (Pahlow et al., 2003). These bacteria have occasionally been isolated from silages. However, propionibacteria can not tolerate the acidic environment of silages (Weinberg et al., 1995). Propionic acid are frequently detected in silages (< 10 g kg^{-1}), but that does not necessarily indicate the presence of propionic acid bacteria, since other bacteria (e.g. *Clostridium propionicum*) can also produce propionic acid (McDonald et al., 1991; Pahlow et al., 2003).

Although propionic acid bacteria have been shown to ferment lactate to propionate in low pH silages and to prefer lactate over sugar as a substrate, it is unlikely that the activities are intensive enough to result in silage spoilage, especially since their action on lactate involves little or no loss of acidity (Woolford, 1975b).

2.2.3.5. Enterobacteria (coliforms)

Enterobacter are facultative anaerobes that ferment glucose and other carbohydrates to organic acids and gas; their optimum growth temperature is 30 to 37°C. Several species are opportunistic pathogens (Holt et al., 1994).

The enterobacteria, or more specifically the coliform bacteria subgroup, have long been of interest because they include several important pathogens. Some enterobacter species contain endotoxin in the outer cell membrane and have been associated with feeding problems and cases of mastitis (Lindgren, as cited in McDonald et al., 1991; Pahlow et al., 2003)

Enterobacteria are usually the second most numerous bacteria of the epiphytic micro-flora active in the silo and thus the most important in their competition with the predominant LAB flora (Pahlow et al., 2003). Lin et al. (1992) identified epiphytic micro-flora on alfalfa and corn; enterobacteriaceae

were the predominant micro-flora in both alfalfa and corn with the LAB constituting less than 0.5% of the total microbial population in both crops. For anaerobic growth, enterobacteria are strictly dependent on fermentable carbohydrates and compared with other relevant epiphytic groups, they are generally less fastidious in nature, which enable them to survive unfavourable conditions such as cold and dry winter periods (Pahlow et al., 2003).

The presence of enterobacteria in silage is undesirable. Because of their facultative anaerobic nature, they are able to compete with the LAB for nutrients prior and during silage fermentation. If clostridia or enterobacteria dominate the fermentation, they may increase the buffering capacity of the ensiled mass and counteract the intended rapid lowering of pH. Losses of DM and energy will be much greater than if the LAB had been dominant. Such high losses arise from the extensive production of CO_2 and H_2 from the fermentation of lactate or hexoses and from deamination and decarboxylation of amino acids (McDonald et al., 1991; Pahlow et al., 2003).

Better than any other single parameter, low levels of enterobacteria in silage reflects the presence of good ensiling conditions, moderate temperatures, the availability of nutrients and water, an efficient conversion of those nutrients to fermentation products and a low pH produced by LAB (Pahlow et al., 2003).

2.2.3.6. Competition between bacteria

As mentioned earlier, efficient silage fermentation is based on predominance of LAB. However, during the complicated ensiling process, many other micro-organisms can compete with LAB on substrates.

Fast initial acidification during ensiling is the key to control the growth of enterobacteria and clostridia; these competitors will continue to grow until an inhibitory concentration of undissociated acids and / or a sufficiently low pH have been achieved (Pahlow et al., 2003). Competition from anaerobic species, particularly clostridia, can be a serious problem, especially in wet forages, since they destroy lactic acid and amino acids, producing butyric acid, amines and NH_3 which are toxic, the pH rise and the silage becomes unstable; enterobacteria and

yeasts are other groups of micro-organisms which can be active, and cause spoilage, under certain conditions (McGechan, 1990).

2.2.4. Fungi

Fungi are eukaryotic organisms which are strictly aerobic, although some can grow under anaerobic conditions (McDonald et al., 1991). Yeasts and molds, account for more than 30% of the epiphytic micro-flora on corn (Lin et al., 1992). When the silo is opened for feeding, growth of aerobic microbes such as yeasts and molds takes place. Yeasts are considered the main micro-organisms responsible for the initiation of aerobic deterioration of silages (Moon, 1983; Spoelstra et al., 1988; Woolford, 1990; Kung et al., 1998). Yeast and mold growing on aerobically exposed silages ferment lactic acid which causes an increase in pH and DM losses. Heat generated from lactic acid fermentation cause rise in silage temperature. Furthermore, acetic acid is a stronger antimytotic than lactic acid (Kung and Ranjit, 2001)

2.2.4.1. Yeasts

Nearly all species of yeasts will grow within pH and temperature range of 3 to 8 and 0 to 37°C, respectively (McDonald et al., 1991).

They are less sensitive to dry conditions than most bacteria and their survival during storage is dependant on factors such as degree of anaerobiosis, pH, concentration of organic acids and ambient temperature. The main fermentation products of yeasts are ethanol and CO_2 (Woolford and Wilkie, 1984; Muck et al., 1991; Pahlow et al., 2003). Yeasts are the most important micro-organisms implicated in the initiation of aerobic deterioration of silages.

The role of yeasts in the aerobic deterioration of silages was first noted in 1964 by Beck and Gross (as cited in McDonald et al., 1991) who showed that silages with high yeast populations were more unstable on exposure to air than silages with low yeasts populations. When silage with high yeast population is exposed to air, the yeasts resume respiratory activity and oxidise the preserving fermentation acids (e.g. lactic and acetic acids). This will result in aerobically

deteriorated silage with high pH and temperature and significant DM losses (Middelhoven and Franzen, 1986).

Yeasts are most active under high pH conditions at the early stages of fermentation (McGechan, 1990). Yeast population in most silages can increase dramatically from < 10^2 to 10^{12} cfu g^{-1} DM within three days of ensiling (Woolford, 1984b), with highest counts after 2 to 14 days post-ensiling (Middelhoven and van Baalen, 1988). After two weeks of ensilage, the anaerobic and acidic environment becomes hostile to yeasts and their counts decrease significantly (Middelhoven and van Baalen, 1988; Pahlow et al., 2003). However, some yeasts are able to grow at low pH (e.g. 4.5) and in the presence of short-chain fatty acids (Moon, 1983). Daniel et al. (as cited in Spoelstra et al., 1988) stated that silages with yeast population of at least 10^5 cfu g^{-1} DM are very susceptible to aerobic spoilage. This was supported by the aerobic deterioration models developed afterwards (Courtin and Spoelstra, 1990; Pitt et al., 1991). On the other hand, Woolford (1984b) suggested that yeasts benefit corn silage in the long term by inhibiting other fungi.

2.2.4.2. Molds and mycotoxins

Molds are eukaryotic fungi, which grow as multi-cellular filamentous colonies (McDonald et al., 1991). Molds can grow within a wide range of pH. For example, Penicillium species have a minimum tolerance pH of 1.2 to 1.4 and an optimum pH of 4.3 to 8.8 (Panasenko, as cited in Muck et al., 1991). The occurrence of molds in silages is usually restricted to the surface layers and often indicates poor sealing and / or compaction. They develop also during the advanced stages of aerobic deterioration of silage (Woolford, 1990). Pitt et al. (1991) found that aerobic stability was largely unaffected at mold populations less than 10^4 g^{-1} DM, and was highly affected at populations above 10^6 g^{-1} DM.

Molds presence is undesirable since they not only break down sugars and lactic acid via normal respiratory pathways, but also hydrolyse and metabolise cellulose and other cell wall components (McDonald et al., 1991). The detrimental impact of molds is not solely restricted to DM losses and reduction of

palatability. Visibly molded areas of silage underestimate the much larger surrounding areas of silage containing invisible mycelium (Pahlow et al., 2003), as well as the high probability of various mycotoxins, produced by several silage molds.

Mycotoxins are fungal secondary metabolites. They can contaminate foods and feeds, and exhibit toxic effects in higher organisms that consume the contaminated commodities. The most obvious negative economic impact of mycotoxins is an outright loss of crops and affected animals, particularly when a severe outbreak occurs (Arora, 2004). Associated effects may include feed refusal, reduced animal performance, increased abortions, hormonal imbalances, suppressed immune system and in some cases toxicity and death (Pitt and Muck, 1993; Pahlow et al., 2003; Arora, 2004). The most important mycotoxigenic mold species are: *Penicillium roqueforti, Aspergillus fumigatus, Byssochlamys nivea* and Fusarium spp. (Pahlow et al., 2003). Since the initial discovery of aflatoxin in 1961, more than 300 different mycotoxines were documented but the effect of those on ruminant animals is still very much unclear (Mahanna and Chase, 2003).

For the studies in which molds growth were measured, there was a negative correlation between yeast and mold counts (Jonsson, 1991; Pitt et al., 1991). Muck et al. (1991) showed that maximum growth rates of yeasts were greater than those for molds by a factor of two or three. The authors also indicated that domination of one group over another will also depend on the initial population of each group.

2.2.5. Aerobic stability and deterioration of silage

Aerobic stability is defined by Muck (2004) as the time until the silage begins to heat during feed-out.

When silage is exposed to air, the anaerobic environment is changed to an aerobic one. Yeasts, molds and aerobic bacteria, present in the silage but dormant under anaerobic conditions, begin to flourish and respire residual WSC and organic acids. This, in turn, will result in an increase in silage pH and temperature, high yeast and mold counts and possible production of mycotoxins

and growth of pathogenic species. Aerobic deterioration is usually accompanied by CO_2 production, loss of digestible DM and reduction in silage palatability (Ohyama et al., 1975; Lindgren et al., 1985; Honig and Woolford, as cited in Spoelstra et al., 1988; McDonald et al., 1991; Muck et al., 1991; Muck and Pitt, 1994; Weinberg et al., 2001; Pahlow et al., 2003).

Air exposure can also take place during the storage phases if the silo is inadequately sealed (Woolford, 1990). Silages show a large variability in response to air; some silages remain unaffected for some weeks while others start deteriorating shortly after aerobic exposure (Spoelstra et al., 1988; Courtin and Spoelstra, 1990). Woolford (1984b) differentiated between losses at the surface of silos, occurring as a result of air infiltration into the silo during storage, and those which occur when the silo is opened for feeding.

Several factors can affect aerobic stability of silage. These include water activity, concentration of undissociated organic acids and number of fungi and acetic acid bacteria (Courtin and Spoelstra, 1990). A model of aerobic fungal growth in silage listed the parameters that most affect the aerobic stability in interaction with pH. These include temperature, fungal population, and WSC and organic acid concentrations (Pitt et al., 1991). Kung et al. (1998) found a negative correlation between the number of yeast and aerobic stability in corn silage (Figure 2-3).

According to the model of Pitt et al. (1991) the silage of greatest aerobic stability was predicted to be aerobically stable silage which is highly buffered, of low DM content, and contain sufficient WSC before ensiling to allow complete fermentation to the lowest possible pH with little or no residual WSC after fermentation. Cereal silages are in general, less aerobically stable than legume silages due to their higher residual WSC content (McAllister and Hristov, 2000).
The aerobic deterioration of silages has great economic significance (Williams, 1994). Alfalfa and corn are two major silage crops in the United States and Canada. Whole-plant corn is recognized as an ideal crop for preservation by ensiling, but normally its aerobic instability is high once the silo is opened.

Attempts to make silages more aerobically stable must consider yeasts, molds and acetic acid bacteria growth (Muck and Pitt, 1994).

However, there are several other interdependent factors which influence aerobic deterioration such as the: botanic origin, DM of the ensiled forages, ambient temperature and fermentation substrate (Woolford, 1990).

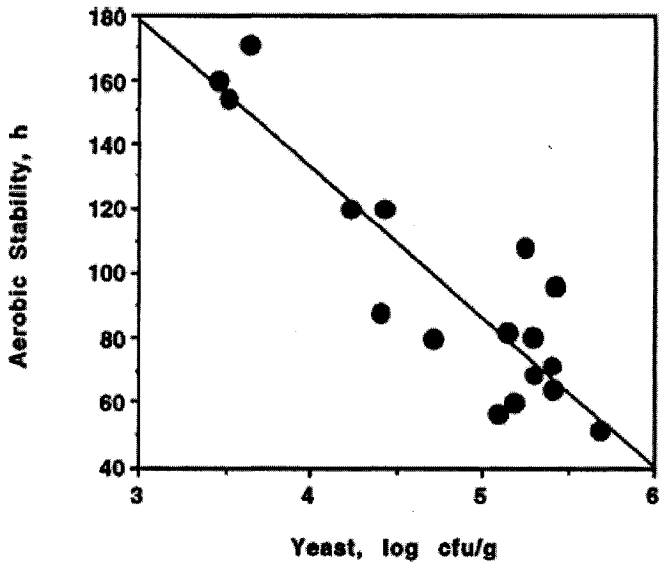


Figure 2-3: Relationship ($y = 315.4 - 45.7x$; $r^2 = 0.79$) between numbers of yeast in silage and hours of aerobic stability (Kung et al., 1998).

2.3. Biochemistry of ensiling

The ensiling process is an anaerobic fermentation of sugars by the micro-flora present on the ensiled crops. These microbes metabolize WSC to obtain energy for growth and release organic acids together with other end products. In the majority of silage fermentations, the main substrates are fructose and glucose. Once chopped, the total WSC concentration in pre-ensiled forages tends to decrease, because of the continuous plant respiration and the microbial fermentation (Woolford, 1984b; Rooke and Hatfield, 2003).

2.3.1. Water soluble carbohydrates

As previously mentioned, silage is produced by the process of a natural lactic acid fermentation. The initial reaction is the anaerobic conversion of

pyruvate to lactate, which lowers the pH. The basic and desired reaction completed by the homolactic LAB could be summarised as:

$$\text{Glucose (or fructose)} + 2\text{ADP} + 2\text{Pi} \longrightarrow 2 \text{ lactate} + 2\text{ATP} + 2\text{H}_2\text{O}$$

The two pyruvate molecules from either sugar source are subsequently reduced to two molecules of lactic acid by lactate dehydrogenase (McDonald et al., 1991). This pathway results in no DM, and negligible energy losses. However, under conditions of low glucose concentration, other bacteria present in the silage, can convert pyruvate to other products such as acetate, CO_2, formate and ethanol (McDonald et al., 1991; Rooke and Hatfield, 2003). Under normal conditions, WSC content of ensiled forages are depleted within few days of ensiling (Figure 2-4).

In general, forages with less than 80 g kg^{-1} WSC (DM basis) may not reach a pH low enough to produce a stable silage (Kunkle et al., 2006). However, high levels of residual WSC may reduce aerobic stability as a result of increased fungal growth (Ohyama et al., 1975; Pitt et al., 1991). At the beginning of aerobic exposure, aerobic micro-organisms will utilize WSC in preference to lactic acid. However, as WSC depleted, lactic acid becomes the main energy source for these microbes (Weinberg et al., 2001).

Water soluble carbohydrate content of ensiled forages is affected by several factors. These include forage species, DM content, wilting and weather conditions (Pahlow et al., 2003). Due to their higher WSC content (Table 2-2) and lower buffering capacity, cereal forages are usually easier to ensile than legumes (McAllister and Hristov, 2000). In corn, the pre-ensiling WSC concentration ranges from 80 to 310 g kg^{-1} with a DM content of 25 to 35% (Lindgren et al., 1985; Weinberg et al., 2001). However, exact measurements of WSC losses arising from plant enzyme activity are difficult to determine, as sugars lost through respiration may be partly replaced by sugar released from hydrolysis of plant structural carbohydrates (McDonald et al., 1991).

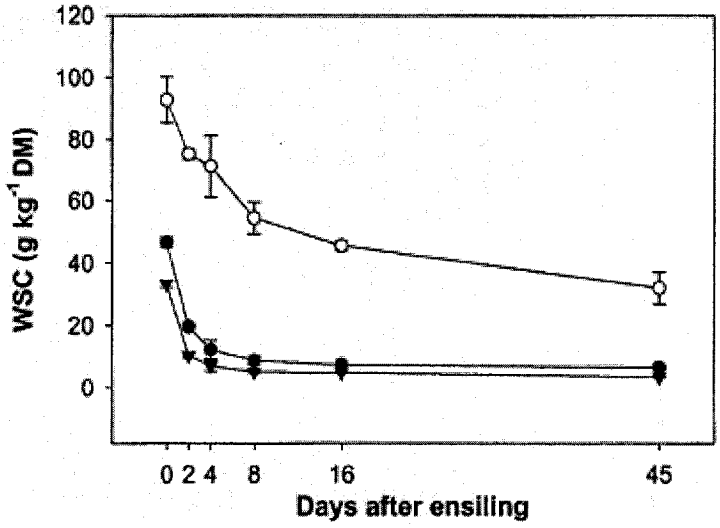


Figure 2-4: Changes in WSC of ensiled whole-crop forage faba bean (●), pea (○), and soybean (▼) (Mustafa and Seguin, 2003).

Table 2-2: **Water soluble carbohydrates content in different crops**

	Crop	Harvest (DM %)	WSC (DM %)
Warm-season annuals	Corn	28-35	10-20
	Forage sorghum	20-35	10-20
	sorghum-sudan, millet	15-30	10-15
	Soybeans	25-40	2-4
	Cowpea	15-30	5-8
Cool-season annuals	Rye, oats, wheat, triticale	20-30	8-12
	Ryegrass	15-30	8-12
Warm-season perennials	Bermudagrass, stargrass	18-30	2-4
	Bahiagrass	20-30	< 5
	Limpograss	20-30	< 5
	Perennial peanut	18-30	1-4
Cool-season perennial	Alfalfa	22-35	4-7

(Adapted from Kunkle et al., 2006)

2.3.2. Respiration

Respiration is an oxidative degradation of organic compounds by which plants obtain energy for growth and maintenance. Respiration and photosynthesis in the intact plant are mainly responsible for the synthesis and utilization of hexose sugars. During wilting of chopped forages and ensiling, the results of plant respiration can be summarised as:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 0.69 \text{ Mcal mol}^{-1} \text{ glucose}$$

The consequences for the ensiling process are a decrease in the amount of substrate available for fermentation and, in the silo, a rise in temperature (McDonald et al., 1991; Muck, 1996; Rooke and Hatfield, 2003).

Plant respiration is useful in that it removes oxygen from the silo which creates an anaerobic environment. However, excessive respiration is undesirable because it reduces the energy content of the silage, may lead to excessive heating and may not leave enough sugar for fermentation by LAB (Muck, 1996).

Factors that influence aerobic plant respiration include temperature, pH and DM content (Meidner, as cited in Rooke and Hatfield, 2003). However, McDonald et al. (1991) stated that the rate of respiration is controlled mainly by temperature, because of the effect of temperature on enzyme reactions, and thus the two are interdependent. In the silo, the amount of O_2 trapped in the ensiled mass is the most important factor, followed by other factors such as silage pH and temperature (Rooke and Hatfield, 2003). When the O_2 in the silo is exhausted, plant respiration can continue anaerobically.

2.3.3. Silage pH

As a result of WSC fermentation by LAB, the pH of ensiled forages declines rapidly within few days post-ensiling (Figure 2-5). Hristov and McAllister (2002) reported that rate of pH, decline for various barley silage treatments, ranged from 0.32 to 0.53% h^{-1}. Throughout the literature emphasis is placed on the importance of reaching a low pH in early stages of the ensiling

process to reduce the risk of early growth of clostridia and other undesirable micro-organisms (Woolford, 1984b). Consequently, it was indicated that a rise in a pH can be a useful criterion to assess silage deterioration(Ohyama et al., 1975). However, Pitt et al. (1991) reported that stability increases as pH decreases from 6 to 3, but at the same time, in a pH typically found in silage, i.e. 3.7 to 5, pH had a very minor effect on stability.

Acidic environment, such as that of silages, is hostile for fungi. Short-chain fatty acids such as propionic and acetic acids are known to inhibit yeasts at low pH (Moon, 1983). In principle, the lower the pH the higher the ratio of undissociated to dissociated short-chain fatty acids. Undissociated organic acids can diffuse into the fungal cells and lower the intracellular pH by releasing H^+ ions (see 2.5.5.). This process will rapidly kill the yeast cell, unless it is counteracted by an active, energy-requiring mechanism for removal of H^+ ions (Warth, as cited in Pahlow et al., 2003).

Another aspect of the silage pH is the enzymatic activity. In general, enzymes are only active over a limited range of pH and, in most cases, for each enzyme there is a pH value at which the rate of activity is optimal. These changes may affect the rate of reactions, like intensity of respiration in the silo, and thus the fermentation process (McDonald et al., 1991). A rapid decline in silage pH will reduce the activity of plant enzymes and therefore decrease the breakdown of protein into non-protein nitrogen (Mckersie, 1985). The pH is usually lower in wetter than in drier silages (Thomas, 1978).

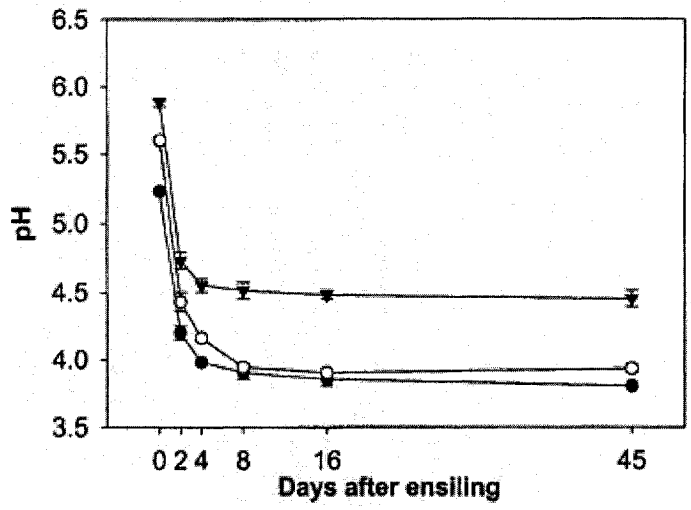


Figure 2-5: Changes in pH of ensiled whole-crop forage faba bean (●), pea (○), and soybean (▼) (Mustafa and Seguin, 2003).

2.4. Silage quality

Several factors are known to influence silage quality. These include WSC content, forage buffering capacity, DM concentration, temperature during ensiling and storage, rate of harvest, and air exposure during harvest, storage and feeding (Kunkle et al., 2006).

Flieg, and later Zimmer, (as cited in Woolford, 1984b) developed schemes upon which points are awarded according to the relative proportions of the various organic acids in the silage. According to those schemes, the highest quality silage will be one with less than 1.5 and 15% butyric and acetic acid, respectively, and more than 75% lactic acid content. Cherney and Cherney (2003) defined high quality silage as one with: pH < 4.2, butyric acid < 10 g kg^{-1} DM, lactic acid > 30 g kg^{-1} DM, and low anaerobic spore counts (< 10^2 cfu g^{-1}).

2.4.1. Management factors

As previously mentioned, creating and maintaining an anaerobic environment in the silo, is a critical factor in producing high quality silage and in avoiding negative impacts of plant respiration, plant proteolysis and aerobic microbial activity (Muck, 1988).

Air infiltration of ensiled forages during silo filling, storage and feed-out phases can lead to significant DM losses which consist of both invisible losses

through oxidation of nutrients and visible silage spoilage due to serious oxidation or overheating (McGechan, 1990). Air allows the respiration process to continue using WSC, which produces heat that increases silage temperature. Air exposure during storage leads to yeast and mold growth on and beneath exposed surfaces. Air exposure at feeding also results in: rapid mold growth, heating, loss of nutritive value, reduced palatability, and production of potential of toxins, which involves health hazard to livestock and personal handling the feedstuff (Woolford, 1990; Kunkle et al., 2006).

The density of silage is an important factor both for farm management and fermentation biology (Muck et al., 2003). Silage is a porous material, and O_2 can move through the pore spaces when the silo is opened. Diffusion is the primary mechanism of O_2 movement on silage (Muck and Pitt, 1994). Silage porosity and microbial respiration activity are the most important silage characteristics influence O_2 flow (Pahlow et al., 2003). The porosity (or density) of the silage depends on forage compaction in the silo which is a function of forage DM content, compacting method and machinery, forage structure and maturity and chopping length (McGechan, 1990; Weinberg and Ashbell, 1994). The modern practice of chopping forages to lengths of 50 mm or less enable ensiled forages to be compacted to higher densities than was possible with longer particle lengths (Rees et al., 1983). However, others believe that shorter chopped forages offers less resistance to gas movement than longer ones, despite their higher density (McGechan, 1990). In addition, excessive densities will increase effluent losses (Muck et al., 2003).

Obviously, the top layer of the ensiled forages is more susceptible to air penetration than the lower layers (1994). After opening the silo, exposure to oxygen is inevitable (Honig, 1984; Honig, 1985; McDonald et al., 1991; Pitt and Muck, 1993). The rate and extent of deterioration at the open face of the silo are known to depend on silage chemical composition and the dynamic of gas and heat transfer at the face of the silo (Pitt and Muck, 1993). Silages with poor aerobic stability can be found on many dairy farms because of slow filling rates or inadequate packing densities at the time of ensiling (Kleinschmit et al., 2005).

According to Muck and Huhnke (1995), During feed-out, air can penetrate 1 to 2 m behind the silage face, so that exposure to O_2 may be prolonged. Therefore, Kunkle et al. (2006) suggested that silage exposed to air should be fed within 24 to 48 h. To minimize the effects of air, Woolford (1990) suggested a rapid silo filling, consolidation of the ensiled forage in the silo, application of adequate seal and the use of effective additives.

2.4.2. Assessing silage quality

Most analytical procedures, which are used to assess silage quality, are quantitative. While some quality evaluation parameters such pH, volatile fatty acids (VFAs) and DM content are well standardized, others such as aerobic stability and DM losses, vary between different studies. Silage quality can be assessed after ensiling or aerobic exposure. The most difficult aspect is relating quality measurements to animal performance (Cherney and Cherney, 2003). In general, a good quality silage is characterized by low pH, NH_3 and acetic acid concentrations, high lactic acid content and undetectable levels of butyric acid (Kung et al., 1998; Hristov and McAllister, 2002).

2.4.2.1. Temperature of silage and aerobic stability

In general, heating is confined to the region near the open face (Muck and Pitt, 1994). Ohyama et al. (1975) found that silages tended to be less stable at 25 to 30°C than at 10 to 15°C ambient temperature, while at 5 to 10°C no deterioration occurred. High temperatures (37 to 41°C) during ensiling of whole crop corn and wheat, led to higher pH, less lactic acid and greater losses, than cooler temperatures (24 to 28°C). Warmer silages might also be more susceptible to aerobic deterioration, especially if the ambient temperature is high (Muck and Huhnke, 1995; Weinberg et al., 2001). According to Rooke and Hatfield (2003) a rise in silo temperature is the most reliable indicator for aerobic deterioration.

In the laboratory, aerobic stability is defined as the time required for the temperature of aerobically exposed silage to rise by 2°C above ambient temperature (Pitt et al., 1991; Kung et al., 1998; Ranjit et al., 2002; Muck, 2004; Kleinschmit et al., 2005). However, others used 1°C rise above ambient

temperature (Driehuis et al., 1999; O'kiely and Muck, as cited in Cherney and Cherney, 2003). In some cases, for a more detailed evaluation, other parameters such as: silage pH, yeast and mold populations, organic acid concentrations, and gas production can be used to assess the quality of aerobically exposed silages (Honig, 1990; Cherney and Cherney, 2003). The rise in silage temperature is a simple and reliable system to determine aerobic stability, and thus suggested as a standard procedure for silage evaluation (Honig, 1990).

In some silages, the growth of molds follows that of yeasts and this is often reflected in the appearance of two thermal peaks which can be detected during aerobic deterioration (McDonald et al., 1991). The first peak, which may occur within two-three days of aerobic exposure, is usually caused by yeasts, while the second thermal peak, occurring about three to four days later, can be attributed to molds (Yamashita and Yamazaki, as cited in McDonald et al., 1991). Lindgren et al. (1985) found that stability fell below 3 h when mold populations were above $10^{6.5}$ g^{-1} DM or yeasts were above $10^{8.5}$ g^{-1} DM. Muck et al. (1991) suggested that the molds will succeed the yeasts as temperature rises during deterioration.

Aerobic instability of corn silage can be a major concern for farmers, particularly in warm weather, and considered as a common trouble even with good silage management (Muck, 2004). Pitt et al. (1991) found that aerobic stability decreased as initial temperature increased from 10 to 40°C, due primarily to the increase in fungal growth rates with temperature; at lower and higher temperatures (< 10 or > 40°C) stability increased dramatically because of inhibition and death of fungal growth. Susceptibility of yeasts to high temperatures (> 40°C) was supported later on (Lindgren et al., 1985; Yamashita and Yamazaki, as cited in McDonald et al., 1991; Weinberg et al., 2001). Silage additives were developed to inhibit fungal growth and minimise silage deterioration will be discussed later.

2.4.2.2. Silage DM losses

Dry matter losses as a result of aerobic exposure during storage and feed-out phase accounts for most DM losses associated with the ensiling process. About 50 to 70% of DM losses of ensiled forages can be attributed to aerobic exposure (Honig, 1984; Holland and Kezar, 1990). Buckmaster et al. (1989) showed that the DM losses from aerobic processes during storage and feed-out account for over 70% of the DM losses associated with storing crops by ensiling. Factors that contribute to DM losses other than air exposure include silage compaction, and ambient temperature (Pitt and Muck, 1993).

Dry matter losses could be measured by calculating the ratio of DM content of the silage before and after specific period of aerobic exposure (Muck, 2004). Production of CO_2 and / or O_2 consumption during aerobic exposure can also be used as indicators of silage DM losses. Linear relationships between DM losses and CO_2 production or O_2 consumption have been reported by Honig (1990).

Research showed that nearly 50% of the silage DM losses occur from secondary aerobic decomposition, which takes place on any surface of the silage that is exposed to O_2, while in storage or in the feed-bunk (Holland and Kezar, 1990). In the USA, during the warm summer, it is recommended that at least 15 cm d^{-1} of silage will be removed to mitigate aerobic deterioration (Figure 2-6).

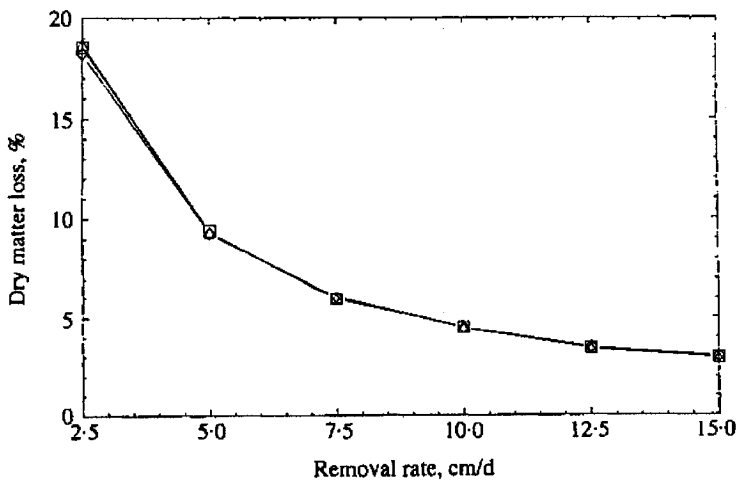


Figure 2-6: Dry matter loss vs. rate of silage removal (Pitt and Muck, 1993).

2.5. Silage additives

Silage additives are used mainly to improve the fermentation characteristics of the ensiled forages, to improve feeding value of silages, and / or to increase the shelf life of silage (Carr et al., 1984; Kunkle et al., 2006). An ideal silage additive should reduce DM losses, improve the hygienic quality of the silage, limit secondary fermentation, improve aerobic stability, increase silage feed efficiency, give the farmers a return greater than the cost of the additive, and be safe to handle (Merensalmi and Virkki, as cited inHenderson, 1993).

Different classification systems of silage additives can be found in the literature. Any system may have some degree of overlapping with others. For instance, a given additive can posses properties common to two or more classes (Woolford, 1984b). In general, silage additives can be classified into fermentation stimulators and spoilage inhibitors (Thomas, 1978). Furthermore, spoilage inhibitors can be subdivided into fermentation inhibitors and aerobic stability enhancers (Kung et al., 2003). Microbial inoculants and enzymes (i.e. biological additives) are considered fermentation stimulators, while the inhibitory additives are mainly organic acids, nitrogen sources (i.e. chemical additives) and, to lesser extant, other chemicals such as gases and inorganic acids.

Microbial inoculants have been added to silages to improve fermentation efficiency while organic acid additives have been added to improve aerobic stability. There are two primary reasons for using a preservative to improve the aerobic stability: 1) to prevent heating and DM losses associated with this process and 2) to prevent a reduction in animal performance from the feeding of spoiled silage. An efficacious silage preservative would prevent one or both of these from occurring (Kung et al., 1998; Kung et al., 2004).

It is possible to add both chemical and biological additives to ensiled forages in order to promote adequate fermentation patterns, especially under sub-optimal conditions (Weinberg and Muck, 1996). Any additive intended as a preservative should aim at the total suppression of micro-organisms rather than of any specific group.

2.5.1. Bacterial inoculants

Bacterial inoculants are part of biological additives which also include enzymes. They are a popular means to increase the quality of preserved plant materials and to enhance the aerobic stability of silages. Biological inoculants have advantages over chemical additives because they are safe (non-hazardous), easy-to-use, non-corrosive to farm machinery, do not pollute, and are regarded as natural products. Selection of fast-growing bacterial strains for inclusion in a silage inoculant is the principal factor that will influence the impact of the product on silage fermentation and subsequently, animal performance (Weinberg and Muck, 1996; McAllister and Hristov, 2000; Kunkle et al., 2006).

Inoculants containing propionibacteria are usually used to enhance aerobic stability of silages due to the antifungal properties of propionic acid (Woolford, 1975b). It is anticipated that such inoculants would produce propionic acid in the silage and inhibit yeasts and moulds upon aerobic exposure. Inoculants containing propionibacteria have been effective in situations where the decline in pH was slow and / or when the final silage pH was relatively high, e.g. > 4.5 (Weinberg and Muck, 1996; Kung et al., 2003). However, in other studies, the addition of propionic acid bacteria failed to improve fermentation characteristics or aerobic stability of silages (Weinberg and Muck, 1996; Higginbotham et al., 1998). Inconsistency of propionibacteria inoculants is likely due to their relative poor acid tolerance, strict anaerobic requirements and slow growth rates (Kung et al., 2003). The direct method of adding propionic acid to silages (Kung et al., 1998) has been more effective in improving aerobic stability.

Silage inoculants containing principally LAB have become the dominant additives in many parts of the world not only because of convenience and safety, but also because they are expected to control microbial events during silage fermentation. Their function has been to promote rapid and efficient utilization of WSC of the ensiled forages, which results in intensive production of lactic acid and a rapid decrease in pH, with a lower final value. Silages treated with adequate

homo-fermentative LAB should consist of higher lactic acid content and lower pH, acetic acid and NH_3 (Weinberg and Muck, 1996).

Several species of LAB have been used to produce silage inoculants. These include *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Enterococcus faecium*, *Pediococcus acidilactici* and *Pediococcus pentosaceus*. However, other species such as *Streptococcus thermophilus*, *Serratia rubidaea* (Phillip and Fellner, 1992) and *Lactobacillus buchneri* (Weinberg and Muck, 1996; Oude Elferink et al., 2001; Ranjit et al., 2002) have been used.

Thus far, most commercial silage inoculants contain homo-fermentative or facultative hetero-fermentative LAB (Oude Elferink et al., 2001). Homo-fermentative LAB are used: to supplement the natural population of LAB in forages, to ensure a rapid silage fermentation that is higher in lactic acid and lower in acetic acid and ethanol concentrations, and also to reduce DM losses (McDonald et al., 1991; Driehuis et al., 1997; Muck, 2004). Several studies found that Homo-fermentative LAB inoculants improved silage quality, in terms of pH reduction and lower clostridial counts (McAllister and Hristov, 2000; Hristov and McAllister, 2002; Mayrhuber et al., 2005).

Hetero-lactic inoculants contain hetero-fermantative bacteria (e.g. *Lactobacillus buchneri*) which, in addition to lactic acid, can also produce other organic acids such as acetic acid (Oude Elferink et al., 2001; Ranjit et al., 2002; Muck, 2004). Because acetic and propionic acids are stronger antimytotics than lactic acid, silages inoculated with hetero-lactic inoculants are more aerobically stable than silages inoculated with homo-lactic inoculants (Moon, 1983; Kung et al., 2003). Inoculants containing *L. buchneri* treatment has been shown to be consistent in improving aerobic stability of various ensiled crops. Yeast population was reduced during both fermentation and aerobic exposure (Driehuis et al., 1999; Oude Elferink et al., 2001; Ranjit et al., 2002; Muck, 2004; Kleinschmit et al., 2005; Kunkle et al., 2006). However, homo-lactic fermentation of sugars should result in no DM loss, whereas losses from hetero-fermentation can be significant (Muck, 1996), since their fermentation process consists of waste products such as CO_2 and alcohol (Wilkinson et al., 2006).

A major concern with inoculants containing *L. buchneri* is the possible negative impact of high acetic acid concentration on animal performance (Kung et al., 2003; Muck, 2004). However, Taylor et al. (2002) reported that feeding barley silage treated with *L. buchneri* had no negative effects on intake or performance of dairy cows.

Recently developed inoculants, contain multiple bacterial species, based on evidence that growth of one bacterial species may facilitate the growth of another (Muck, 1996; McAllister and Hristov, 2000). Novel inoculants which contain both homo- and hetero-lactic bacteria have been developed to enhance the fermentation process as well as the aerobic stability of silags. Recent studies have confirmed their effectiveness on corn silage (Kunkle et al., 2006).

Although silage inoculants are the most common type of silage additive, they have not been consistently effective in improving silage quality (Thomas, 1978; Muck, 2004; Kung et al., 2004), digestibility or feed intake (Carr et al., 1984; Weinberg and Muck, 1996). Reasons for inoculant's ineffectiveness can be divided into two categories; 1) failure to dominate fermentation due to high natural LAB population and / or low WSC, and 2) failure to inhibit adverse microbial activity due to high phage activity (Weinberg and Muck, 1996; Muck, 1996). Weinberg and Muck (1996) reported that 25% of the silage samples contained infectious phages, mainly in high moisture silages, and their presence was associated with poor quality silages. Low ambient temperature, and low DM content, can also reduce the effectiveness of silage inoculants. Driehuis et al. (1999) reported that the use of hetero-lactic inoculants increased DM loss during fermentation due to the formation of CO_2 on conversion of lactic acid to acetic acid; the DM loss increases with inoculation level and length of ensiling.

The rapid decline in pH during the ensiling process as a result of inoculation is more pronounced in legume and grass silage than in cereal silages where the pH usually falls below 4 without inoculation (McAllister and Hristov, 2000).

A major drawback of homo-lactic acid inoculants is related to silage aerobic stability. It is well documented that silages treated with homo-lactic acid

inoculants deteriorate faster than untreated silages upon aerobic exposure. Cereal silages inoculated with homo-lactic acid inoculants have higher pH, DM losses and yeast and mold populations compared with untreated silages (Weinberg et al., 1993; Weinberg et al., 1996; Filya, 2003). Inoculating silages with homo-fermentative LAB improved aerobic stability in some studies (Wohlt, 1989) but make it worse in others (Kung and Ranjit, 2001). Based on previous studies it seems that homo-lactic acid inoculants are more effective in legume than in cereal forages.

New approaches for developing new silage inoculants should consider fermentation efficiency, aerobic stability and animal performance. Other factors such as crop specificity, DM content of the target silage and silo type should also be considered (Weinberg and Muck, 1996).

2.5.2. Enzymes

When WSC are limiting, enzyme additives, such as amylases, cellulases, and pectinases can be added to the ensiled forages to break down complex carbohydrates into simple sugars. Most of the commercially available enzyme additives are mixtures of several enzymes and are expected to improve fermentation characteristics by releasing soluble sugars for the use by LAB during the ensiling process. This will result in silages with a lower final pH and higher lactic acid content. Enzyme additives may also degrade portions of the plant cell wall during storage, thereby reducing fibre content and potentially improving animal performance (Thomas, 1978; Sheperd and Kung, 1996; Kunkle et al., 2006). Plant cell wall degrading enzymes, are more effective with immature and low DM silages (Spoelstra, as cited in Henderson, 1993).

But enzyme inoculants tend to be inconsistent, and in some cases ineffective. Chen et al. (1994) concluded that the treatment of corn silage with an experimental enzyme-inoculant mixture (carbohydrase) did not improve silage preservation or composition, ruminal degradation of silage DM or NDF, or feed intake by early lactation cows; experiments to demonstrate possible increases in digestibility, have found them not effective (Thomas, 1978; McDonald et al.,

1991). Enzyme-inoculant treatment had no effect on in-vitro NDF digestion, and ADF or NDF concentration (Sheperd and Kung, 1996; Ranjit et al., 2002, respectively). Enzymes are also known to increase effluent flow, and hence should be avoided, where there is a risk of pollution (Henderson, 1993).

2.5.3. Gases

Formaldehyde is a well-known sterilising agent and is commercially available as formalin, which contain 40% of the gas in aqueous solution (McDonald et al., 1991). The interest in formaldehyde as a silage additive is due to its bacteriostatic properties, and because it is known to protect plant proteins from degradation during ensiling and in the rumen.

Treating grass silages with Formaldehyde led to the inhibition of LAB (Ohyama and McDonald, 1975), unfavourable acid composition, and high DM losses (Theune and Honig, 1979). When applied at high levels, formalin depresses DM digestibility and intake, whereas at low application levels it tends to encourage growth of clostridia (McDonald et al., 1991). Therefore, commercially available products contain mixtures of acids and formalin (Ohyama and McDonald, 1975; McDonald et al., 1991; Henderson, 1993). Although formalin-acid mixtures are very effective in ensiled grasses, the potential health risks of exposure to formaldehyde together with its high volatility, led to the banning of these additives in many countries (McDonald et al., 1991).

2.5.4. Non-protein nitrogen

Ammonia and urea are practical and relatively inexpensive sources of non-protein nitrogen which can be used to increase the protein concentration of low protein forages such as corn silage (Carr et al., 1984; Kunkle et al., 2006). Due to their antifungal properties, both urea and NH_3 appear to stabilise aerobically exposed silages, increase silage shelf life and therefore reduce DM losses (Honig, as cited in McDonald et al., 1991). Ammonia has been used as an additive in silage making mainly to improve the nitrogen content of the product (McDonald et al., 1991). The rapid rise in the pH reduces plant enzymatic activity and decreases protein breakdown by approximately 30% (Holland and Kezar, 1990).

Ammonium bicarbonate and NH_3-N inhibits yeasts and molds; the NH_3 treatment markedly improved the stability and lowered the peak temperatures of aerated corn silage, due to its fungicidal properties, and thus increased bunk life. Ammonia toxicity is related to a high pH that maintained the undissociated form of NH_3 which is toxic to fungal cells (Britt and Huber, 1976; DePasquale and Montville, 1990; Kung et al., 2000; Kunkle et al., 2006).

Adding urea to corn silage at ensiling can improve its relatively low protein content. However, this additive produces silages with high pH owing to its high buffer capacity (McDonald et al., 1991). Silages treated with NH_3 also had high initial and final pH and consequently LAB growth is delayed (Kung et al., 2000).

Feeding urea and NH_3 treated silages to ruminants yielded inconsistent results. This can be attributed to factors such as clostridial fermentation (in grass silages), or (in corn silage) inability to apply the product uniformly at the right rate (Kung et al., 2003). Major drawbacks of NH_3 application is its volatility, corrosion to equipment, and operator risks (McDonald et al., 1991; Kung et al., 2000; Kunkle et al., 2006).

2.5.5. Organic acids

Organic acids have been extensively used as forage and grain preservative (Czarnecka et al., 1991). Organic acids improve silage quality by accelerating the decline in pH during the early stages of the ensiling process and by acting as antifungal agents. Chain length of organic acid is directly related to their antifungal properties and inversely related to their ability to reduce silage pH (Woolford, 1975b). Addition of sufficient amounts of organic acids lead to a rapid decline in pH, inhibits the growth of most bacteria and yeasts and reduces DM losses and proteolysis (Thomas, 1978; Moon, 1983).

Weak organic acids are more effective as antifungal agents at low pH (i.e. undissociated form) with the greatest effectiveness at or below the pKa of the acid. Lambert and Stratford (1999) showed that inhibition of microbial growth by weak-acids involves rapid diffusion of undissociated molecules through the

plasma membrane. The dissociation of these molecules within cells liberates protons, thus acidifying the cytoplasm and preventing fungal growth (Figure 2-7). Only uncharged weak acid molecules (HA) can diffuse freely across the plasma membrane. Charged anions (A^-) and protons (H^+) are retained within the cell. Cytoplasmic protons are expelled by the membrane-bound H^+-ATPase.

By modelling preservative action of organic acids using a thermodynamic and kinetic approach, it was possible to demonstrate that: (i) inhibition depends more on the degree to which individual preservatives are concentrated within cells rather than on undissociated acid concentration per se, (ii) it is entirely feasible for microbes to pump protons out of the cell during extended lag phase and raise internal pH despite further influx of preservatives, (iii) the duration of the lag phase can be predicted from the model, using a Gaussian fit of proton-pumping H^+-ATPase activity against pH, (iv) theoretical ATP consumption for proton pumping can be directly correlated with the reduction in cell yield observed in glucose-limited cultures (Lambert and Stratford, 1999). As operation of the pump requires a high energy input, resistant to preservatives will be greatest with high glucose concentrations (Warth, 1977).

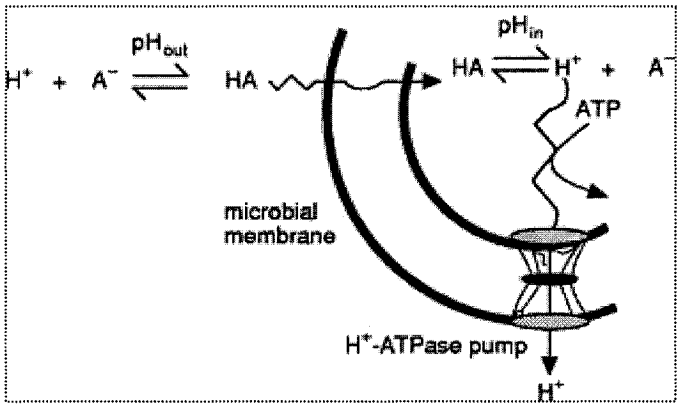


Figure 2-7: Predicted medium of cytoplasmic weak-acid and anion equilibria (Lambert and Stratford, 1999).

The antifungal properties of aliphatic acids, with 2 to 6 carbon atoms are well known (Lacey et al., 1981). High levels of formic acid may delay the onset of deterioration, inhibit plant respiration and proteolysis, and improve DM recovery and feed intake. However, yeasts have been found to be particularly

tolerant to formic acid whereas LAB were inhibited. In some cases, formic acid even increased silage deterioration (Ohyama and McDonald, 1975; Britt et al., 1975; Thomas, 1978; McDonald et al., 1991; Henderson, 1993). In addition, where there is a risk of pollution, formic acid, which increases effluent flow, should be avoided (Henderson, 1993).

Valeric and caproic acids can effectively inhibit the aerobic deterioration of silages (Ohyama and McDonald, 1975; Theune and Honig, 1979). Butyric acid can increase the aerobic stability of the silage (Ohyama and McDonald, 1975; Theune and Honig, 1979). Nevertheless, it is a weak acid and a larger quantity would be required to reduce pH to a level which suppress the spore-bearing bacteria (Woolford, 1975b). However, butyric, valeric and caproic acids are usually more expansive, and associated with silage which has undergone a clostridial fermentation (Woolford, 1975b).

Propionic or butyric acid are effective in reducing the growth of *Aspergdlus flavus* and subsequent aflatoxin production (Ghosh and Haggblom, 1985). It has been suggested that propionate is a more powerful antifungal agent than other organic acids at the same pK_a (Moon, 1983). In addition to diffusion through micro-organism plasma membrane, propionic acid functions by standing active on the surface of micro-organisms and by competing with amino acids for space on active sites of enzymes or through altering the cell permeability (Kung et al., 2003). Freese et al. (1973) demonstrated that low concentrations of propionate inhibited the transport of amino acids across membrane vesicles of bacteria and was therefore more toxic than other acids.

Undissociated lactic and acetic acids are strong inhibitors of fungal growth. Acetic acid is a stronger antimytotic than lactic acid. However, because acetic acid is weaker than lactic acid, it will be more undissociated at any given pH. On the other hand, lactic acid may play a greater role in silage fermentation because of its higher concentration and its greater ability to reduce pH (Pitt et al., 1991; Muck et al., 1991; Kung and Ranjit, 2001).

Antifungal compounds, such as benzoate, propionic and sorbic acids cause corrosion. Acid salts, are less corrosive and reduce health risks, but at the same

time are less effective than the equivalent acid. Therefore, they must be applied at higher rates to obtain a similar effect (Thomas, 1978; Woolford, 1984a; McDonald et al., 1991; Henderson, 1993; Kung et al., 2000).

Treating corn silage treated with 0.09% sorbic acid or potassium sorbate improved aerobic stability probably by inhibiting yeast growth (Alli et al., as cited in Kleinschmit et al., 2005). In other studies sodium benzoate and potassium sorbate + EDTA have been effective at inhibiting the growth of yeasts and molds and improving the aerobic stability of silages (Woolford, 1975a; Kleinschmit et al., 2005); Sodium benzoate was found to posses antimicrobial properties and to be most effective at low pH values (McDonald et al., 1991).

In the 1980s there was some interest in the ensilage of whole crop cereals treated with sodium hydroxide (Deschard et al., 1987; Tetlow et al., 1987). Tetlow et al (1987) found that 50 to 70 g NaOH kg^{-1} DM not only increased the digestibility and intake by sheep, but also appeared to enhance the aerobic stability of the silage. On the other hand, Deschard et al. (Deschard et al., 1987) observed the occurrence of extensive primary and secondary fermentation in wheat silage treated with NaOH.

Several buffered acid products which are less corrosive and hazardous are now commercially available (Kunkle et al., 2006). Acetic acid in combination with lactic acid has been found to inhibit yeast (Moon, 1983) and buffered propionic acid-based products with active ingredients containing ammonium and sodium propionate, propionic, acetic, benzoic, and sorbic acids, inhibited fungi growth and improved aerobic stability (Kung et al., 2000; Kung et al., 2004).

2.5.5.1. Propionic acid and propionic acid additives

Relative to the other short chain fatty acids, propionic acid had the greatest antifungal activity (Moon, 1983). The antifungal property of propionic acid increases as pH declines, which makes it an ideal preservative for cereal forages (Kung et al., 2003). Propionic acid bacteria ferment lactate acid to propionate and acetate which inhibit yeast and mold growth that are responsible for aerobic deterioration. However, propioni-bacteria have difficulty surviving the ensiling

process and therefore additives containing propionic acid have been developed as alternatives (Higginbotham et al., 1998). Additives based on propionic acid consistently decrease silage temperatures during fermentation and feeding, reduce fungal counts and CO_2 production with minor impact on fermentation end products (Huber and Soejono, 1976; Britt and Huber, 1976; Thomas, 1978; Kunkle et al., 2006) and have some action against endospore-forming bacteria (Woolford, 1975b). Propionic acid can also be used as an emergency treatment for silages which had already begun to deteriorate in the open bunker (Woolford, 1975b).

Propionic acid is volatile, corrosive, unpleasant to handle and relatively expensive. Once naturalized or buffered, it becomes less volatile and corrosive, and hence safer to handle (Lacey et al., 1981; Kung et al., 1998). Therefore, acid salts (e.g. calcium, sodium and ammonium propionate) became commercially available. The efficiency of propionic acid and its salts, such as calcium sodium and ammonium propionate, is closely related to their solubility in water. The stronger the bond between the acid and the base, the less soluble the product, and thereby it is less effective in inhibiting molds. Among these salts, ammonium propionate is by far, the most soluble in water, followed by sodium and calcium propionate (900, 250 and 50 g l^{-1}, respectively) (Kung et al., 2003). Woolford (1984a) found that at any given pH, ammonium dipropionate was the most effective antimycotic among propionate salts followed by ammonium propionate and propionic acid, respectively.

A propionic acid additive (contain 56% active ingredient) applied at a rate of 2 g kg^{-1} of fresh forage has been found to improve fermentation of barley silage, resulting in a higher concentration of lactic and acetic acids (Kung and Ranjit, 2001). However similar responses have not been reported for corn silage (Kung et al., 1998; Kung et al., 2000). Several studies have shown that propionic acid and propionic acid-based additives are more effective than inoculants in improving silage aerobic stability (Fellner et al., 2001; Kung et al., 2004). In some studies, despite substantial improvement in aerobic stability (i.e. number of hours before silage starts to spoil), significant differences were not reported between

untreated and propionic acid treated silages (Kung and Ranjit, 2001). Kung et al. (2000) found that improvement in aerobic stability of corn silage was achieved by high (3 g kg^{-1}) but not moderate (2 g kg^{-1}) or low (1 g kg^{-1}) concentrations of propionic acid-based additive.

Kung et al. (2004) showed that buffered propionic (BP1) acid was significantly more effective than microbial inoculant (MI) when compared (Table 2-3). However, in some circumstances when used together, these two additives can result in improvements in silage fermentation and aerobic stability.

Table 2-3: Composition of high moisture corn after 0 and 120 d of ensiling.

	Fresh high moisture corn, after treatment but before ensiling			Composition of high moisture corn, after 120 d of ensiling		
Item	Control	BP	MI	Control	BP	MI
pH	6.11^{a}	5.62^{bc}	6.21^{a}	4.13	4.17	4.23
Propionic acid, %[1]	0.01^{c}	0.33^{a}	0.01^{c}	$<0.01^{c}$	0.29^{a}	0.01^{c}
Aerobic stability[2]				122	161	216
Yeast, log_{10} cfu g^{-1}	6.02	4.7	5.36	4.16^{a}	0.57^{c}	3.92^{a}

[1]DM basis
[2]Hours until 2°C rise in the silage, above ambient temperature
[a,b,c] Means in columns with unlike superscript differ ($P < 0.05$)
BP = buffered propionic acid-based additive (0.2%)
MI = Microbial Inoculant (LAB)

(Adapted from Kung et al., 2004)

Mills and Kung (2002) found that buffered propionic acid based additives can partially compensate for poor silo management (e.g. delayed ensiling). Several propionic acid additives can also be added to open silos and silages or total mixed diets just prior to feeding to prevent heating and spoiling in the feed bunk (Kung et al., 1998).

However, Kung et al. (1998) indicated that controlling yeasts at the time of ensiling is more efficient that try to control their numbers in the feed bunk. They determined effects of preservative based on propionic acid on the

fermentation and aerobic stability of corn. These authors found that low levels of propionic acid additives applied prior to ensiling had no effect on fermentation end products but delayed the onset of heating and improved the aerobic stability of silages. When applied in the feed bunk, propionic acid additives were effective in reducing pH and temperature of total mixed diets after 24 h in the feed bunk.

The optimal application rate of propionic acid additive varies according to moisture content, length of storage, desired use and formulation with other preservatives. High levels (~10 g kg^{-1}) of propionic acid can improve stability but may restrict silage fermentation. Propionic acid additives, mixed with other organic acids, have recommended use rates of 0.5 to 1 g kg^{-1} of fresh weight (Kung et al., 2003).

Buffered propionic acid-based additives applied at 2 to 3 g kg^{-1} (but not at 1 g kg^{-1}) of fresh forage weight have decreased the forage pH, lowered the peak temperatures during spoilage, and improved the aerobic stability of corn silage (Kung et al., 1998; Kung et al., 2000; Kleinschmit et al., 2005). At pH 5, propionic acid would be expected to restrict growth of actinomycetes and fungi at a concentration of 84.5 mmol L^{-1} which approximates to an application of 2.5 L ton^{-1} to hay with 40% DM (Woolford, 1984a).

Several studies evaluated propionic acid and NH_3 as well as their combination and found both additives highly effective, in improving silage characteristics and animal performance (Yu and Thomas, 1975; Britt and Huber, 1976; Thomas, 1978; Woolford, 1984a; Kung et al., 2000; Kung et al., 2004). Thomas (1978) reported that propionic acid and ammonium isobutyrate decreased fungal numbers, increased feedable DM and protein by 25%, while reducing temperature and acid detergent insoluble protein and hence enhancing the digestible protein content. Kung et al. (2000; 2004) used buffered propionic acid-based preservative, with ammonium and sodium propionate as the active ingredients; they found it significantly effective in inhibiting fungal growth and improving aerobic stability. Woolford (1984a) concluded that Ammonium propionate appears more effective than the free acid.

2.6. Effects of adding silage additives on animal performance

Considerable effort has been devoted to understanding how additives affect animal performance, because such improvements are in many cases the principle economic justification for their use. One also have to remember that studies without positive results tend not to be published (Kung et al., 2003). Since bacterial inoculants are more common, further information is available in the literature, concerning their effects on animal performance.

In a survey conducted by Dhiman et al. (as cited in Kung et al., 2003) which included 16 studies with homo-fermentative LAB silage inoculants, there was no animal response, unless the inoculation increased the number of LAB by 10 fold. Treating silages with bacterial inoculants improved total tract DM and fibre digestibilities in some studies (Weinberg and Muck, 1996; Fellner et al., 2001) which resulted in improved animal growth rate (Fellner et al., 2001). The latter study found that increase in growth rate with bacterial inoculation was achieved without changes in feed intake or nutrient digestion, suggesting that the response may be related to improved efficiency of metabolisable energy utilisation.

Ruminal nutrient degradability is usually not affected by microbial inoculation. Hristov and McAlliste (2002) found that inoculating barely silage with three different inoculants had no effect on ruminal degradability. Similar findings have been reported for alfalfa silage (Rizk et al., 2005). However, other studies reported improved ruminal DM degradability of whole crop barely (McAllister et al., 1995) or grass (Mandebvu et al., 1999) silage as a result of inoculation.

Several studies have reported positive (Kent et al., 1988; Gordon, 1989) or no (Keady and Murphy, 1997) effects of silage inoculation on milk yield. Improvement in milk yield was attributed mainly to the increase in metabolic energy intake, either because of increased DM intake or improved DM digestibility (Martinsson, 1992). The effects of silage inoculation on milk composition are minimal whether inoculation improved (Kent et al., 1988; Martinsson, 1992) or failed to enhance animal performance (Kung et al., 1987;

Kent et al., 1988). However in few studies, inoculation with homo-lactic acid bacteria increased milk fat (Mayne, 1990) and milk protein (Kung et al., 1987).

Reasons for improved animal performance as a result of silage inoculation are not fully understood. One hypothesis is a probiotic effect, in which specific LAB strains interact with rumen micro-organisms to enhance rumen fermentation (Petit and Flipot, 1990). Another hypothesis is the involvement of a variety of antimicrobial substances such as bacteriocins, which produced by LAB (Weinberg and Muck, 1996; Aksu et al., 2004). Bacteriocins are usually defined as proteins which produce intra-species antagonistic effects. Recently the inhibitory spectrum of these molecules produced by LAB has been widened to include various Gram-negative bacteria and food pathogens (Vandenbergh, 1993). Some researchers attributed the positive impact of inoculation on animal performance to improved fibre digestibility (Kung et al., 1993; Meeske et al., 1999). However, other offered no explanantion (Gordon, 1989; Rooke and Kafilzadeh, 1994).

Limited and inconsistent information is available on the effects of propionic acid-treated silage on animal performance. Despite improvement in aerobic stability of total mixed diets, propionic acid additive did not improve performance of dairy cows (Kung et al., 1998). These authors attributed the lack of improvement to the fact that untreated silage was not spoiled to an extent that would have affected feed intake.

Producers have experienced drastic drops in milk production when cows are fed spoiled and hot feed (Kung et al., 2000). Hoffman and Ocker (as cited in Kung et al., 2000) reported that cows fed spoiled high moisture corn produced 3.2 kg less milk than cows fed fresh feed during a 14 d period. Lower feed value and higher fungal biomass in dry hay resulted in lower intake and DM and fibre digestibilities (Wittenberg, 1994). Undi and Wittenberg (1996) reported that hay preference declined as fungal biomass increased; when calves have a choice of feedstuffs, molded hay caused greater feed sorting and lower intake. A better understanding of the degree of silage spoilage, necessary to reduce animal performance and rate of cattle growth, is needed.

3. Materials and Methods

3.1. Forage preparation

Whole-plant corn (Pioneer 38A24; 2900 CHU) was harvested at one-half milkline (31% dry matter), rolled and cut to a theoretical length of 0.75 cm with a forage harvester (New Holland 900, New Holland). Chopped forages were left untreated or treated with a silage additive - Solution Foin (Agro-Bio Contrôle inc., Saint-Charles-Sur-Richelieu, QC, Canada) to obtain a final application rate of 5 L ton^{-1} of fresh forage weight. The active ingredient in Solution Foin is ammonium propionate (minimum 70% of propionic acid). Alternate loads of the chopped forage were either treated or left untreated, and then packed into six plastic silo bags (three each) using press bagging machine. The amount of forage per silo bag was 10 ± 1 ton of fresh weight. The six silo bags were opened one day (d 1) after ensiling.

3.2. Ensiling parameters

Representative forage samples (3 to 5 kg) were taken daily from each silo bag and kept frozen for later analysis. Temperature was measured daily for 30 d by inserting a glass thermometer 2 and 20 cm inside the ensiled forages, as well as ambient (field) temperature. Temperature was measured at four locations for each depth. Dry matter of ensiled corn was determined by drying 500 g samples in a forced-air oven at 60°C for 48 h. Fifty g samples of ensiled corn from each silo bag were homogenized for 10 min in 500 mL of distilled water and the pH of the water extract was immediately measured, using pH meter (Accumet Basic, model AB15 plus, USA). Lactic acid and water soluble carbohydrates (WSC) were determined on the water extract using calorimetric methods, according to (Barker and Summerson, 1941) and (Dubois et al., 1954) respectively.

3.3. Microbial population

Fifty g of forage samples taken on d 0, 1, 3, 5, 7, 10, 12, 14, 17, 21 and 26 post-ensiling were homogenized in 500 mL of sterile peptone (0.1%) water in a blender for 1 min and used for culturing. Subsequent serial 10-fold dilutions were

made (with the same diluter), to obtain 30 to 300 colonies per dish. Lactic acid bacteria (LAB) was enumerated in triplicate by pour plating of Rogosa SL agar (Difco, 0480-17-0) with one to three mm overlay (to acquire anaerobic conditions). After autoclaving, cycloheximide solution (0.01% w v^{-1}) was added to the media to prevent fungal growth. Yeasts and molds were enumerated in triplicate pour plates of malt extract agar (Difco, 0112-17-6) with 0.25% lactic acid (used for pH adjustment) (Woolford and Cook, 1978). Culture plates for both LAB and fungi were incubated for three d at 30°C. Plates from appropriate dilutions were counted.

3.4. Volatile fatty acid determination

Water extract used for microbial population was also used for volatile fatty acid analysis. One mL of the extract was combined with 200 µL of 0.5N H_2SO_4 and centrifuged for 15 min at 10,000 x *g*. Supernatant was mixed with 10% internal standard (2-ethylbutiric acid) and 0.1g Dowex resin (Sigma, 69011-20-7) for demineralization. Extracts were filtered using 0.2µm filters and were analyzed for acetic, propionic, and butyric acid by gas chromatography (Hewlett Packard model 5890 series II, equipped with flame ionization and model 7673 auto injector; Hewlett Packard, Palo Alto, CA) fitted with 15 m Nukol fused silica capillary column (Supleco, Bellefonte, PA, USA). Column temperature was fixed at 150°C for a run time of eight min. Injector and detector temperatures were 180 and 200°C, respectively. Gas flows were 30, 300 and 30 mL min^{-1} for He, air and H_2, respectively.

3.5. Aerobic stability

Samples (120 g) of ensiled corn taken on d 0, 5, 10, 15 and 20 were used for aerobic stability determination. After been thoroughly shaken to ensure air exposure and prevent lumps, samples were packed loosely in 500 mL plastic containers. Samples were covered with double-layered cheesecloth to prevent drying and contaminations, and incubated for seven d at 25°C. Four small holes were made on top and bottom of each container to permit air exchange. An

additional container filled with water to detect ambient temperature. Thermal insulator was wrapped around the sides of each container to prevent heat loss. Thermocouple probes were placed in the geometric center of the containers to measure temperature. Temperature was measured using a Hotmux data logger (DDC Corporation, Pennsauken, NJ) with temperature recorded every two min and averaged every two h. Aerobic stability was defined as time required to raise temperature by 2°C (Pitt et al., 1991).

3.6. Dairy production study

Two isonitrogenous and isocaloric diets were formulated to meet the requirements of lactating dairy cows. The diets were formulated as total mixed diets with 50% forage and 50% concentrate. In both diets, treated or untreated ensiled corn was the main source of forage. Equal amounts (about 120 kg) of ensiled corn were removed daily from each silo bag, and the silo bags were sealed after. Dry matter of ensiled corn was determined weekly and diet formulations were adjusted accordingly to account for changes in DM levels. Diets were offered twice a day (08:00 and 16:00 h) and feed quantity was adjusted every two days to allow weigh back of 5 to 10% of intake.

Thirty lactating Holstein cows (550 ± 100 kg body weight) were blocked by parity (one to four) and days of lactation (178 ± 55 days in milk), and randomly assigned to one of the dietary treatments (15 cows each) for 30 d period. Animals within each dietary treatment were subdivided into three groups in such a way that every five cows were fed forage from the same silo bag. Animals were housed in tie stalls with continuous access to water. Cows were milked three times a day at 04:00, 12:00 and 18:00 h. Milk yields were recorded at each milking and milk samples were collected once a week from the three milkings, for milk component analysis. The quantities of feed offered and refusals were measured daily for each cow to determine daily feed intake. Feed efficiency was calculated on a daily basis, as milk yield divided by feed intake.

Samples of total mixed diets and silages were collected weekly, dried for 48 h at 55°C in a forced-air oven and pooled monthly. Samples were later ground

through a 1-mm screen, using a Wiley Mill before chemical analysis. Ground samples of total mixed diets were analyzed for DM according to the procedures of the Association of Official Analytical Chemists (AOAC, 1990). Neutral (NDF) and acid (ADF) detergent fibre were determined using the ANKOM System (ANKOM 2000 Fibre Analyzer and F57 filter bags, ANKOM Technology, Fairport, NY, USA). Crude protein (CP; N x 6.25) was determined using a LECO Nitrogen System FP-428 (LECO Corp. St-Joseph, MI, USA).

Milk samples were analyzed for fat, protein, lactose, milk urea nitrogen and somatic cell count at the Dairy Herd Analysis Service (Programme d'analyse des troupeaux laitiers du Quebec) with an infrared system using an electric Milk-O-Scan 4000 (Foss-Food technology, Hillerød, Denmark). Daily milk yield and DM intake were averaged monthly, and values were obtained.

3.7. Statistical Analysis

All microbial data is presented on a logarithmic scale, and on a wet weight basis. Chemical data are presented on a DM basis. Statistical analyses were performed using the GLM procedure of SAS (SAS Institute, 1999) for a completely randomized design. Chemical composition data of the ensiled corn was analyzed using a completely randomized model with 3 replications. Dairy data was analyzed using the mixed model procedures of SAS (SAS Institute, 1999) with cows as a random effect.

4. Results and Discussion

4.1. Ensiling characteristics

4.1.1. Silage temperature

Silage temperature was measured at two depths from the surface of the ensiled forage (i.e. 2 and 20 cm). Changes in temperature during ensiling were similar for both depths (Figure 4-1a and 4-1b). For both depths, temperature of treated and untreated forages increased ($P < 0.05$) between d 1 and d 4 post-ensiling form 24°C to >30°C. Temperatures stabilized between d 4 and d 14 post-ensiling and then declined ($P < 0.05$) between d 16 and d 30 post-ensiling.

Silage additive had minimal effect on daily temperature at both depths. Significant differences ($P < 0.05$) were only observed on d 21 of the 2cm depth (Figure 4-1b). However, the overall effect for the 20cm measurement was stronger than for the 2 cm one ($P < 0.0001$ and $P < 0.05$, respectively) throughout the ensiling period (Table 4-1), and this is most likely due to the relatively cold ambient temperatures (e.g. 8 to 14°C on average) in the field.

Silage temperature is determined by the balance between the rate of heat generation by microbial activity and the heat losses by conduction, radiation, evaporation and convection (Williams et al., 1997). The initial increase in temperature of the ensiled forage is likely due to plant proteolytic enzymes and aerobic bacteria, which produce heat by metabolizing plant sugars and O_2 trapped in the packed forage (Muck, 1988; Kunkle et al., 2006) until those, depleted from the silo. As the temperature increases, microbial growth rates increase until the optimum temperature for microbial growth (~30°C) is reached (Williams et al., 1997; Ashbell et al., 2002). Rees (1982) concluded that for every 10°C temperature rise, a DM loss of 17 g kg^{-1} d^{-1} was expected. Hence, lower silage temperatures, between 15 to 30°C are desirable and indicate more stable forage with less microbial population, as will be discussed later.

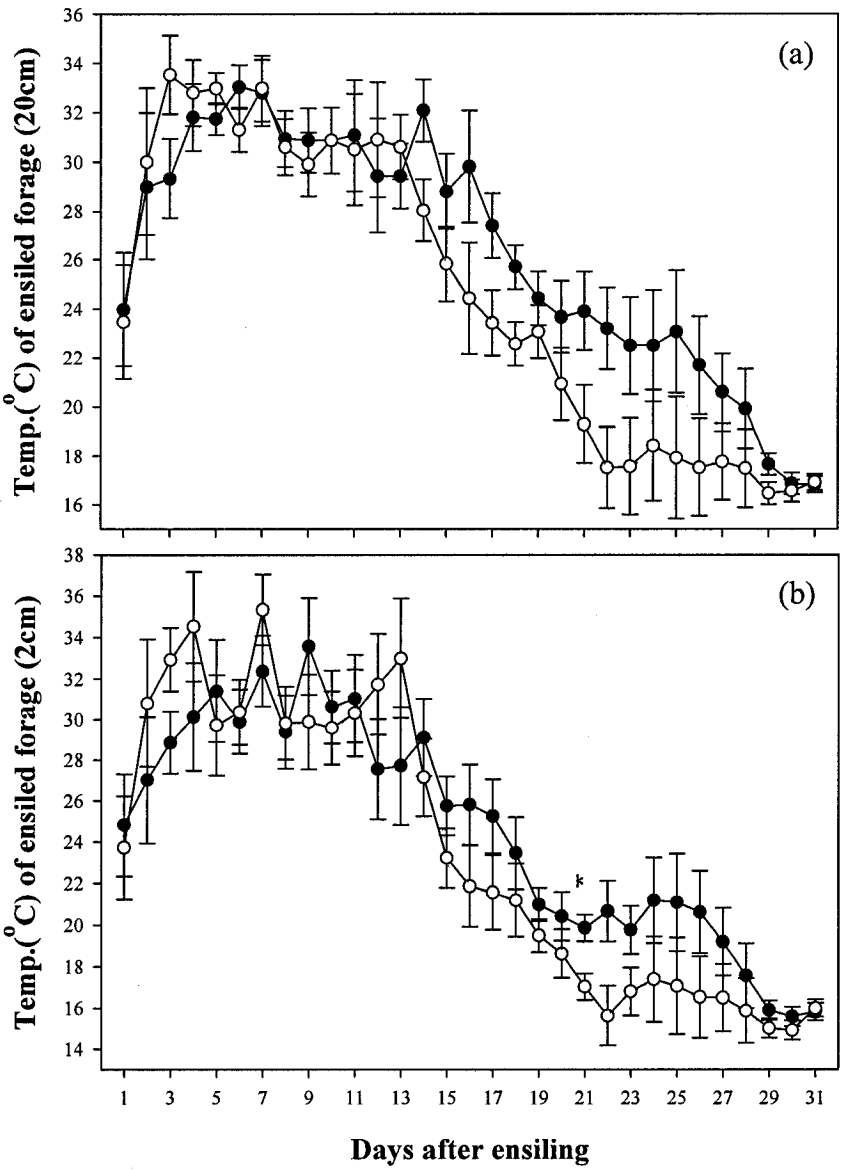


Figure 4-1: Changes in temperature of treated (○) and untreated (●) ensiled corn (2 and 20cm from silo face).
Vertical bars represent ± SE; * represent significant difference ($P < 0.05$).

Table 4-1: Effects of Solution Foin on ensiling characteristics of ensiled corn

Silage values	Untreated	Treated	SE
Dry Matter (g kg^{-1})	321	317	6.9
Lactic Acid (g kg^{-1} DM)	10.42b	14.65a	1.01
Acetic Acid (g kg^{-1} DM)	0.75	0.78	0.07
Propionic Acid (g kg^{-1} DM)	0.00b	1.04a	0.06
Water Soluble Carbohydrates (g kg^{-1} DM)	19.02b	23.83a	1.32
Temperature (^{o}C, 2cm)	24.60a	23.67b	0.31
Temperature (^{o}C, 20cm)	26.29a	24.59b	0.28
pH	4.11	4.10	0.03
a-b Means within row followed by different letters are different ($P < 0.05$).			

4.1.2. pH

The treated pre-ensiled forage pH was lower ($P < 0.01$) than the untreated one (Figure 4-2). The difference is most likely due to the addition of the propionic acid additive to the treated forage. These results are in agreement with Kung et al. (1998) who reported lower pH in corn forage treated with propionic acid-based additive. The corn forage pH values for d 0 were similar to other studies (Kung et al., 1998; Driehuis et al., 1999; Muck, 2004).

For both forages, pH declined rapidly ($P < 0.05$) between d 0 and d 4 post-ensiling (Figure 4-2). The pH then fluctuated until d 16. Reasons for the substantial rise and fall in pH between d 12 and 14 are unknown. However, others (Stokes and Chen, 1994; Sebastian et al., 1996) also showed fluctuation in silage pH during ensiling. Both treatments reached low and stable pH by d 18, indicating a well fermented silage. The 30 d pH values of ensiled corn are in good agreement with other studies (Stokes and Chen, 1994; Kung et al., 2000; Weinberg et al., 2001). The lack of effects of Solution Foin on silage pH (Table 4-1) is in agreement with other studies (Kung et al., 1998; Kung et al., 2004) which showed no effect of propionic acid-based additive on silage final pH.

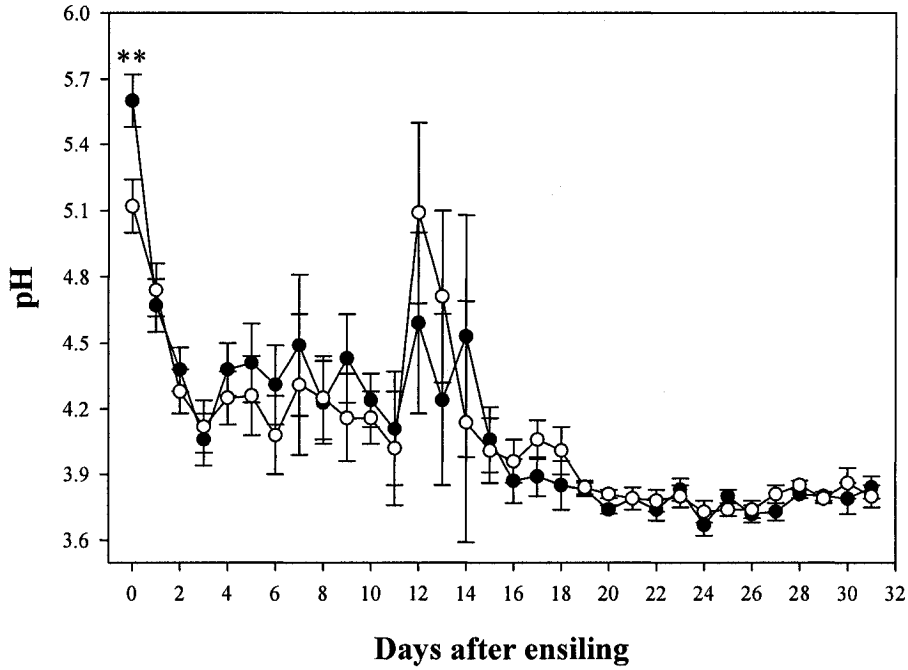


Figure 4-2: Changes in pH of treated (○) and untreated (●) ensiled corn. Vertical bars represent ± SE; ** represent significant difference ($P < 0.01$).

4.1.3. Water soluble carbohydrates

The WSC content of pre-ensiled corn was on the lower end of the range (80 to 310 g kg^{-1}) reported for corn with a DM content of 25 to 35% (Lindgren et al., 1985; Weinberg et al., 2001). However, it was significantly higher than 56 g kg^{-1} which was reported by Weinberg et al. (2002). Water soluble carbohydrates declined ($P < 0.05$) rapidly between d 1 and d 3 post-ensiling (Figure 4-3). The concentration of WSC dropped below 18 g kg^{-1} DM by d 5 post-ensiling. The pattern of WSC decline in this study is similar to that reported before (Mustafa and Seguin, 2003). In disagreement with other studies (Kung et al., 2004; Kleinschmit et al., 2005), propionic acid treatment had an overall treatment effect with higher ($P < 0.02$) residual WSC concentration in the treated than untreated ensiled forage (Table 4-1). In addition, the daily comparison analysis found d 7 to be significantly higher ($P < 0.001$) in WSC, for the treated ensiled forage (Figure 4-3).

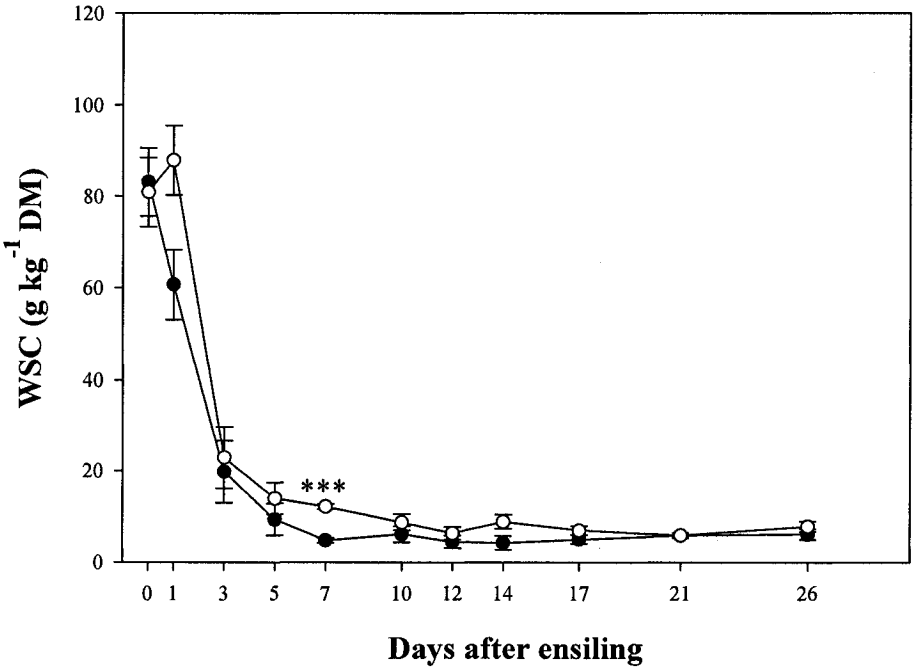


Figure 4-3: Changes in water soluble carbohydrates of treated (○) and untreated (●) ensiled corn.
Vertical bars represent ± SE; *** represent significant difference ($P < 0.001$).

4.1.4. Volatile fatty acids

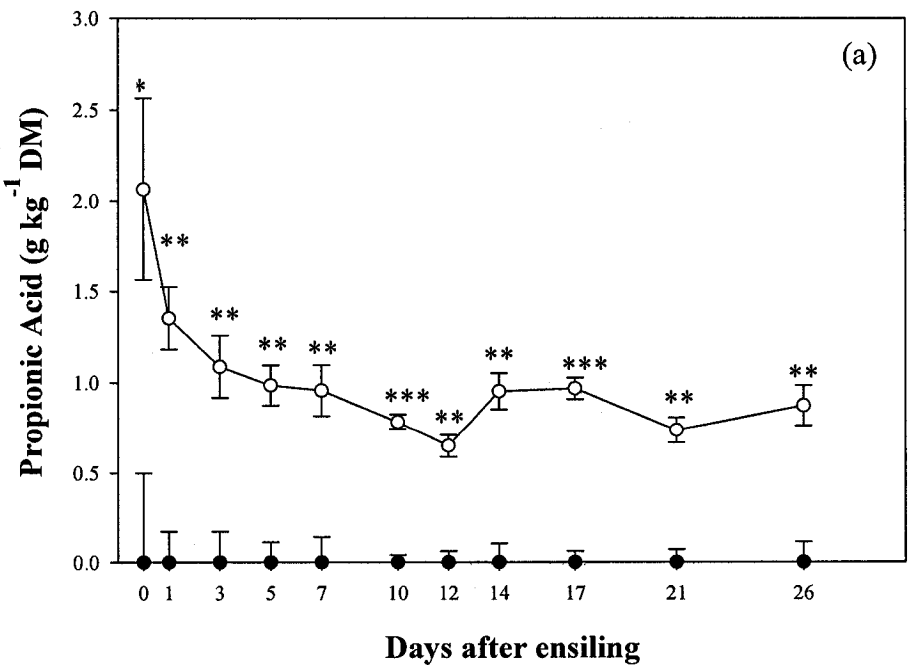
As expected, propionic acid concentration was higher for treated than untreated ensiled corn both overall ($P < 0.0001$; Table 4-1) and on a daily basis comparison (Figure 4-4a). The concentration was highest in d 0 (i.e. > 2 g kg^{-1}) and decreased with ensiling time (i.e. < 1 g kg^{-1} from d 10 post-ensiling). Propionic acid concentration in untreated silages is usually < 10 g kg^{-1} DM (McDonald et al., 1991) to undetectable (Kung et al., 1998; Kung et al., 2000; Kleinschmit et al., 2005).

Lactic acid concentration increased ($P < 0.05$) rapidly between d 1 and d 3 post-ensiling for both untreated and treated forages (Figure 4-4b). Lactic acid concentration reached a high and stable level by d 17 post-ensiling which coincided with the lowest pH (Figure 4-2). Differences in lactic concentration during ensiling between untreated and treated forages were minimal (Figure 4-

4b). However, overall, treated ensiled forage had significantly higher ($P < 0.01$) lactic acid content during the trial (Table 4-1). Lactic acid increasing content showed a typical trend (Stokes and Chen, 1994; Mustafa and Seguin, 2003) as ensiling time prolonged, with rather lower values compared with other studies (Stokes and Chen, 1994; Weiss et al., 2003). However, McDonald et al. (1991) indicated that lower concentrations may occur when acid additive are applied.

Acetic acid content increased with ensiling time, with similar values for treated and untreated ensiled forages, except for d 7 which was significantly higher ($P < 0.05$) in treated than untreated ensiled forage (Figure 4-4c). Acetic acid concentration stabilized (~1.2 g kg^{-1} DM) at d 17 post-ensiling and was lower than the average value (26 g kg^{-1} DM) reported by McDonald et al. (1991). However the same authors showed that acid additive decreased acetic acid content significantly. From d 3 on, at any given ensiling time, acetic acid concentration was significantly ($P < 0.05$) lower than that of lactic acid. Overall, silage additive had no effect on acetic acid concentration (Table 4-1) which agrees with other studies (Kung et al., 1998; Kleinschmit et al., 2005).

No butyric acid was detected in any forage sample.



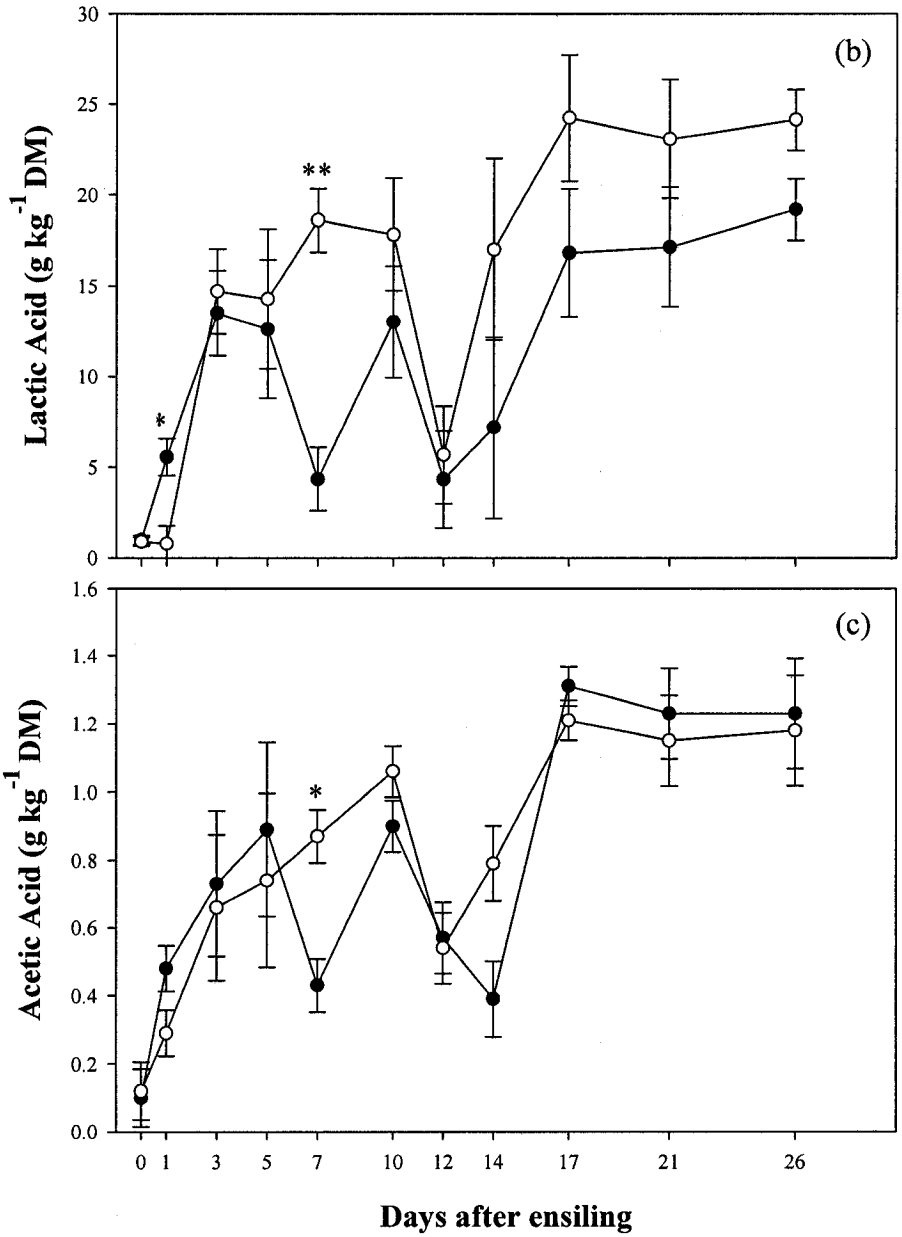


Figure 4-4: Changes in VFAs concentration of treated (○) and untreated (●) ensiled corn. Vertical bars represent ± SE; *, ** and *** represent significant differences ($P < 0.05$; 0.01 and 0.001, respectively).

4.2. Microbial population

4.2.1. Lactic acid bacteria

Counts of epiphytic LAB (d 0) were within the range (10^1 to 10^7 cfu g^{-1}) reported by Pahlow et al. (2003). Population of LAB increased ($P < 0.05$) between d 0 and d 12 post-ensiling with some fluctuations, from ~ 4 (d 0) to > 8 log cfu g^{-1} (d 12) (Figure 4-5), with no overall treatment effect (Table 4-2). The significant increase in LAB in early stages of the ensiling process is expected in any typical ensiling process during the anaerobic fermentation phase. The LAB population declined rapidly ($P < 0.05$) between d 14 and d 21 post-ensiling.

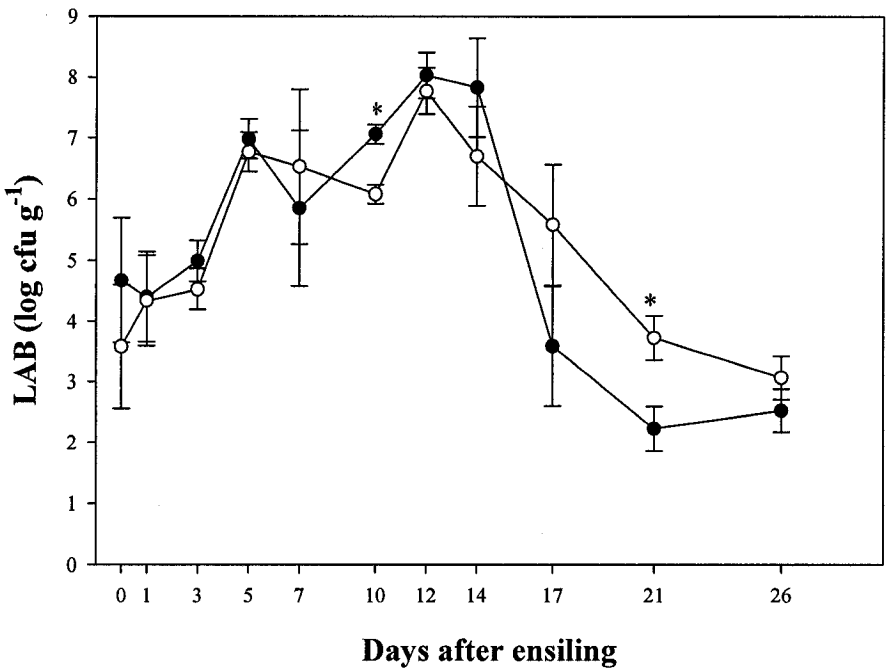


Figure 4-5: Changes in LAB population of treated (○) and untreated (●) ensiled corn. Vertical bars represent ± SE; * represent significant difference ($P < 0.05$).

After two weeks of ensiling, the ensiled forage was fermented. The pH values decreased below 4 and the LAB population decreased correspondingly. Lack of substrate and / or accumulation of organic acids could explain this decline. Lactic acid bacteria population (Figure 4-5) and pH profile (Figure 4-2) showed a typical inverse relation, as described by Roth and Undersander (1995).

On the daily basis comparison, untreated ensiled forage in d 10 had higher ($P < 0.05$) LAB counts, but on d 21 the treated one was significantly higher ($P < 0.05$). However, treated corn silage LAB counts tended to decrease at a slower rate and was characterised by numerically higher counts between d 14 and d 26 post-ensiling. These differences correspond to the higher lactic acid content during the same period (Figure 4-4b) as well as the overall treatment effect (Table 4-1). Sebastian et al. (1996) showed a numerically higher LAB counts for propionic acid treated corn silage, compared with the untreated one.

4.2.2. Fungi

Epiphytic fungi populations (d 0) were 3 to 4 fold lower compared with other studies (Sebastian et al., 1996; Kung et al., 1998; Ranjit et al., 2002). Low epiphytic micro-flora counts could be attributed to the cold weather in the field (i.e $< 15^{\circ}C$), which is usually characterised by lower microbial populations (Pahlow et al., 2003). Yeast population in untreated ensiled corn increased ($P < 0.05$) rapidly between d 5 and d 10 post-ensiling (Figure 4-6a). A similar increase was observed for mold population between d 1 and d 7 post-ensiling, with another sharp increase in d 14 post-ensiling (Figure 4-6b). Solution_Foin notably reduced both yeast and mold populations between d 5 and d 14 post-ensiling (Figures 4-6a and 4-6b). The inhibition of fungi growth by the buffered propionic acid-based additive is in agreement with other studies (Kung et al., 1998; Kung et al., 2000; Kung et al., 2004). The populations of yeast and mold declined sharply between d 14 and d 17 post-ensiling with no effect of the silage additive. Statistical analysis also found a strong overall inhibition effect ($P < 0.0001$) of both yeast and mold populations (Table 4-2).

Lambert and Stratford (1999) showed that inhibition of microbial growth by weak-acids involves rapid diffusion of undissociated molecules through the plasma membrane. The dissociation of these molecules within cells liberates protons, thus acidifying the cytoplasm and preventing fungal growth. Kung et al. (2003) concluded that the antifungal property of propionic acid increases as pH declines, which makes it an ideal preservative for cereal forages. Therefore, a

reasonable explanation for the lack of differences in fungal populations between d 0 to d 5 could be attributed to the fairly high pH during that period (Figure 4-2) and make the additive more active, in terms of undissociated:dissociated ratio. After d 14, the ensiled forage became more fermented, and the lower pH presumably inhibited the fungi growth in the untreated silage too.

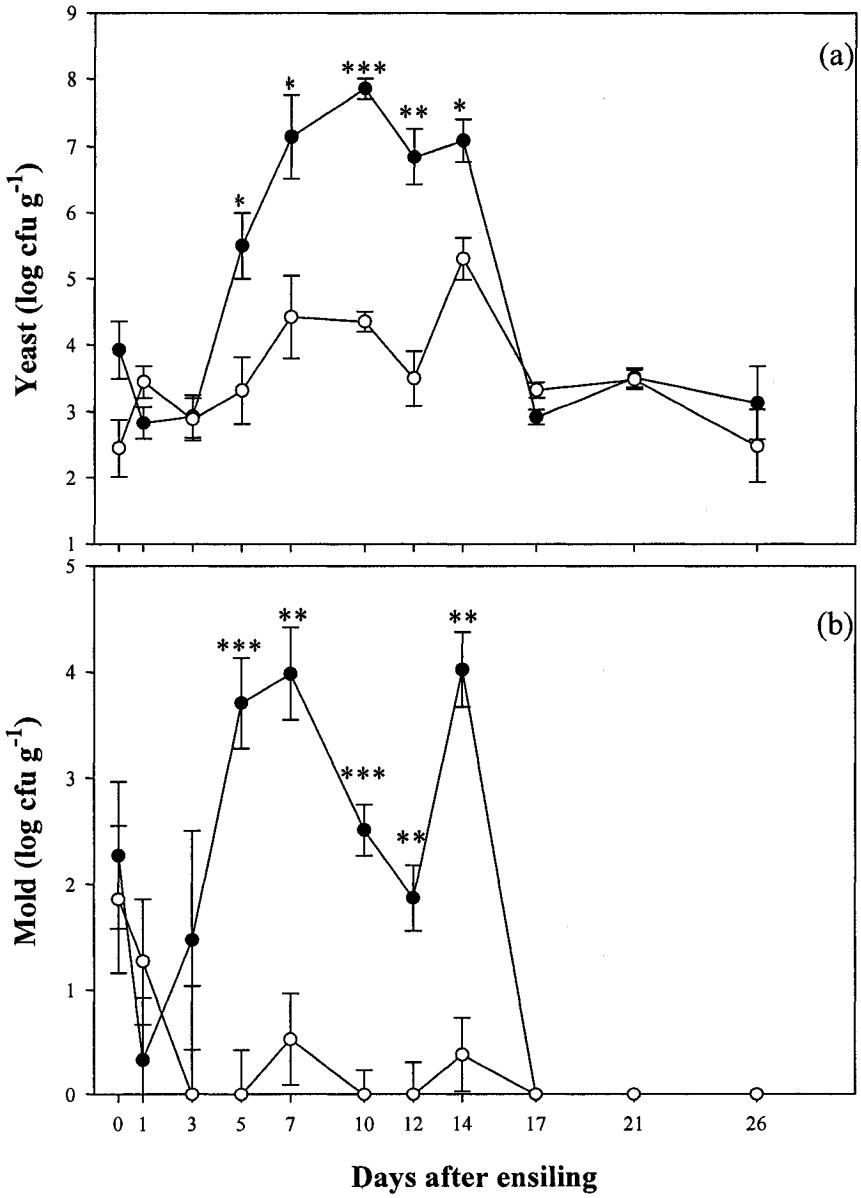


Figure 4-6: Changes in fungal population of treated (○) and untreated (●) ensiled forage. Vertical bars represent ± SE; *, ** and *** represent significant differences ($P < 0.05$; 0.01; and 0.001, respectively).

Table 4-2: Effects of Solution Foin on overall microbial population of ensiled corn

Microbial population	Untreated	Treated	SE
LAB (cfu g^{-1} DM)	5.29	5.33	0.19
Yeast (cfu g^{-1} DM)	4.88a	3.54b	0.10
Mold (cfu g^{-1} DM)	1.83a	0.37b	0.13
a-b Means within row followed by different letters are different ($P < 0.0001$).			

Among the molds that were found in the ensiled corn, four main species were isolated and identified as: *Diheterospora hyphomycetes*, *Penicillium hyphomycetes*, *Rhizomucor zygomycetes* and *Paecilomyces hyphomycetes*. The last two species are well-known for producing toxins, with health concerns to human and animals (Dannaoui et al., 2003; Lugauskas et al., 2005, respectively).

4.3. Aerobic Stability

Solution Foin improved ($P < 0.01$) aerobic stability of ensiled forage at d 0, 5 and 10 post-ensiling with the highest effect noted at d 0 (Figure 4-7). This is likely due to the high initial concentration of the propionic acid (Figure 4-4a) additive. On d 15 post-ensiling, treated ensiled corn was numerically more stable than untreated one, however, the silage additive had no effect on aerobic stability at d 20 post-ensiling.

After 20 d of ensiling, both treated and control silages remained stable for more than a week (Figure 4-7), most likely due to the same silage maturity progression, towards the stable phase, which characterized by anaerobic and low pH environment, which is hostile to most of the micro-organisms (Muck, 1996; Pahlow et al., 2003; Kunkle et al., 2006).

When the silo is opened for feeding, growth of aerobic microbes such as yeasts and molds takes place. Yeasts are considered the main micro-organisms responsible for the initiation of aerobic deterioration of silages (Moon, 1983;

Spoelstra et al., 1988; Woolford, 1990; Kung et al., 1998). The growth of molds follows that of yeasts, which is often reflected by the appearance of two thermal peaks which can be detected during aerobic deterioration (McDonald et al., 1991). The inhibitions of fungi population (Table 4-2) corresponding with the aerobic stability results, mainly during the first two weeks after ensiling.

The strong action of Solution Foin on aerobic stability during the first 10 d of ensiling, and in particular on d 0 ($P < 0.0001$), suggests that the additive could be added to prevent heating and spoiling of silage or total mixed rations (TMR) in the feed bunk. Kung et al. (1998) mentioned propionic acid products, which have been designed to be added to silages or TMR just prior to feeding, for that matter. These results are in agreement with previous studies (Kung et al., 1998; Kung et al., 2000) that found buffered propionic acid additives to be effective in inhibiting fungi growth and improving aerobic stability of corn silage.

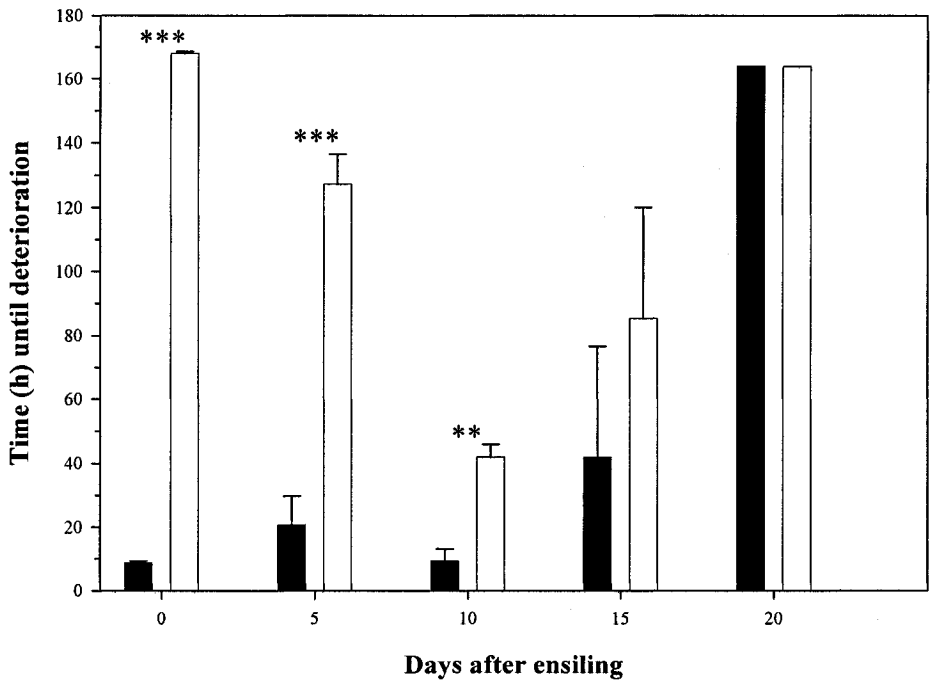


Figure 4-7: Changes in aerobic stability of treated (□) and untreated (■) ensiled corn. Deterioration is defined as the time until the aerated ensiled corn rises in 2°C above ambient temperature. Vertical bars represent ± SE;
** and *** represent significant differences ($P < 0.01$, and 0.001, respectively).

4.4. Dairy production study

4.4.1. Fibre analysis

As expected, the fibre analysis of the ration, given to dairy cattle during the study, showed no differences in the fibre (NDF, ADF and ADL) and crude protein content (Table 4-3).

Table 4-3: Ingredients and chemical composition of diets in the dairy cow trial

Diet ingredients (% of DM)	Untreated	Treated
Corn silage	33.1	33.1
Alfalfa hay	24.5	24.5
Crack corn	30.8	30.8
Commercial protein supplement	6.1	6.1
Soya bean meal	3.9	3.9
Mineral mix[b]	0.7	0.7
Sodium bicarbonate	0.9	0.9

Chemical composition (% of DM)	Untreated	Treated	SE
Dry matter	47.86	49.56	0.57
Crude protein	16.61	16.45	0.40
Natural detergent fibre	30.28	28.00	1.24
Acid detergent fibre	16.54	14.92	1.82
Acid detergent lignin	2.15	2.12	0.10

[a] Contained (%) 89 DM, 50 DF, 6 ADF, 10 NDF, 11 NFC, 4 FAT, 50 Protein, 10 UIP, 2.5 Ca, 1.5 P, 0.8 Mg, 0.3 Na, 0.5 K, 0.75 NaCl, 0.5 S; (Mcal kg^{-1}) 1.65 NEL, 1.9 NEM, 1.3 NEG; (mg kg^{-1}) 700 Fe, 370 Zn, 370 Mn, 125 Cu, 1.2 Co, 7.4 I, 1.9 SE; (kUI kg^{-1}) 39.5 vitamin A, 11.85 vitamin D, 0.16 vitamin E; -216 DCAB mEq kg^{-1}.

[b] Contained (%) 1 Na, 18 Ca, 9 P, 8.0 Mg, 1.2 S; (mg kg^{-1}) 144 I, 7,500 Fe, 2,400 Cu, 7,200 Mn, 7,200 Zn, 24 Co, 500 F; (kUI kg^{-1}) 750 vitamin A, 225 vitamin D, 3 vitamin E.

4.4.2. Dairy performance

The silage additive had no effect on feed intake of dairy cows (Table 4-4). This is in agreement with other studies, which showed no improvement in DM intake of lactating cows fed silage treated with propionic acid-based additives (Kung et al., 1998). Milk yield and milk composition were also similar for cows fed treated and untreated corn (table 4-4). This is in agreement with the same study, which showed no improvement in milk yield and milk composition of lactating cows fed silage treated with propionic acid-based additives (Kung et al., 1998). Broderick et al. (as cited in Kung et al., 2003) reported that high DM alfalfa silage treated with propionic acid had no effect on DM intake and milk yield of dairy cows.

Table 4-4: Effects of Solution Foin on dairy cows performance

Dairy values	Untreated	Treated	SE
Feed intake (kg d^{-1} DM)	22.8	23.4	0.9
Milk yield (kg d^{-1})	28.7	29.3	1.7
Milk fat (%)	3.13	3.39	0.17
Milk protein (%)	3.51	3.41	0.08
Milk lactose (%)	4.56	4.64	0.08
Milk urea (%)	10.32	11.05	0.66

5. General Discussion and Conclusions

To our knowledge, no other studies on the effects of additives on the short term ensiling of forages, are available in the literature. Upon aerobic exposure of silage, yeasts, molds and aerobic bacteria, present in dormant condition under anaerobic conditions, begin to flourish and respire residual WSC and organic acids (McDonald et al., 1991; Pahlow et al., 2003; Weinberg et al., 2001). This will result in aerobically deteriorated silage with high pH and temperature and significant DM losses (Middelhoven and Franzen, 1986), as well as possible production of mycotoxins. Therefore, the higher residual WSC and lactic acid content in the treated relative to untreated ensiled forage in the current study could be attributed to the anti-fungal properties of the silage additive. By restraining yeast and mold growth, more WSC will be available to be utilized by the LAB, which in turn can produce more lactic acid. Lower aerobic microbial populations also utilize less lactic acid, which is known as another source of energy for aerobic microbes (Kung and Ranjit, 2001).

As expected, our results found no effect of Solution Foin on the ensiled forage pH, after d 0. However, once the pH sufficiently declined few days post-ensiling, the propionic acid additive became more undissociated and hence more active in inhibiting fungal cells, by penetrating their membranes and releasing toxic ions (Lambert and Stratford, 1999). Once the ensiled forage is fully fermented, the anaerobic and acidic environment becomes hostile to most of the micro-organisms including fungi. Therefore, Solution Foin is expected to be most effective in inhibiting yeast and mold growth during the period where the pH is sufficiently low to allow for dissociation of propionic acid until the pH is too low for any fungal growth.

Aerobic instability of corn silage can be a major concern to farmers, particularly in warm weather, and is considered a common problem even with good silage management (Muck, 2004). Pitt et al. (1991) found that aerobic stability decreased as initial temperature increased from 10 to 40°C, due primarily to the increase in fungal growth rates with temperature. Therefore, the inhibition of the fungi population (i.e. less production of heat from microbial activity)

attained by the additive, could explain the result that the treated ensiled forage was colder than the untreated one.

Another aspect of silage temperature is the aerobic stability. Our results showed that Solution Foin substantially improved the aerobic stability of the ensiled corn during the early days of the ensiling process when the silage is not well fermented. This was consistent with our findings for the fungal and mold populations. The lack of differences in aerobic stability between untreated and treated ensiled corn at later phases of ensiling was also in agreement with the results obtained for the microbial populations.

Overall, the propionic acid-based additive was found to be effective in inhibiting yeast and mold growth for more than 10 days post-ensiling. In turn, lactic acid and residual WSC contents were considerably higher in the treated than untreated ensiled forage. In addition, in terms of temperature, both in the field and in the laboratory (aerobic stability), the treated ensiled corn forage was colder, and hence significantly more stable.

The addition of Solution Foin to ensiled corn had no effect on the performance of dairy cows. Limited and inconsistent data are available on the effects of propionic acid treated silage on animal performance. Despite improvement in aerobic stability of total mixed diets, propionic acid additive did not improve performance of dairy cows (Kung et al., 1998). These authors attributed the lack of improvement to the fact that untreated silage was not spoiled to an extent that would have affected feed intake. Producers have experienced drastic drops in milk production when cows are fed spoiled and hot feed (Kung et al., 2000). Hoffman and Ocker (1997) reported that cows fed spoiled high moisture corn produced 3.2 kg less milk than cows fed fresh feed during a 14 d period, and found inverse relationship (R^2=0.63) between mold population and milk yield. Lower feed value and higher fungal biomass in dry hay resulted in lower intake and reduced DM and fibre digestibility (Wittenberg, 1994). Weinberg et al. (2001) concluded that in a warm climate, special care should be taken during silage making and storage in order to avoid heating as much as possible. In addition, in a warm climate, silages are more susceptible to aerobic

deterioration. Thus, it is most likely that under poor conditions (i.e. warmer temperatures and / or poor management), the spoilage will be more momentous and hence the additive will most likely be more effective.

In conclusion, the propionic acid-based additive was found to be effective in inhibiting fungal growth and improving ensiled forage profile and stability. Therefore, Solution Foin can effectively be used as a silage additive, especially in case of silage shortage, which forces farmers to open the silo before the forage is fermented, while it is more susceptible to microbial deterioration. However, a better understanding of the degree of ensiled forage spoilage, necessary to reduce animal performance and rate of cattle growth, is needed, in order to prove the additive worthwhile economically.

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