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Effects of silage inoculants on silage fermentation, aerobic stability and animal performance

by

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DEDICATION

This thesis is dedicated to my beloved wife, Ernestina Addah and to my children, Kaweh Anita and Atungdeweh Eleazer for their selflessness that allowed me to stay away from home for all these years.

Abstract

Ferulic acid constitutes a major constraint to ruminal fibre digestibility. The overall objective of this study was therefore to determine the effects of a first (non-fibrolytic) - or a third (ferulic acid esterase-producing) - generation inoculant on the fermentation characteristics, aerobic stability and nutritional value of silages. In experiment 1, barley and corn silages were inoculated with a first-generation inoculant containing *Lactobacillus plantarum*, *Enterococcus faecium* and *Pediococcus acidilactici* in a 2 × 2 factorial design. Inoculation induced a more homolactic fermentation in barley than in corn silage but did not improve aerobic stability, DM intake, *in situ* digestibility or growth performance of growing feedlot steers. Aerobic stability of barley silage, and DM intake and growth performance of steers fed barley silage were improved as compared to corn silage. In experiments 2 and 3, barley silage was inoculated with a third-generation inoculant containing ferulic acid esterase-producing *Lactobacillus buchneri* in combination with *Lactobacillus plantarum* and *Lactobacillus casei*. The inoculated silages had higher concentrations of acetic acid and were more aerobically stable than uninoculated silage. Inoculation increased *in situ* fibre digestibility (experiment 2) and feed efficiency for growing feedlot steers (experiment 3). In the final experiment (experiment 4), barley silage was chopped to a theoretical chop length (TLC) of approximately 1.0 (SC) or 2.0 cm (LC) and inoculated without or with the same inoculant used in experiments 2 and 3 in a 2 × 2 factorial design. Inoculation increased the concentration of acetic acid in the LC silage and improved its aerobic stability, but decreased the concentration of

acetic acid and had no effect on the aerobic stability SC silage. Growth performance of finishing feedlot steers were neither affected by TLC, inoculation nor their interactions, however, the proportion of saleable meat and rib eye area of steers was greater for SC than for LC. In conclusion, this study demonstrated that third-generation inoculants could be used to improve the nutritional value of barley silage. Third-generation inoculants could also allow TLC to be increased from 1 to 2 cm without adverse effects on silage fermentation or quality.

Key words: Barley, beef steers, corn, silage inoculant, digestibility, esterase-producing inoculant.

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List of Abbreviations

ADF	Acid detergent fibre
ADIN	Acid detergent insoluble nitrogen
CFU	Colony–forming units
DM	Dry matter
DMD	Dry matter disappearance
FAE	Ferulic acid esterase activity
FC	Efficiency of silage fermentation
IN	Inoculated silage
IR	Infrared
LAB	Lactic acid producing bacteria
MRS	deMan–Rogosa–Sharpe
NA	Nutrient agar
ND	Not detected
NDF	Neutral detergent fibre assayed with heat–stable amylase and expressed inclusive of residual ash
NDFD	Neutral detergent fibre disappearance
NGR	Non–glucogenic ratio
peNDF	Pysical effective NDF
SDA	Sabouraud's dextrose agar
TMR	Total mix ration
TB	Total culturable bacteria
UN	Uninoculated silage
VFA	Volatile fatty acid
WSC	Water–soluble carbohydrates (glucose equivalent)

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CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.0. GENERAL INTRODUCTION

Silage is a product formed when forage or other material of sufficiently high moisture content liable to spoilage by aerobic microorganisms is stored anaerobically (Woolford 1984). Ensiling aims to preserve forage nutrients and the technologies associated with this practice have improved considerably over the years. More often than not, however, the natural process of silage fermentation proceeds uncontrollably and production of quality silage cannot be fully guaranteed (Woolford 1984; Merry and Davies 1999; Kung and Ranjit 2001). This is largely due to variations in the populations and efficacies of epiphytic lactic acid bacteria (Bolsen et al. 1992; Lin et al. 1992a), chemical composition of the forage being ensiled and the ensiling environment (Garcia et al. 1989; McAllister and Hristov 2000; Hargreaves et al. 2009).

The addition of silage inoculants to freshly harvested forage offers a means of increasing the predictability of the ensiling process and increases the chances of obtaining good quality silage. First-generation silage inoculants contained homolactic lactic acid-producing bacteria (LAB), mainly *Lactobacillus plantarum* and were developed to accelerate the decline in silage pH as a result of increased production of lactic acid. The lower pH attained inhibited the growth of clostridia and enterobacteria thereby reducing dry matter (DM) and nutrient losses during fermentation (McDonald 1981; Woolford 1984; Muck 1988). However, the high amount of lactic acid produced by these inoculants served as a substrate for lactic acid assimilating yeasts upon exposure of the silage to air during feed-out (Woolford 1990; Inglis et al. 1999). A second-generation of silage inoculants that included *Propionibacteria* spp. and *Lactobacillus buchneri* were therefore subsequently developed. *Lactobacillus buchneri* possesses a heterolactic pathway of anaerobic fermentation capable of degrading lactic acid accumulated during the initial primary fermentation, into acetic acid which extends the aerobic stability of the silage by inhibiting growth of yeasts and moulds (Driehuis et al. 1999; Oude Elferink et al. 2001; Reich and Kung 2010).

However, the rapid decline in silage pH that is characteristic of first-generation silage inoculants and the increased production of acetic acid associated with second-generation inoculants did not address one of the main constraints to forage utilization in ruminants; poor digestibility of forage fibre. In a review of studies (1989-2009; n = 38) on the effects of first-generation inoculants on silage nutrient digestibility, inoculation had no effect on neutral detergent fibre (NDF) digestibility and growth performance in 63% and 80%, respectively, of the studies examined (Adesogan et al. 2009). Studies on the effects of second-generation inoculants on digestibility showed that NDF digestibility was not affected across a range of silages (Filya 2003; Rizk et al. 2005; Zhang et al. 2009). This was because these inoculants lacked any proven fibrolytic activity during ensiling that could potentially improve ruminal fibre digestibility (McAllister et al. 1998; Filya 2003; Adesogan et al. 2009).

Silage utilization by ruminants can be constrained by poor fibre digestibility resulting in a lower digestibility of NDF and ADF. Ferulic acid (FA) is one of the most abundant hydroxycinnamic acids in cell walls of cereal crops that play a significant role in inhibiting fibre digestion in ruminants (Yu et al. 2005; Hatfield et al. 2010) through inhibiting the attachment (Akin et al. 1988) and growth (Varel and Jung 1986) of major fibrolytic ruminal bacteria. Arabinoxylans and glucuronoarabinoxylans, the major digestible components of forage fibre may be ester-linked to FA or cross-linked to each other by other hydroxycinnamic acids. Ferulic acid also forms ether linkages with lignin, further reducing fibre digestibility. Hydrolysis of these ester linkages would therefore be expected to increase ruminal fibre degradation, providing energy and improving animal performance. The use of third-generation silage inoculants with proven FA esterase (FAE) activity has previously been shown to improve the fibre digestibility of corn and ryegrass silages (Nsereko et al. 2008), but the response in corn appears to be hybrid-dependent (Kang et al. 2009).

Treatment of forages with exogenous FAE enzyme has been shown to hydrolyze feroulylated polysaccharides and increase the release of water-soluble carbohydrates (WSC; Krueger et al. 2008). The use of a third-generation inoculant

with FAE could increase the solubility of fibre during ensiling while at the same time increasing the availability of WSC for microbial fermentation. Improvements in fermentation through inoculation with a third-generation silage inoculant could potentially allow the chop length of forage to be increased to take advantage of the benefits of physically effective fibre without compromising fermentation and digestibility.

1.1. LITERATURE REVIEW

1.1.1. The Ensiling Process

The process of silage fermentation is achieved under suitable anaerobic conditions by lactic acid-producing bacteria (LAB) that convert plant sugars into organic acids. These end-products of fermentation then inhibit the growth of spoilage microorganisms thereby preserving the silage. Even though the achievement of suitable anaerobic conditions in the silo is usually practically challenging, the objective of ensiling is to maintain the nutritional value of the ensiled product vis-à-vis that of the original forage prior to being ensiled (Muck 1988; Charmley 2001). Two major pathways of fermentation may occur during ensiling; homolactic fermentation in which glucose or fructose is reduced to two moles of lactic acid in the presence of an aldose enzyme (Embden-Meyerhof glycolytic pathway) and heterolactic fermentation in which glucose or fructose is reduced to equimolar amounts of lactic acid, acetic acid, carbon dioxide and ethanol in the presence of a phosphoketolase enzyme (Table 1.1.; McDonald 1981; Woolford 1984). Both homo- and hetero-lactic bacteria have flexible metabolic pathways and are also capable of fermenting pentose (xylose and arabinose) sugars into mainly lactic and acetic acids via the pentose-phosphate pathway (Table 1.1.; McDonald 1981; Woolford 1984). Genetically modified *Lactococcus lactis* (Shinkawa et al. 2011) and *L. plantarum* (Okano et al. 2009) have both been engineered to produce greater amounts of lactic acid and only trace amounts of acetic acid from xylose and arabinose respectively, by blocking the phophoketolase pathway and enhancing the pentose-phosphate pathway. These

strains present an opportunity to improve the ensiling of forages that are lower in hexose sugars such as mature tropical grasses.

Ensiling is a natural microbially driven process and it is possible for undesirable biochemical transformations to occur such as deamination, decarboxylation and oxidation/reduction of amino acids by clostridia and enterobacteria (McDonald 1981; Table 1.1.).

Table 1.1. Fermentation pathways and estimated DM and gross energy (GE) losses from various substrates (modified from McDonald 1981)

Microbial Substrate	Type of fermentation	Moles of end-products formed	ATP[z]	DM loss (%)	Energy loss (%)
Sugars					
Desirable fermentation by Lactic acid bacteria					
Glucose	Homolactic	2 lactic acid	2	0	0.7
Fructose	Homolactic	2 lactic acid	2	0	0.7
Glucose	Heterolactic	Lactic acid, ethanol and CO_2	1	24	1.7
Fructose (3 moles)	Heterolactic	Lactic acid, acetic acid, 2 mannitol and CO_2	2	4.8	1.0
Undesirable fermentation by yeasts and clostridia					
Glucose	Yeasts	2 ethanol and 2 CO_2	2	48.9	0.2
Glucose	Clostridial	butyric acid, 2 CO_2 and $2H_2$	3	–	–
Organic acids					
Desirable fermentation by lactic acid bacteria					
Lactic acid (2 moles)	Heterolactic[y]	0.5 acetic acid, 0.04 ethanol, 0.48 1,2 propionediol and 0.5 CO_2	1	1.5-7.1	–
Undesirable fermentation by lactic acid bacteria					
Citric acid (2 moles)	Heterolactic	Lactic acid, 3 acetic acid, and CO_2	1	29.7	+1.5
Malic acid	Homolactic	Lactic acid, CO_2	nil	32.8	+1.8
Lactic acid (2 moles)	Clostridial	Butyric acid, 2 CO_2 and $2H_2$	1	51.1	18.4
Amino acids					
Undesirable fermentation by Clostridia					
Arginine and lysine	Deamination	Ammonia, butyric acid and acetic acid	–	–	–
Lysine, arginine and tryptophan	Decarboxylation	Amines (e.g. putrescine, histamine, tryptamine) and CO_2	–	–	–
Alanine, glycine and leucine	Oxidation/ reduction	Ammonia, acetic acid and CO_2	1	–	–

[z]Moles of ATP produced for microbial growth

[y]Fermentation by *L. buchneri* (Driehuis et al. 1999; Oude Elferink et al. 2001; Ranjit et al. 2002).

Several factors can affect the fermentation process. For example the characteristics of the forage at harvest, the predominant LAB and management practices such as the design of the silo and the rate at which the silo is being filled can affect the pathway of fermentation and ultimately influence the outcome of the ensiling process (McAllister and Hristov 2000). Other biological processes that are likely to adversely affect the ensiling process include plant respiration and enzyme activity, and clostridial and yeast fermentation (Muck 1988). The biochemical changes that occur at each stage of ensiling are a reflection of the ecology of the predominant microflora participating in the fermentation process (Woolford 1984). The process of silage fermentation can be divided into four major phases based on the distinct biochemical activities occurring at each stage of the ensiling process (McAllister and Hristov 2000).

1.1.1.1. Phase I: The Aerobic Phase

The aerobic phase can be subdivided into two periods. The first is the period immediately after the plant is harvested and as the silo is being filled with the plant material (aerobic). The second is the period of heterolactic fermentation by facultative anaerobic LAB as trapped oxygen is gradually depleted.

The first period of phase I is characterized by plant and microbial enzyme activities. Fresh forage introduced into the silo has a pH (6.0-6.8) that is suitable for a host of plant enzymatic activities. Two main types of enzymatic activities occur in the forage material during this period; the enzyme activity during plant respiration and anaerobic plant enzymatic hydrolysis of structural carbohydrates to monosaccharides independent of plant respiration (Muck 1988; Charmley and Veira 1991). The later process may also include protease activity that converts protein nitrogen into nonprotein nitrogen (Muck 1988). The first period of phase I often lasts approximately 48 h and the main end products include water, CO_2 and heat with temperatures and pH usually being 18.2-22.0^{o}C and 6.0-6.5, respectively (McCullough 1984).

During the first period of phase I, oxygen present within and between fresh plant materials is consumed by aerobic and facultative microorganisms as

well as the plant itself through respiration. If the silo is rapidly filled and packed with the fresh forage, the available oxygen is quickly utilized and depleted with minimal loss of WSC resulting in the production of less water and heat in the silage mass. It is desirable that the duration of this period is short as possible to prevent the loss of WSC through respiration which can be conserved or used by anaerobic LAB during the active fermentation phase. There is usually an unavoidable initial loss of DM due to aerobic microbial activities that consume trapped oxygen, but these losses are estimated to be small and range from 0.12% for a high-quality silage to 3.9% for a low-quality silage (Muck 1988). This phase is important because it eliminates oxygen from the silo, creating an anaerobic environment for silage fermentation to proceed, thereby contributing to a rapid pH decline, conservation of DM and an avoidance of heating which leads to the formation of Maillard's products and a reduction in the digestibility of silage (Muck 1996; 1988; Chen and Weinberg 2009). The predominant microorganisms in naturally fermented silages at this period include fungi and enterobacteria (Weinberg and Muck 1996; Lin et al. 1992b).

Management practices that can help to reduce the length of this period are those that aid in quick exclusion of air from the silo. These practices include harvesting the forage at optimum DM content and chopping it to an optimum and uniform length that will increase packing density and avoid the occurrence of air pockets in the silo. Slow filling and sealing of the silo delays the breakdown and release of plant cell contents by plasmolysis thereby delaying the release of WSC, the formation of lactic acid and the rapid decline in pH (McDonald 1981; Muck 1988; Charmley 2001). Clostridial fermentation of leads to higher pH and temperatures (Woolford 1984; Muck 1988), and greater concentrations of acid detergent fibre (ADF) and acid detergent insoluble nitrogen (ADIN, Ruppel et al. 1995) in the silage through the utilization of soluble components of fibre. Extensive clostridial fermentation of amino acids may also lead to the formation of higher concentrations of toxic products such as biogenic amines (Table 1.1.). Prolonged exposure of barley forage to air before ensiling increased the population of yeasts on forage by more than 10^3 CFU g^{-1}, decreased WSC

concentration by more than 50%, increased ammonia-N concentration by 40%, and increased pH by more than 1 unit (Mills and Kung 2002). This subsequently resulted in silages with 45% less lactic acid and higher ADF and NDF contents compared to those ensiled immediately after harvesting and processing.

Good harvesting and silo filling techniques in phase I will help to minimize WSC losses and in turn spare more WSC for lactic acid fermentation in phase II (Oude Elferink et al. 2000). The second period of phase I represents the time when oxygen is almost depleted within the silo. During this time, the microbial ecology consists predominantly of facultative bacteria (enterobacteria). Enterobacteria produce acetic, propionic and lactic acids, ethanol and CO_2 from the fermentation of hexoses and pentoses (McDonald 1981; Woolford 1984). The activities of these thermo-tolerant bacteria further increase the temperature of the silage. Even though the predominant bacteria during this period are inefficient fermenters, the acids they produce initiate the drop in pH of the silage to ~5.0. This decline subsequently inhibits the growth of these organisms and creates a suitable environment for the growth of the more acid-tolerant LAB. Hence the final stages of this period represent the time when the population of LAB begin to multiply rapidly, a process that occurs within 24-72 h after ensiling (McCullough 1984). In a study of the microbial succession during ensiling, enterococci represented about 71% of all microorganisms enumerated in chopped forages prior to ensiling. This population decreased and disappeared within 24-72 h of ensiling while *L. plantarum* which represented only 43% of the microbial population prior to ensiling, accounted for 92% of the population after 24-72 h of ensiling (Lin et al. 1992b). The growth of the more acid-tolerant *L. plantarum* during this period of ensiling also often corresponds with the death of enterococci as pH declines to < 4.5 (Cai 1999).

Management practices that can be employed to reduce the length of this phase include treatment of forages with silage inoculants that contain bacterial strains tolerant to air, higher temperatures and pH. For example *Enterococcus faecium, Pediococcus acidilactici* and *Lactococcocus lactis* are fast growing strains of homolactic LAB that predominate the fermentation process at a higher

pH and temperature (Fitzsimons et al. 1992; McAllister et al. 1998; Cai 1999). Inoculation of silages with these bacteria can therefore reduce the length of phase I of ensiling and accelerate the rate of pH decline to a level that allows acid-tolerant LAB to predominate at a lower pH. When it is impractical to immediately ensile or seal chopped forages such as in a large commercial bunker silo, treatment of forages with additives that inhibit the growth of yeasts and moulds prior to delivery to and sealing of the silo could minimize spoilage during ensiling, and upon silage feed-out. Prolonged aerobic exposure of forages prior to ensiling increases ammonia N and butyric acid concentrations, and lowers *in situ* DM digestibility. Direct treatment of the silage with buffered priopionic acid can inhibit spoilage microorganisms at this initial stage of ensling (Mills and Kung 2002).

1.1.1.2. Phase II: The Active Fermentation Phase

The first period of phase II is a short-lived transitional phase that lasts about 24-48 h after ensiling (Seglar 2003). As the pH is gradually lowered, the populations of enterobacteria and facultative heterofermentative LAB are replaced by strictly obligate homofermentative LAB. This phase is characterized by the production of large amounts of lactic acid and the predominant microflora is an efficient group of homofermentative LAB. The population of the more efficient LAB multiplies rapidly and the production of lactic acid further decreases silage pH. A rapid decline in pH during this period is critical if high quality silage is to be produced. Insufficient production of lactic acid at this time, will create conditions favourable for opportunistic clostridia to multiply and cause saccharolytic and proteolytic deterioration of the silage (Woolford 1984; Muck 1988).

While the rapid decline in pH due to accumulation of lactic acid is often considered as the main reason for the disappearance of the less acid-tolerant colonizers such as enterobacteria, staphylococci, streptococci and leuconostocs, the production of unknown inhibitory substances other than organic acids has also been proposed to contribute to the microbial succession often observed between phase I and phase II of ensiling (Woolford 1984; Marciňáková et al. 2008). The

lower pH attained at this stage of fermentation does not only help to preserve nutrients but also plays a significant role in increasing the availability of soluble carbohydrates from complex carbohydrates for further microbial fermentation. This process is described as acidic hydrolysis. Acidic hydrolysis is a slow chemical process that involves the interaction of hydrogen ions and fibre fractions when pH is very low (Muck 1996). It has been found to have significant impact on cellulose, hemicellulose and pectin degradation during ensiling thereby contributing to the amount of WSC available for microbial fermentation (Keady et al. 1994; Rotz and Muck 1994; Muck 1996; Charmley 2001).

In silage ensiled without inoculants, *L. plantatrum* may constitute up to 100% of the population of LAB during phase II (Lin et al. 1992b). Only a few acid-tolerant proteases and carbohydrases, and some specialized heterolactic LAB, such as *L. buchneri* or *L. brevis* are metabolically active during this period (Oude Elferink et al. 2000; Kung et al. 2003). This phase is the longest of the ensiling process and depending on the characteristics of the forage being ensiled, could last up to 10-21 d in forage ensiled without inoculants a duration that can be reduced to 3-10 d with inoculation (Seglar 2003).

1.1.1.3. Phase III: The Stable Phase

At this time, the silage is stable and no significant biochemical changes in its composition are expected if the anaerobicity is maintained. The pH attained at this phase depends on the type of forage ensiled and the microbial composition of the silage inoculant. Addition of heterolactic inoculants will almost always result in a relatively higher pH compared to homolactic inoculants (Driehuis et al. 2001; Nishino 2004). Also, legumes and grasses will produce silage with a higher pH than cereals, because of a higher buffering capacity and lower WSC in the former. Among cereal silages, those with relatively higher protein content such as barley also tend to have higher pH and hence higher buffering capacity than those with a lower protein content such as corn (McDonald 1981; Woolford 1984).

In silages treated with *L. buchneri* at ensiling, the last stage of this phase involves the degradation of accumulated lactic acid into acetic acid, 1,2-

propanediol, and ethanol (Driehuis et al. 1999; 2001; Oude Elferink et al. 2001). Some strains of *L. plantarum* are also capable of degrading lactic acid to formic acid (Lindgren et al. 1990). Fermentation of citric and malic acids into acetic acid, ethanol and formic acid by *Enterococcus faecium* and *E. faecalis* under anaerobic conditions may also occur at this time (McDonald 1981; Woolford 1984). Such secondary anaerobic fermentation account for the shifts in microbial populations (Woolford 1984) and the slight rise in pH occasionally observed in silages that have already reached stability (Lin et al. 1992b; Driehuis et al. 2001). Degradation of fermentation acids during this time depends on the type of LAB present in the silage and their tolerance to low pH (Oude Elferink et al. 2001). Studies on microbial succession have shown a shift from 85% homoferementative bacteria at 4 d of ensiling to 75% *L. brevis* and 97% *L. buchneri* after 142 d of ensiling, both of which are heterofementative (Woolford 1984). Similar studies with *L. buchneri* documented significant changes in the biochemistry of silage after the silage had obtained terminal pH, as this bacterium becomes metabolically active when accumulation of lactic acid is highest and pH the lowest (Driehuis et al. 1999; 2001; Oude Elferink et al. 2001).

1.1.1.4. Phase IV: The Feed-out Phase

The feed-out phase is the phase when the silage is exposed to air during opening of the silo and feeding. It is the phase characterized by re-activation of spoilage organisms and the decomposition of the silage. The biochemistry of silage deterioration involves the metabolism of WSC and/or lactic acid into carbon dioxide, water and ethanol leading to a loss of feedable nutrients (Woolford, 1990; McDonald, 1981). The predominant microorganisms during aerobic deterioration of silage include yeasts, moulds and other bacteria such as bacilli and enterobacteria (Woolford 1990; Inglis et al 1999; Muck and Pitt 1994). In an experiment to determine the origin of spoilage organisms in aerobically deteriorating silage, silage exposed to sterilized air still supported the growth of yeasts and moulds, suggesting that these microorganisms were inherent in the silage itself and survived the ensiling process (McDonald 1981). This implies that

the initial population of epiphytic spoilage organisms may subsequently influence the stability of the silage when it is exposed to air during feed-out. Of all the losses associated with ensiling, those that occur as a result of aerobic deterioration constitute the greatest percentage, accounting for 10-30% of silage DM (McDonald 1981; Woolford 1990).

The principal factors affecting aerobic deterioration of silage are the population and type of yeasts present in the silage, the concentration of residual WSC and/or lactate that serve as readily available substrates for spoilage microorganisms and the concentration of inhibitory organic acids such as acetic, propionic and butyric acids (McAllister et al. 1995; Woolford 1990). In cereal silages, yeasts are the initiators of silage deterioration (Lindgren et al. 1985; Inglis et al. 1999; Woolford 1990) with 10^5 CFU g^{-1} DM being being the threshold for spoilage (Woolford 1990). Muck and Pitt (1994) predicted that the threshold population of yeasts for initiation of temperature increase in aerobically exposed silage was 3×10^8 CFU g^{-1}. Yeasts isolated from deteriorating silages can be grouped into lactic acid- and WSC- utilizers and the susceptibility of silages to deterioration upon exposure depends on the nature of the yeasts present (Woolford 1990). Unlike the activities of WSC-degrading yeast (e.g. *Torulopsis*) which often dominate the microflora of aerobically exposed low-pH silages (Ashbell et al. 2002), the degradation of lactic acid by lactic acid-utilizing yeasts (e.g. *Candida*), is usually accompanied by an accelerated rise in pH and a loss of stability (Woolford 1990). Lactic acid-degrading yeasts therefore dictate the pace of aerobic silage deterioration especially in silages with low WSC concentration (Woolford 1990; Inglis et al. 1999). In other studies with corn silage, enterobacteria were found to be the initiators of aerobic deterioration in silage collected from the the silo face (Muck and Pitt, 1994). Deterioration by enterobacteria occurs only when yeasts populations are overwhelmed by the enterobacteria population (Driehuis et al. 1996).

The loss of DM during aerobic deterioration of silages represents a loss of potentially digestible nutrients. The metabolism of WSC and lactic acid into CO_2, water and ethanol by aerobic yeasts results in higher pH accompanied by the

evolution of heat (McDonald 1981). The higher pH and depleted concentrations of WSC and lactic acid stimulates the growth of acetic acid bacteria which is then followed by bacilli and moulds that are capable of utilizing more complex sugars (Lindgren et al. 1985; Muck and Pitt 1994; Woolford 1990). The depletion of these potentially digestible nutrients during feed-out has been found to decrease the nutritive value of silage by as much as 16% compared to its value at time of opening of the silo (Tabacco et al. 2011b). Exposing wheat silages to air for 7 d increased silage temperature and CO_2 concentration, and reduced DM and NDF digestibility of the silage by 14% and 7%, respectively (Chen and Weinberg 2009). Pathogenic bacteria such as *Listeria* may also multiply following this succession (Woolford 1990).

Even though gradual depletion of residual WSC and fermentation acids in silage undergoing deterioration may account for the replacement of yeasts by bacilli and moulds owing to their ability to utilize complex carbohydrates, higher temperatures (>40^{o}C) around this time may also reduce yeasts (Lindgren et al. 1985). Surprisingly, treatment of both wheat and sorghum silages with exogenous yeasts at ensiling failed to enhance deterioration upon aerobic exposure, possibly due to acetic acid inhibiting yeasts (Weinberg et al. 1999).

Cereals have higher WSC and lower protein content than legumes implying readily available substrates for lactic acid production. Unlike cereal silages, legume silages are therefore more resistant to aerobic deterioration because they have less residual WSC and lactic acid available for yeasts upon exposure of the silage to air (Ruppel et al. 1995; Adesogan and Salawu 2004).

Management strategies to reduce DM loss upon exposure to air include achieving an optimal packing density earlier during silo packing to reduce air penetration and subsequently air pockets in the silage, a good management of the feed- out face such as keeping the silo face even and reducing the amount of loose silage at the base of the feed-out face (Ruppel et al. 1995). Direct addition of chemical additives such as acetic acid or propionic acid can also inhibit yeasts and improve aerobic stability. Finally, inoculation of silage with heterolactic LAB such as *L. buchneri* (Driehuis et al. 1996; Muck 2004; Reich and Kung 2010) and

some selected strains of homolactic bacteria with bacteriocin-producing capabilities such as *Enterococcus faecium* (Marciňáková et al. 2008) have been shown to improve the aerobic stability of silage. Marciňáková et al. (2008) found that treatment of grass silage with a bacteriocin-producing *E. faecium* EF9296, reduced populations of *Escherichia coli*, enterobacteria, staphylococci and bacilli-like bacteria within the first 7-14 d of ensiling.

1.1.1.4.1. Methods of Assessing Aerobic Stability of Silage

Conventionally, aerobic stability has been defined as the number of hours that the temperature of a silage exposed to air, remains $1^{o}C$ (Driehuis et al. 1999) to $2^{o}C$ (Kung et al. 2004) below ambient temperature. However, the suitability of this method for assessing aerobic stability of silages stored in farm silos has recently been questioned by Borreani and Tabacco (2010) given the fluctuation of ambient temperature. These reseachers proposed using the core temperature of the silage, 20 cm from the surface as a reference temperature.

A recent technology that could be explored for assessing the aerobic stability of silages is the use of thermal imaging analysis. Thermal imaging is a technique used to convert the invisible radiation pattern of an object into visible images or thermograms that show the thermal distribution over the surface of a body (Vadivambal and Jayas 2011). It allows temperature mapping of any particular region of interest to be obtained quickly (50-60 images per s; Gowen et al. 2010) in real-time. Aquistion of data in this manner is not possible with thermocouples or other temperature sensors which can only record temperature at a single site (Vadivambal and Jayas 2011). Single-dimension virtual thermograms constructed with spot-measurements of temperature using thermocouples (Borreani and Tabacco 2010) are limited as compared to two-dimensional thermograms indicating the temperature profile of an entire region of the silo face. Temperature measurements taken from several locations within the face of a silo have been used to construct virtual thermograms to document temperatures over the face after aerobic exposure (Borreani and Tabacco 2010). Even though this method offers an opportunity to estimate heat distribution, it is practically

impossible to directly measure the temperature distribution over the entire surface face. Consequently, temperature at regions of the face that have not been measured directly with thermometers is estimated through interpolation. However, the use of thermal cameras offers an opportunity to directly measure heat distribution over the entire face of the silo in a two-dimensional manner.

Thermal imaging has been used to monitor the quality of agricultural products such as meat, fruits and vegetables (Gowen et al. 2010), and in grading wheat (Manickavasagan et al. 2010). It also has been used to detect spoilage in grain silos (Manickavasagan et al. 2006). The advantages of thermal imaging include: 1) quick visual appraisal of heat distribution in the silage in real-time 2) temperature measurements are not based on voltage-to-temperature relationship which are potentially non-linear (Benington et al. 1996) 3), non-contact measurement of silage temperature reduces the possibility of cross-contamination that may occur during sampling in conventional approaches used to assess aerobic stability and 4) application of the technology may also reduce costs associated with personnel and chemical reagents used for conventional assessment of aerobic stability of silage.

Table 1.2. Major phases in natural silage fermentation

Major phase	Period	Biochemistry/end-product	Temperature (^oC)	pH	Microorganism	Management practices
Aerobic (0-3 d)	I	Cell respiration and enzymatic hydrolysis	20.6	6.0-6.5	Moulds, yeasts	Rapid silo filling, optimum and uniform packing density
	II	Acetic acid, lactic acid, ethanol, CO_2, heat and water	32.2	5.0	LAB and enterobacteria	1. Inoculation with fast growing LAB, 2. Avoid air infiltration
Active Fermentation (10-21 d)	III	Lactic acid	28.9	5.0-4.0	LAB	Avoid air infiltration
	IV	Lactic acid	28.9	4.0	LAB	Avoid air infiltration
Stable (after 21 d)	V	Anaerobic fermentation of organic acids into acetic acid[z]	28.9	4.0	LAB (*L. buchneri* and *L. brevis*)	Avoid air infiltration
Feed-out (Duration depends on feed-out rate)	VI	Aerobic decomposition. Products include butyric acid, CO_2, heat and water	28.9	7.0	Moulds, yeasts	1. Silage inoculation 2. Good silo face management

[z]Fermentation of lactic acid is into acetic acid, 1,2 propionediol and carbon dioxide by *L. buchneri.* Modified from McCullough (1984).

LAB, lactic acid-producing bacteria

1.1.2. Factors Affecting Silage Quality

1.1.2.1. Impact of Forage Type on the Ensiling Process

The dynamics of natural silage fermentation is dependent on the interactions among the characteristics of the forage being ensiled, microbial population and the ensiling environment (McAllister and Hristov 2000). The chemical characteristics of the forage that influence its ensilability include DM and chemical composition such as WSC and protein, and the epiphytic microbial composition. The changes observed in silage compared to the original forage therefore emanate mainly from the fermentation of plant carbohydrates and proteins, and organic acids such as malic and citric acids (Woolford 1984; McDonald 1981; Muck 1988). Microbial factors include the relative populations of desirable (LAB) and undesirable (yeasts and moulds, enterobacteria, clostridia) microorganisms. Successful ensiling is dependent on achieving anaerobic conditions in the silo.

1.1.2.2. Dry Matter and Chemical Composition

The constituenets of dry matter of a forage is influenced by species, variety, stage of growth (maturity), agronomic practices and environmental conditions. It is however, often difficult to conclusively isolate the effects of environment such as temperature from biological factors such as maturity as the environment obviously plays a role in plant maturation. In temperate regions, temperature rises progressively with the growing season. At lower temperatures, the concentration of storage carbohydrates in the leaf increase, while with increasing temperatures cellulose, lignin and silica concentrations increase and hemicellulose decreases (Nelson and Moser 1994).

Stressors such as frost, drought and excessively high temperature may also affect the maturation of forage and influence its DM concentration through effects on photosynthesis and partitioning of photosynthates (Nelson and Moser 1994). Maturity of forage for silage making should therefore be based on physiological characteristics of the plant rather than calendar date or time after seeding (McAllister and Hristov 2000). Nonetheless in non-stressed forages,

maturity is linearly related to DM content and the optimum DM for harvesting the crop is often a trade-off between biomass and nutrient yield (McAllister and Hristov 2000; Hargreaves et al. 2009). The recommended optimum DM for ensiling forage ranges from 35% to 45% (Hargreaves et al. 2009). Forage maturity also affects the end-products of silage fermentation. Lactic acid concentration increased and acetic acid, ethanol and ammonia concentrations decreased in alfalfa silage as the DM content of the forage increased from 31% at the late-bud stage to 44% at 50% bloom (Bolsen et al. 1992).

Higher DM impedes exclusion of trapped air (anaerobiosis) and achievement of higher packing density during silo filling. This leads to lower population of lactic acid-producing bacteria because lower water activity reduces bacterial growth (Rizk et al. 2005; Whiter and Kung 2001). Studies have shown that the population and growth rate of LAB is reduced, lactic acid concentrations are lower and pH does not decline as rapidly in silages with higher DM (53-54%) as compared to those with optimum DM (30-32%; Whiter and Kung 2001). Even though moisture by itself is important, it is efficiency to which water can participate in biochemical reactions (water activity) rather than moisture content *per se* that indicates how much moisture is really available for microbial activity (Albert et al. 1989). In high DM silage, harvest losses are always higher and decline in pH often comes too late to ensure optimal silage quality (Muck 1988).

Glucose, fructose and fructosans are the primary WSC in most preserved silages. Forage WSC is affected by species, variety and stage of maturity as well as agronomic practices such as planting density, and fertilizer application (McDonald 1981).

In cereal silages, the two most important chemical components that affect silage quality are WSC and crude protein (CP) concentration of the forage prior to ensiling (Hargreaves et al. 2009). Even though glucose and fructose are the major substrates used by bacteria during fermentation, other sugars may also be metabolized. Most bacteria have flexible pathways for utilization of a wide range of carbohydrates, thereby allowing other sugars to be used as alternatives or in conjunction with glucose and fructose. Some strains of *L. plantarum* have been

shown to have a preference for sucrose and lactose even in the presence of excess glucose or are capable of using glucose, sucrose and lactose simultaneously (Plumed-Ferrer et al. 2008). This may partially explain why high concentrations of fermentation acids have been measured in silages even with low initial glucose concentrations (Woolford 1984; Charmley and Veira 1991; Kung et al. 2004).

Readily fermentable substrates such as glucose, sucrose and dextrose can be added to forages at ensiling to increase the initial rate of fermentation. Bolsen et al. (1992) applied dextrose (2% of forage DM) alone or in combination with an inoculant containing *L. plantarum* and *P. cerevisiae*, to alfalfa forage at ensiling. They observed an increase in lactic acid concentration and in pH decline as compared to control silage, but terminal pH did not differ between the two silages.

The concentration of CP and WSC, as well as fibre digestibility decrease as the forage matures due to fibre and phenolics (Jung and Allen 1995; Hargreaves et al. 2009) accounting for an increasing proportion of silage DM. Starch deposition in cereal silages similarly increases with maturity, but this often does not offset the adverse effects of the reduction in fibre digestibility associated with with advancing forage maturity (McAllister and Hristov 2000; Hargreaves et al. 2009). Concentrations of plant proteins and organic acid in forages may also affect silage fermentation. Organic acids (e.g. sulfates and nitrates) account for 68-80% whereas plant proteins account for 10-20% of the buffering constituents of most ensiled forages (Woolford 1984). The efficiency of silage fermentation (FC) is therefore a function of WSC and DM concentration, and buffering capacity of the forage at the time of ensiling (Weissbach and Honig 1996).

In barley forage, WSC concentration increases with maturity up to the milky kernel stage and thereafter declines (McGregor and Edwards 1968; Hargreaves et al. 2009). The amount of forage WSC entering the silo does not always correspond with the amount of organic acids formed from fermentation. The concentration of fermentation acids in the silage sometimes exceeds the acid-production potential of fermentable sugars in the original forage. It has long been considered that proteins, amino acids and other organic acids may be alternative substrates for formation of fermentation acids (McDonald 1981; Woolford 1984).

Charmley and Veira (1991) found the concentration of WSC in γ-radiated lucerne silages to be nearly double the initial concentration in the forage. They observed that this increase in WSC was associated with a corresponding decrease in hemicellulose concentration and attributed the phenomenon to hemicellulase activity that was independent of that produced by microbes. Hydrolysis of plant polysaccharides due to plant enzymatic and/or acidic hydrolysis has been responsible for increases in WSC concentration in grass (McDonald and Henderson 1974; Heron et al. 1986) and other cereal (Weinberg et al. 1993; Filya et al. 2000) silages. An increase in WSC concentration from 124 g kg^{-1} DM in ryegrass forage to 204 g kg^{-1} DM after 153 d of ensiling has been reported (Heron et al. 1986). The phenomenon of acidic hydrolysis of plant structural carbohydrates during ensiling has also been documented in studies in which silage with either low pH (Weinberg et al. 1993) or treated with acid (McDonald and Henderson 1974) had higher WSC concentrations than the original forage. In earlier studies with whole-crop barley, no xylose or arabinose could be detected in the original forage, but appreciable amounts of xylose and trace amounts of arabinose were recovered after ensiling (McGrgor and Edwards 1968). Consequently, the role of WSC as the major substrate for microbial fermentation has been questioned with some researchers concluding that WSC *per se* may not be as critical to silage fermentation as previously proposed (Woolford 1984; Charmley and Veira 1991; Charmley 2001).

1.1.2.3. Epiphytic Microbial Populations of the Forage

The population of epiphytic microflora varies among forages. Bolsen et al. (1992) found higher numbers of epiphytic organisms on corn compared to alfalfa forage. Lin et al. (1992a) also studied the effects of six categories of epiphytic microorganisms on microbial succession during the ensiling of of corn and alfalfa. The numbers of streptococci, Enterobacteriaceae, yeasts and moulds, lactic acid-utilizing yeasts, and carbohydrate-fermenting clostridia were higher on corn than on alfalfa. Even among corn hybrids, distinct differences in epiphytic populations and fermentation characteristics have been observed (Bolsen et al. 1992).

Environmental factors such as variations in daily temperature, precipitation and humidity prior to harvesting may also affect epiphytic populations on the forage (Lin et al. 1992a).

A lack of response of corn forage to inoculants has therefore been attributed to higher epiphytic LAB populations which overwhelm the bacteria within the inoculant and negate favorable responses (Kung et al. 1993; Bolsen et al. 1992). For an inoculant to be effective, Pahlow (1991) proposed that it must deliver a LAB population that will exceed the epiphytic population by at least a factor of two. However, it is often the growth rate during ensiling rather than the theoretical LAB inoculation rate that determines whether an inoculant will overwhelm the epiphytic population and cause favourable shifts in fermentation (Muck 1998).

1.1.2.4. Effects of Silo Type: Laboratory vs Large Farm-Scale Silos

Factors such as silo design, packing density, as well as temperature, radiation, wind and precipitation at the time ensiling may all affect the ensiling process (Garcia et al. 1989; McAllister and Hristov 2000). In most experiments, silages are stored in laboratory-scale silos. The main advantages of laboratory silos over large farm silos have been noted (Lesins and Schulz 1968) and include: 1) the preparation of a large number of silos enabling easy replication of treatments 2) smaller silos reduce the formation of micro-environments that may not be indicative of overall ensiling outcomes 3) easier sampling for laboratory analyses and 4) only small amounts of forage are required for filling silos. However, results from mini silos should always be field-tested using commercial silos before being applied at the producer level as results in laboratory silos can not always be extrapolated to farm-scale ensiling methods (Woolford and Sawczyc 1984; Cherney et al. 2004). According to McAllister et al. (1998) laboratory silos may be useful for assessing silage inoculants when large compositional changes are induced, but when the changes are less obvious, these techniques may offer little insight into the possible effects of inoculant application on variables related to animal performance. Laboratory silos do not also allow the assessment of the

effects of factors such as silage feed out rate and face management on aerobic stability (Ruppel et al. 1995).

Quick filling, air-tight sealing and small size of the experimental silos ensure rapid achievement of anaerobosis thereby reducing the likelihood of observing a difference between inoculated and uninoculated silages. This is contrary to experiments involving large-scale silos where it is practically impossible to ensile large quantities of herbage in as short of period of time as is with laboratory silos. There is usually therefore substantial variability in the quality of forage entering these silos thereby making it difficult to attribute any observed reponse solely to inoculation. However, similarities in fermentation characteristics of *L. buchneri*-treated grass silage stored in farm-scale silos and those stored in laboratory-scale silos have led some researchers into concluding that *L. buchneri* had similar effects under both laboratory- and farm- scale conditions (Driehuis et al. 1999; 2001).

1.1.3. Silage Intake, Digestibility Rumen Fermentation and Animal Performance

A major limitation to practical application of forage quality information is the lack of a uniform quantitative definition or expression of what constitutes quality (Moore 1994). Confusion in the literature over the definition of terms such as "forage quality" and "forage nutritive value" has further compounded the problem. "Forage quality" encompasses the inherent characteristics which determines its voluntary intake while "forage nutritive value" is a measure of the inherent characteristics of the forage consumed and includes nutrient concentration, digestibility and the nature of the end products of digestion and their impact on animal performance (Moore 1994). Based on economic considerations, the quality of forage is best evaluated by its impact on efficiency of ruminant production (Moore 1994; Cherney 2000). The nutritive value of silage therefore depends on its voluntary intake, its digestibility and efficiency of utilization of digestible components by the animal (Woolford 1984).

A comparison between silage and hay conserved from forage of the same maturity suggest that voluntary intake of silage is usually about 17% lower than hay (Thiago et al. 1992a). This suggests that end products of the ensiling process may limit intake. The intake of silage may be influenced by fermentation characteristics through either their pre-ingestive effects on palatability (Shaver et al. 1985; Buchanan-Smith 1990; Rook et al. 1990) or post-ingestive effects on ruminal VFA concentrations (Phillip et al. 1981; Oba and Allen 2003).

Silages treated with homolactic inoculants are often characterized by higher lactic acid concentration and lower pH while those treated with heterolactic inoculants may contain higher concentrations of acetic acid (e.g., *L. buchneri*) or propionic acid (e.g., *Propionibacteria*) and comparatively higher pH.

In general, increases in forage NDF digestibility lead to increased DMI (Oba and Allen 1999), however, this hypothesis should be limited to high-producing animals where intake is restricted by rumen fill. Increased DMI due to increased forage NDF digestibility is not expected if rumen fill is not limiting intake (Jung and Allen 1995). Ruminal metabolism of silage such as the production of VFA, changes in osmolarity and lower pH may also act alone or in combination to depress silage intake. Silage inoculation can cause shifts in the concentration of ruminal VFAs (Keady and Steen 1994; Weinberg et al. 2003). The absorption and utilization of these VFAs can directly influence satiety and affect feed intake (Allen 2000; Oba and Allen 2003). Intraruminal infusion of sheep with extracts of silage increased osmolarity resulting in a liner decrease in feed intake as compared to extracts from fresh forage. However, adjustment of both extracts to equal osmolarity using sodium chloride resulted in no difference in DMI, suggesting that ruminal osmaolarity rather than the products of silage fermentation were responsible for short-term decreases in silage intake (Phillip et al. 1981). Higher ruminal solubility of silage DM may also lead to a rise in concentration of ruminal VFAs which stimulates chemo-receptors to inhibit intake (Thiago et al. 1992b). Inoculation of forages prior to ensiling may increase ruminal propionic acid concentration (Keady and Steen 1994; Sharp et al. 1994; Steen et al. 1989) and as intraruminal infusion of propionic acid decreases DM

and ME intake of dairy cows (Oba and Allen 2003), silage may elicit a similar response.

Another factor that is particularly peculiar to estimating silage DM intake is the laboratory procedure for determination of DM concentration. Because the concentration of volatile compounds in silages is influenced by inoculation, methods of silage DM determination that increase volatilization of these compounds may underestimate actual DMI as these compounds are lost during drying. Determination of silage DM by oven-drying tends to underestimate DM concentration and lead to underestimation of silage DM and digestible energy intake (Clancy et al. 1977; Porter et al. 1995). The volatility of silage compounds increases with increasing temperature, and is generally higher for VFAs (0.9) than for lactic acid (0.4) at 100^{o}C (Porter and Murray 2001). The average estimated loss of DM of whole-crop corn silages decreased from 10% for samples oven-dried at 100^{o}C for 48 h to 4% for those oven-dried at 70 ^{o}C for 48 h (Brahmakshatriya and Donker 1971). Porter et al. (1995) developed robust empirical equations for correction of volatilization of VFA, lactic acid and ammonia in silages where DM was determined by oven-drying. Nonetheless, a comparison of determining DM concentration using oven-drying (65^{o}C for 72 h) to toluene distillation showed that there was no difference in DM concentration of grass, legume or mixed silages. These researchers concluded that oven-drying was acceptable for determining DM if large number of samples were used (Petit et al. 1997).

The greatest challenge to the utilization of forages in ruminant diets is poor ruminal fibre digestibility and efficiency of utilization of the end products of digestion (Allen and Mertens 1988). Pre-treatment of forage as opposed to modification of the ruminal ecology offers an opportunity to increase the efficiency of ruminal digestion of forages. Compared to chemical treatments, microbial inoculation of silage is a safer method than chemical treatment for enhancing the nutritional value of forages prior to feeding. Chemical additives may be corrosive to farm equipment and also detrimental to the environment,

hence the move towards biological as opposed to chemical approaches for improving silage quality.

Compared to hay, silage has a shorter fermentation lag time and a greater proportion of ruminally soluble DM (Thiago et al. 1992a). Inoculation also tends to increase the rumen soluble DM content of silages (Keady et al. 1994). The rapid degradation of highly soluble silage DM leads to a rise in concentration of ruminal VFAs which may influence silage intake (Thiago et al. 1992b).

Muck et al. (2007) did not observe any positive effects of inoculation on *in vitro* DM digestibility, but gas production was reduced and a glucogenic pattern of rumen fermentation was observed in the majority of inoculated as compared to control silage, irrespective of whether the inoculant caused any changes in silage fermentation. These changes in rumen fermentation pattern could partly explain some of the reponses observed in animal performance with inoculated silage.

1.1.4. Silage Inoculants

Silage inoculants are microbial silage additives that mainly consist of LAB that have been isolated from epiphytic microbial populations and further selected to enhance the efficiency of fermentation. Even though silage inoculants traditionally consist of LAB, other microorganisms such as *Propionibacteria* (Higginbotham et al. 1998; Filya et al. 2004; 2006) and yeasts (Weineberg et al. 1999) have occasionally been used as microbial silage additives. Whether an inoculant will be effective at improving silage fermentation or not depends on the following:

1. The population of LAB delivered by the inoculant. This should be at least 10% greater and have a faster growth rate than the epiphytic population to overwhelm it. Inoculation rates that are even 1% less than the epiphytic population may have minimal or no effect on silage quality (Muck 1989).
2. The efficiency of fermentation of the inoculant LAB. The amount of fermentation acids produced per unit of WSC fermented by the inoculant LAB should be greater than that of the epiphytic LAB (Muck 1989; Weissbach and Honig 1996).

3. The forage being ensiled. It should have sufficient substrates (e.g. WSC) for fermentation (Muck 1989; 1996; 1998; Weinberg and Muck 1996).
4. Crop-inoculant synergy; some strains of LAB are crop specific, for example LAB originally isolated from corn may not perform as well when applied to another crop such as alfalfa; this hypothesis has led to the suggestion of the need to develop crop-specific silage inoculants (McAllister et al. 1998; Muck, 1996).
5. The LAB should be resistant to phage (Muck 1996).

The population and quality of epiphytic microorganisms are highly variable and the efficiency of fermentation is often too low to guarantee successful fermentation (Merry and Davis 1999). Epiphytic LAB range from 10^2 to 10^6 CFU g^{-1} for alfalfa, 10^3 to 10^9 CFU g^{-1} for corn (Bolsen 1992; Lin 1992a; Filya 2004) and 10^6 CFU g^{-1} for barley (Zahiroddini et al. 2004; Baah et al. 2011). It is estimated that 10^8 CFU g^{-1} of LAB is required for immediate decline in silage pH whereas the recommended rate of application for most commercial silage inoculants is 10^5 to 10^6 CFU g^{-1} of forage (Muck 1988). This suggests that most inoculants do not meet the criteria proposed by Pahlow (1991) that the numbers of LAB in the inoculant should be greater than the epiphytic LAB by a factor of 2 to have a positive impact on fermentation. Considering the wide variability in population, viability and fermentation efficiency of epiphytic LAB (Merry and Davis 1999), an immediate decline in silage pH is often not observed and the the lag in pH decline contributes to a loss in silage DM. This lag may further be prolonged by a delay in anaerobicity within the silo (Muck 1988).

Some production characteristics of the inoculant such as improper packaging and storage conditions, poor viability, unsuitable carrier and the form applied (ie., granules vs liquid) may also affect the efficacy of silage inoculants (Whiter and Kung 2001; Kung 2009). The addition of other non-microbial additives meant to enhance the efficacy of silage inoculants such as surface active agents (surfactants) have therefore been used in combination with silage

inoculants to increase bacterial attachment to forage and improve the fermentation of barley silage (Baah et al. 2011).

1.1.4.1. First-generation Homolactic Silage Inoculants

The production of sufficient lactic acid to cause a rapid decline in pH during ensiling was the original criteria for the selection and development of suitable first-generation silage inoculants (Lesins and Schulz 1968; McDonald 1981). Most early silage inoculants were however inefficient and a rapid reduction in pH was often not observed (Woolford and Sawczyc 1984). Following many years of selection, the inclusion of efficient LAB species and strains in most first-generation silage inoculants have resulted in a consistent rapid pH decline and improvements in DM retention across a range of forages (Table 1.3). The criteria for selection of LAB to be included in silage inoculants were first proposed by Whittenbury as outlined in McDonald (1981):

1. They must be able to grow vigorously and overwhelm the dominant epiphytic population.
2. They must possess a homolactic fermentation pathway to produce sufficient lactic acid that will inhibit the growth of other microorganism while at the same time being tolerant to low pH.
3. They must be able to ferment a wide range of sugars (e.g. hexose, sucrose, fructans and pentoses) but should not metabolize organic acids.
4. They should be capable of enduring and growing at temperatures up to 50^{o}C.
5. They should be able to grow on material of lower moisture content such as wilted forage.

While these criteria still remain the guiding principles for selecting most silage inoculants, recent advancements in the understanding of silage microbiology have shifted research emphasis to the development of inoculants that reduce losses during ensiling and feed-out, while at the same time improving fibre digestibility (Nsereko et al. 2008; Kang et al. 2009).

One strategy to increase the efficacy of first-generation silage inoculants was co-culturing more than one species or strain of the same species, in a single inoculant (Weinberg and Muck 1996). Co-culturing of LAB in a single inoculant is meant to synergise their individual potential to improve the ensiling process. This approach takes advantage of the fact that LAB grow at different rates and have different requirements for nutrients, temperature and anaerobiosis. Mixed-species inoculants have improved DMI, ADG and feed efficiency of feedlot cattle as compared to single-species inoculants, indicating that mixed-species may offer significant advantages (McAllister et al. 1998). Consequently most inoculants in the market are now comprised of multiple species or strains of LAB (Filya et al. 2007). However, others have previously found that co-culturing of different homolactic species of LAB into a single inoculant was immaterial in influencing silage fermentation characteristics (Woolford and Sawczyc 1984) or aerobic stability (Inglis et al. 1999).

Improvements in aerobic stability of silages inoculated with first-generation silage inoculants have generally been poor compared to improvements in fermentation characteristics (Table 1.3). Efficient fermentation patterns result in higher lactic acid and residual WSC in the silage. These substrates are then used by a wide range of aerobic organisms for respiration. Even though the microbiology of deteriorating silages has often been attributed to yeasts and moulds, many species of bacteria have also been shown to play a role in silage spoilage (Woolford 1990; Inglis et al. 1999). This makes it difficult to accurately ascertain the real causative agents of silage deterioration. For example, yeasts populations (10^6 CFU g^{-1}) greater than the threshold population (10^5 CFU g^{-1}) proposed for silage deterioration (Woolford 1990) failed to initiate spoilage of a corn silage-based TMR. This suggests that microorganisms other than yeasts may have been responsible for aerobic deterioration (Nishino et al. 2004).

There are generally few studies that combine silage fermentation with digestibility and production performance. Inoculation of barley silage with a first-generation inoculant containing *Pediococcus* and *Lactobacillus* strains had no effect on growth performance but addition of an exogenous cocktail of fibrolytic

enzymes to the inoculant increased the feed efficiency of growing steers (Zahiroddini et al. 2004). In contrast, Schaefer et al. (1989) found no difference in the growth performance of growing steers fed corn silage treated with an inoculant containing similar species but without exogenous enzymes.

Inoculation of alfalfa silage with *L. plantarum* alone had no effect on DMI, but combining it with *E. faecium* increased DMI by feedlot steers compared to the control (McAllister et al. 1998). The latter therefore suggested that multi-species inoculants may be more advantageous with greater impact on animal performance than single-species inoculants. However, in an earlier study, these authors did not observe any advantage of multi-species inoculants on feed intake of lambs fed barley silage inoculated with *L. plantarum* co-cultured with *E. faecium* (McAllister et al. 1995). Other studies also observed improvements in DMI and weight gains of growing steers fed grass silage inoculated with a single strain of *L. plantarum* (Steen et al. 1989)

Even though inoculation, especially with non-fibrolytic inoculants, is not expected to alter fibre concentration in silages, occasionally, decreases in fibre concentration and an increase in intake have been observed. Inoculation of perennial ryegrass with *L. plantarum* and *E. faecium* decreased NDF concentration and increased silage intake and feed conversion efficiency as compared to untreated silage, but these improvements were attributed to favourable shifts in the fermentation characteristics of the silage (Sharp et al. 1994). Inoculation of grass silage with a single strain of *L. plantarum* also improved silage DM and NDF digestibility even with (Keady and Steen 1994) or without (Keady et al. 1994) significant shifts in fermentation characteristics. However, treatment of barley (Hristov and McAllister 2002) or wheat (Sucu and Filya 2006) silages with an inoculant containing *L. plantarum* and *E. faecium* had no effect on digestibility in cattle or sheep, respectively.

The mechanisms by which first-generation inoculants may enhance silage fibre digestibility have been proposed (Keady and Steen 1994) as: 1) acidic hydrolysis of fibre increases digestibility in the rumen 2) rapid decline in pH leads to more efficient preservation of soluble carbohydrates which are readily digested

in the rumen and 3) synergistic effects of bacterial enzymes on other beneficial enzyme activities produced during ensiling may improve fibre hydrolysis during ensiling. Occasionally, however, silage inoculants have improved digestibility and animal performance without any significant shifts in fermentation characteristics (Steen et al. 1989; Muck 1993; Adesogan et al. 2009). Earlier reports on how first-generation inoculants could improve animal performance without favourable shifts in fermentation suggested that factors other than traditional silage fermentation characteristics may account for this response (Kung et al. 1993). A series of experiments by Weinberg and his colleagues to ascertain the mechanisms by which silage inoculants affected ruminal fibre digestibility and fermentation without marked shifts in in-silo fermentation characteristics have subsequently shown that the LAB in silage inoculants could cause favourable shifts in rumen microbial ecology and fermentation patterns (Weinberg et al. 2003; 2004) and improve DM and NDF digestibility (Weinberg et al. 2007).

Inoculation of alfalfa silage with multiple strains of *L. plantarum* also failed to cause improvements in intake, ruminal DM, CP and NDF digestibilities, or milk production (Rizk et al. 2005). The main reason for the lack of improvement in animal performance was that there was no improvement in fibre digestibility as these inoculants lacked fibrolytic activity. Effects of the interaction between forage types and the efficacy of silage inoculants was clearly demonstrated in studies in which treatment of either grass or whole-crop wheat silages with an inoculant containing a combination of *L. casei, L. plantarum* and *S. lactis* resulted in increased DMI and apparent DM digestibility of grass silage, but not whole-crop wheat silage (Charmley et al. 1996).

The impact of silage composition on rumen fermentation is greater than that of a concentrate whenever silage is included at a level in the diet that is greater or equal to the concentrate (Keady and Mayne 2001). Silage inoculants may therefore influence the pattern of ruminal fermentation and the efficiency of utilization of the end products from ruminal digestion for growth. Inoculation of grass silage with a single-species inoculant containing *L. plantarum* increased the estimated ME content of the diet and shifted the pattern of rumen fermentation

towards increased propionic acid and decreased acetic acid lowering the acetic: propionic acid ratio (Keady et al. 1994). A similar glucogenic ruminal fermentation pattern was also induced by inoculated silages, increasing, the molar proportion of propionic acid and lowering acetic acid and butyric acid (Sharp et al. 1994). Considering that propionic acid is a major precursor for glucose synthesis in ruminants, a glucogenic ruminal fermentation pattern has been associated with a greater ME efficiency for growth in growing cattle (Ørskov 1977).

It can be inferred from these studies that the inability of most first-generation silage inoculants to produce fibrolytic enzymes during ensiling may be responsible for their frequent inability to improve digestibility or animal performance.

Table 1.3. Effects of first-generation homolactic silage inoculants on fermentation and aerobic stability[z]

Inoculant LAB	Crop	Terminal pH	Aerobic stability	DM recovery	Reference
L. casei,	Corn	Higher	Not increased	No effect	Nishino et al. (2004)
E. faecium[y]	Grass	Lower	Increased	Improved	Marciňáková et al. (2008)
L. plantarum and *E. faecium*	Alfalfa	No effect	Reduced	ND	McAllister et al. (1998)
L. plantarum and *E. faecium*	Barley	No effect	Reduced	Not improved	McAllister et al. (1995)
L. plantarum and *E. faecium*	Wheat	Lower	Reduced	Not improved	Sucu and Filya (2006)
L. plantarum and *E. faecium*	Corn	No effect	Reduced by 12 h	No effect	Muck (2004)
L. plantarum and *P. pentosaceus*	Perennial ryegrass	Lower	Not increased	Improved	Driehuis et al. (2001)
L. plantarum, P. acidilactici and *E. faecium*	Barley	Lower	Increased	Not improved	Baah et al. (2011)
L. plantarum, L. bulgaricus and *L. acidophilus*	Barley	Lower	Reduced by ~380 h	Improved	Kung et al. (2004)

[z]Compared to control silages.

[y]Bacteriocin-producing strain EF9296.

ND, not determined.

1.1.4.2. Second-generation Heterolactic Silage Inoculants

Second-generation silage inoculants were developed to reduce losses that occur during fermentation and feed-out while improving silage fermentation characteristics. The growth of silage spoilage organisms such as fungi (yeasts and moulds) and bacteria (enterobacteria and bacilli) is inhibited by the end-products (organic acids) of silage fermentation. The inhibitory effects of organic acids on yeasts and moulds increase with increasing chain length of the acid (Woolford 1975). Of the major volatile fatty acids produced during silage fermentation, butyric acid, a four carbon compound, has a greater inhibitory effect on silage spoilage microorganisms than lactic, acetic or propionic acids. However, increased production of butyric acid is a reflection of the fermentation of sugars and lactic acid, and of the breakdown of proteins and amino acids by clostridia, and is therefore a hallmark of poor quality silage (Table 1.1; McDonald 1981; Woolford 1984; 1991). Consequently, only the production of acetic and propionic acids are desirable for improving aerobic stability of silages. Growth inhibition of spoilage organisms is often a reflection of their adaptation to reduce the diffusion of undissociated acids across their cellular membrane into the cytoplasm, where dissociation increases intracellular acidity, denaturing essential proteins and potentially causing cell death (Booth 1985; Warnecke and Gill 2005).

Two major bacteria used in second-generation inoculants include *L. buchneri* and *Propionibacteria*. *Lactobacillus buchneri* converts lactic acid into mainly acetic acid whereas *Propionibacteria* convert WSC into propionic acid, during ensiling. Both acetic and propionic acids are more anti-mycotic than lactic acid. Moon (1993) has shown that propionic acid inhibited the growth of acid-tolerant yeasts more than acetic acid with a combination of these acids being most effective. The concentration of propionic acid as compared to acetic acid is often too low to appreciably prevent aerobic deterioration of silage. When *Propionibacteria* were applied either to corn or sorghum forage prior to ensiling, the bacteria could not be detected in either silage and there was no measurable increase in propionic acid or improvement in aerobic stability (Filya et al. 2006).

Higginbotham et al. (1998) also showed that the addition of

Propionibacteria inoculant did not significantly influence the fermentation or aerobic stability of corn silage. Inoculation of wheat, sorghum or corn silages with *Propionibacteria* increased acetic and propionic acid concentrations and decreased yeast and mould populations resulting in improved aerobic stability (Filya et al. 2004). However, in other studies these effects on aerobic stability of pearl millet and corn were marginal and no significant production of propionic acid was measured (Weinberg et al. 1995). The latter researchers concluded that the acidic environment in silage was unfavourable for the survival of *Propionibacteria*.

Acetic acid, especially in its undissociated form, prevents the deterioration of silage by reducing the growth of yeasts during ensiling and aerobic exposure (Muck 2004; Driehuis et al. 1999; 2001). Lactic acid bacteria that possess a heterolactic pathway of fermentation use WSC as their main substrate for production of lactic and acetic acids during fermentation. However, *L. buchneri's* use of WSC is marginal and it relies mainly on the conversion of lactic acid into acetic acid for derivation of energy. The degradation of lactic acid into acetic acid by *L. buchneri* therefore occurs only during the latter stages of fermentation when pH is low (pH = 4.3-3.8) and at temperatures between 15-37^{o}C (Driehuis et al. 1999; 2001; Oude Elferink et al. 2001). One major characteristic of all second-generation inoculants is therefore their ability to produce higher concentrations of acetic or propionic acids that inhibit the growth of spoilage organisms.

Aerobic stability of silages treated with inoculants containing *L. buchneri* has been improved across a wide range of forages including; corn (Reich and Kung 2010; Muck 2004; Nishino et al. 2004), barley (Taylor et al. 2002), sorghum and wheat (Weinberg et al. 1999), alfalfa (Zhang et al. 2009), and when silage was a component of a total mixed ration (Taylor et al. 2002; Kung et al. 2003; Nishino et al. 2004). However, there are studies in which *L. buchneri*-based inoculants failed to improve the aerobic stability of silages. The acetic acid concentration of corn (Kleinschmit and Kung 2006) and bi-crop of peas and wheat (Adesogan and Salawu 2004) silages inoculated with *L. buchneri* increased compared to the control but this did not result in a consistent improvement in

aerobic stability. In other studies, acetic acid concentration did not differ between *L. buchneri*-inoculated and untreated silage, but yeasts numbers were reduced and aerobic stability improved (Mari et al. 2009; Tabacco et al. 2011a). The lack of improvements in aerobic stability of silages treated with *L. buchneri* can therefore be attributed to: 1) higher acetic acid concentration in the untreated silage due to heterofermentation by epiphytic microbial population 2) the type of forage being ensiled; legume silages are resistant to spoilage because they have low residual microbial WSC and lactic acid levels for spoilage organisms and 3) aerobic deterioration of silage may be caused by microorganisms other than yeasts that are known to be susceptible to acetic acid. 4) co-culturing of homolactic LAB with *L. buchneri* may reduce the activity of *L. buchneri*.

The potential for yeasts to improve the aerobic stability of silage has also being assessed. Treatment of sorghum and wheat silages with a combination *L. buchneri, L. plantarum* and yeasts (Y-56; isolated from local wheat and *Hansenula subpelliculosa*) showed that neither the addition of yeasts alone nor with LAB at ensiling reduced the aerobic stability of silage, possibly because of increased acetic acid concentration in the silage produced by the yeasts (Weinberg et al. 1999).

Acetic acid by itself may not depress DMI, but because most poorly fermented silages also contain higher concentrations of acetic acid, depression in DMI of such silages is often erroneously attributed to acetic acid concentration (Driehuis et al. 2001). This hypothesis was further supported by studies that found that higher concentrations of acetic acid failed to depress silage DMI. Silage treated with an inoculant containing *L. buchneri* had an acetic acid concentration as high as 5.9% DM compared to 4.0% DM for untreated silage, yet no depression in DMI was observed by dairy cows fed a TMR containing 35% (DM basis) inoculated barley silage plus 15% regular corn silage (Taylor et al. 2002). A similar diet containing *L. buchneri*-treated alfalfa silage had an acetic acid concentration of 6.5% DM, but did not affect the DMI of dairy cows (Kung et al. 2003). Effects of fermentation products on silage intake are variable and inconsistent. Higher concentration of acetic acid alone may depress silage intake

in the short term, but intake may be enhanced when the silage contains of the same concentration of acetic acid in conjunction with other acids such as lactic acid (Buchanan-Smith 1990). Considering that well fermented silages often contain lactic acid as well as all the major VFAs in appreciable amounts, acetic acid is unlikely to depress feed intake under practical production conditions. In a study involving 24 different silages, Krizsan and Randby (2007) found that lactic acid, propionic acid and butyric acids, but not acetic acid concentration of silage, had impact on DMI of silage. However, Shaver et al. (1985) found that higher total hydrogen ion concentration rather than any specific fermentation acid(s) was responsible for lower intake of silage by growing steers. Furthermore, Huhtanen et al. (2002) reported a weak relationship between silage pH and silage DMI, but found that in a two-variable model for predicting silage intake, the depressive effect of acetic acid on DMI for dairy cows was 54% greater than that of lactic acid.

Even though Rook et al. (1990) identified butyric acid as the most important fermentation acid that reduces silage DMI, they were quick to caution that butyric acid was a characteristic end-product of fermentation by saccharolytic and proteolytic clostridia. They also acknowledged that it was also possible that some unmeasured metabolite from clostridial activity could be responsible for intake-depression of silages with higher butyric acid.

As with all forages, silage inoculants may also influence silage intake via their effects on NDF concentration. In the NDF-energy-gut-fill model, intake is often a function of either gut-fill or available energy. When energy is limiting intake, voluntary DM intake is positively correlated with NDF concentration in the feed whereas when fill is limiting intake, intake will be negatively correlated with NDF concentration. Thus the relationship between NDF and gut-fill is directly opposite that of NDF and available energy (Mertens 1994). In forage-based feeding systems, slower ruminal clearance rate increases ruminal feed residency time resulting in greater ruminal digestibility of NDF in low-intake cattle, whereas the opposite effect occurs in high-intake cattle (Shaver et al. 1988; Merchen and Bourquin 1994).

Treatment of corn silage with *L. buchneri* improved DM and nutrient retention both during fermentation and upon aerobic exposure, increasing net energy of gain and potential milk yield (Tabacco et al. 2011b). *Lactobacillus buchneri* also increased average daily gain in sheep, but had no effect on DMI when corn silage was fed (Ranjit et al. 2002). No difference was observed in DMI, milk yield, and composition of lactating cows fed *L. buchneri*-treated or untreated barley silage (Taylor et al. 2002). However when a similar inoculant was used to treat alfalfa, milk yield was greater for cows fed the inoculated silage than for those fed uninoculated silage (Kung et al. 2003).

Treatment of barley silage with an inoculant containing *L. buchneri* increased milk yield in one study (Kung et al. 2003) but not in another (Taylor et al. 2002) when cows were fed barley and alfalfa silage- based diets respectively. The variability in responses of inoculating different forages with the same inoculant has further been demonstrated by studies in which a homolactic inoculant containing *L. plantarum, L. casei* and *S. lactis* improved the efficiency of feed utilization in steers fed inoculated wheat but not those fed inoculated grass silage (Charmley et al. 1996). In that study, improvement in animal performance in response to inoculation was not associated with measured difference in fermentation characteristics between control and treated silage. When alfalfa silage was inoculated with *L. plantarum* alone, no impact on animal growth performance was observed but co-culturing it with *E. faecium* increased daily weight gain and efficiency of feed utilization by growing feedlot steers suggesting an advantage of co-inoculation (McAllister et al. 1998).

The effects of second-generation silage inoculants on feed intake, digestibility and growth performance are variable. The combination of *L. buchneri* with *L. plantarum* increased *in vitro* NDF digestibility of corn silage compared to the control, however, no improvements were observed when it was combined with *P. acidilactici* or *P. pentosaceus* (Reich and Kung 2010). Whereas in in other studies, treatment of alfalfa with *L. buchneri* or *L. plantarum* alone or in combination failed to induce any improvements in DM or NDF *in situ* (Zhang

et al. 2009). These results suggest that the effects of second-generation silage inoculants on animal performance may be inconsistent.

1.1.4.3. Third-generation Esterase-Producing Silage Inoculants

Third-generation silage inoculants contain fibrolytic LAB capable of hydrolysing fibre during ensiling and thereby improving ruminal fibre digestion when fed to ruminants. Arabinoxylans and glucuronoarabinoxylans are the major digestible components of hemicellulose with arabinoxylans being the predominant fraction in cereal forages (Merchen and Bourquin 1994). Arabinoxylans are in turn ester-linked to ferulic acid which may serve as an etherification site for lignin formation (Figure 1.1.; Hatfield and Marita 2010). Ferulic acid is one of the most abundant hydroxycinnamic acids present in cell walls of cereal crops and its presence inhibits fibre digestion in ruminants (Yu et al. 2005; Jung and Phillips 2010).

Like all phenolic acids, FA is synthesized from phenylpropanoids by the phenylpropanoid pathway in the Golgi body and then shuttled to the cell wall in an encapsulated vesicle, via a ferouyl-CoA transferase. However, whether the feroulylation of arabinoxylans occurs in the cytoplasm or in the cell wall matrix is unknown, what is known is that ferouylated arabinoxylans are synthesized and continually deposited throughout plant cell wall development (Hatfield and Marita 2010). Xylans consist of a chain of xylose units that are often substituted at the C2 and/or C3 backbone with arabinose to form arabinoxylans. Muellar-Harvey et al. (1986) found that in barley forage, arabinose is more often linked to FA (7%) than to coumaric acid (3%) with the ratio of FA, arabinose and xylose being 1: 1: 2. During secondary cell wall growth, FA or *p*-coumaric acid are either ester-linked to arabinose at the C5 (monocots) or C2 and C6 (dicots) position (Yu et al. 2005). This arrangement physically blocks the alignment of arabinoxylanase to xylans. These linkages confer the mechanical structure to the plant cell wall, but also impede the ruminal hydrolysis of hemicellulose.

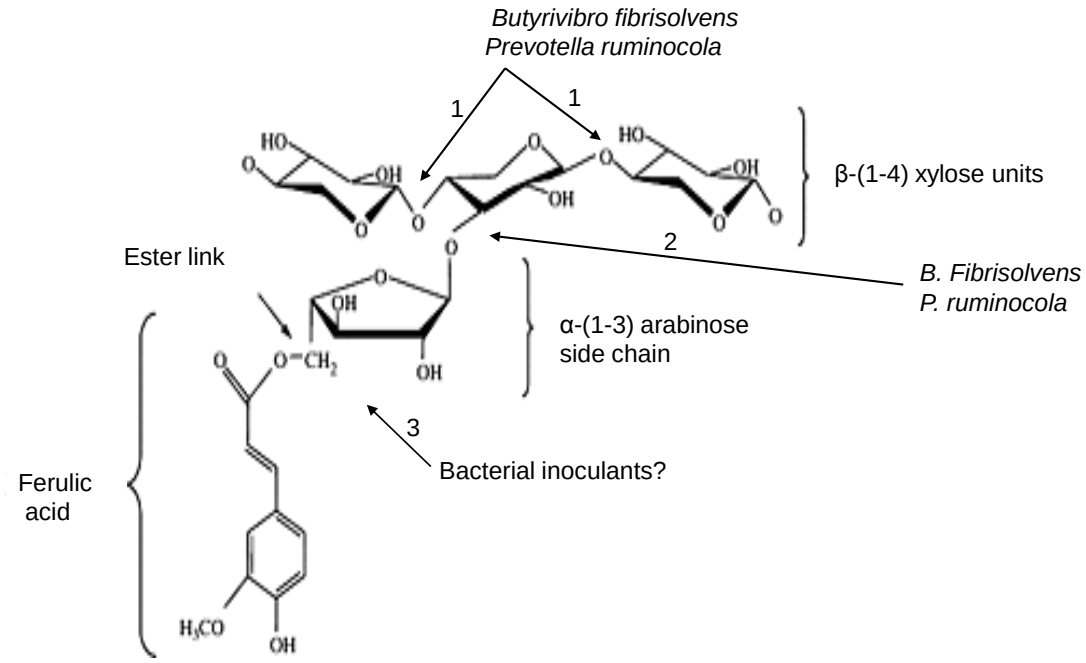

Figure 1.1. Ferouylated polysaccharide indicating linkages liable to hydrolysis by rumen bacteria (linkages 1-2) and inoculants containing ferulic acid esterase activity (linkage 3): Hydrolysis of β-(1-4) xylose units by rumen microbes (*Butyrivibro fibrisolvens* and *Prevottela ruminocola*) with xylanase (linkage 1); Hydrolysis of α-(1-3) arabinose side chain by rumen microbes (*Butyrivibro fibrisolvens* and *Prevottela ruminocola*) with arabinoxylanase (linkage 2); Hydrolysis of ester linkages by first and second-generation silage inoculants may not be possible without ferulic acid esterase (linkage 3). Modified from Niňo-Medina et al. (2010).

Whereas xylans and arabinoxylans could potentially be hydrolyzed by fibrolytic rumen bacteria (Hespel et al. 1995; Figure 1.1), ferouyl esters limit ruminal fibre digestion by inhibiting attachment (Akin et al. 1988) and growth (Varel and Jung 1986) of the major fibrolytic ruminal bacteria. De-esterification of the FA-arabinoxylan ester bond offers an opportunity to increase the utilization of forage fibre (Figure 1.1). The *in vitro* ruminal degradability of NDF increased in corn forage (ferulate ester; *sfe*) developed for its reduced ester linkages as compared to isogenic counterparts (Jung and Philips 2010; Jung et al. 2011). Intake, digestibility and milk production was also higher for cattle fed *sfe* silage-based diets as compared to diets that contained isogenic and control silages (Jung et al. 2011) but this is not commercially available yet.

The effects of FA on digestibility and animal performance was originally thought to be limited to its adverse effects on fibre digestion through its association with plant polysaccharides and other related phenolics such as lignin. However, the formations of FA-protein complexes indicate that FA may reduce the digestibility of protein as well. Rawel et al. (2010) have suggested that under autoxidative conditions, FA may also react with proteins via a semiquinone radical pathway to form protein derivatives with slight changes in structure and physico-chemical properties. They also suggested that hetero-cross-linkages may also occur between protein and arabinoxylans that contain FA moieties esterified to arabinose, which further reduce protein availability.

There are fewer studies that have examined the effects of FAE-producing silage inoculants on silage fibre digestibility. Inoculation of corn and ryegrass silages with third- generation silage inoculants improved ruminal fibre digestibility in steers (Nsereko et al. 2008). However, no significant relationship between the level of FAE activity of the inoculant and NFD digestibility was observed. The authors consequently attributed the improved ruminal digestibility to the ability of the inoculated LAB to grow and become established in the silo during fermentation. A similar consistent result could not however be obtained in a subsequent study as improvement in fibre digestibility was not observed in one of two corn hybrids assessed (Kang et al. 2009).

Currently, the ensiling of high DM forage in the gramminae family is challenging owing to their low soluble carbohydrate concentration as a result of polymerization, and esterification with FA as the plant matures (Jung and Allen 1995). Treatment of forages with exogenous FAE enzyme has been shown to hydrolyze feroulylated polysaccharides and increase the release of WSC (Krueger et al. 2008). It is hypothesized that if esterified hemicelluloses could be hydrolyzed through inoculation of silage during fermentation, free xylose and arabinose could be made available as substrates for fermentation into lactic and acetic acids (McDonald 1981), increasing the availability of fermentable substrates.

Even though Faulds and Williams (1995) showed that the extent to which FA is released from plant cell walls due to decoupling of feruloylated polysaccharides was directly proportional to FAE activity, Nsereko et al. (2008) suggested that release of FA as a result of complete hydrolysis of hemicellulose may not directly reflect FAE activity. This is because FA cross-linkages can be cleaved without releasing ferulic acid. They also proposed that silage inoculants with FAE activity must include homofermentative LAB that elicit a rapid decline in silage pH without inhibiting FAE activity. Therefore, for a fibrolytic inoculant to be effective at improving ruminal fibre digestion, esterase activity should be maintained in the presence of low pH. This will in turn stop further fibre hydrolysis and render hemicellulose partially hydrolysed since extensive hydrolysis during ensiling could increase the level of indigestible fibre in silage and thereby lower its overall digestibility (Thiago et al. 1992a; van Vuuren et al. 1995; Sheperd and Kung 1996) and the amount of substrates available for rumen microbial growth (Charmley 2001). Treatment of corn silage with a cocktail of fibrolytic enzymes at ensiling decreased NDF concentration of the silage but increased the proportion of the indigestible fraction thereby decreasing NDF digestibility *in vitro.*This suggests that the exogenous enzyme cocktail hydrolyzed the digestible components of the NDF during silage fermentation leaving NDF residue of lower digestiblility (Sheperd and Kung 1996). The hall mark of esterase

inoculants should therefore include improved fermentation, extended aerobic stability and improved ruminal fibre digestibility.

1.1.5. Effects of Forage Chop Length on Silage Fermentation and Aerobic Stability

In Canada, barley silage is often chopped to a theoretical length of cut (TLC) of 1.0-3.8 cm (Savoie et al. 1992; Soita et al. 2002; 2003; Zahiroddini et al. 2004). Chopping forage to a TLC greater than 1.9 cm prior to ensiling impedes packing and exclusion of air from the silo, slows down the rate of acidification and enhances penetration of air into the silage upon feedout, thereby increasing the susceptibility of the silage to aerobic deterioration. Chopping forage to a TLC greater than 1.5 cm resulted in silages with higher pH, and lower WSC and DM losses compared to those chopped at 7.5 cm or 15 cm (Panditharatne et al. 1986). In other studies, DM losses were lower for medium (1.3 and 2.5 cm) than for lower (0.63 cm) and higher (3.8 cm) TLC (Savoie et al. 1992). Plasmolysis and release of WSC for fermentation is lower in long-chopped and poorly packed silages as long-chopped forage has smaller surface area for microbial fermentation.

Data from Ruppel et al. (1995) suggest that higher silage packing density was associated with improvements in aerobic stability at the feed-out face of bunker silos. This is because loosely packed silages enhance the rate of ingress of air into the silage thereby exposing silage microorganisms to oxygen and increasing the rate of deterioration and heating of the silage (Wilkinson and Davies, 2012). In most mini silo experiments, aerobic stability is often assessed by placing loose silage samples in a container and exposing it to air hence the effect of chop length in most mini silo experiments is expected to be negligible.

1.1.6. Effects of Silage Chop Length on Feed Intake, Digestibility and Growth Performance

Another method of evaluating the characteristics of forages for ruminant production is to measure its physical effectiveness. Physically effective NDF

(peNDF) is defined as that dietary fibre source, which effectively stimulates rumination and salivation. It is the physical characteristics of fibre (particle size) that influence chewing activity and promote the stratification of ruminal contents (i.e., establishment of the forage mat) and is distinguished from effective NDF (eNDF) which relates to the sum total ability of a feed to replace forage or roughage in a diet without influencing milk fat composition (Mertens 1997).

Forage particle length affects feed intake, rate of digestion and rumen fermentation. Increasing the TLC of a forage increases the physically effectiveness of its fibre. Physical effective fibre is that part of the fibre that stimulates chewing. Chewing is very important in high producing ruminants to promote ruminal health through a reduction in ruminal acidosis and associated metabolic diseases. Copious production of saliva during chewing improves ruminal health because of the buffering effect of sodium bicarbonate in the rumen when the masticated feed is swallowed. The concept of physical effective fibre incorporates chemical (NDF concentration) and physical (particle length) characteristics of a diet and is expressed often in the form of physical effective NDF. Thus, it is not only the NDF content of the diet that matters, but the physical form of the fibre as well.

A method for measuring the physical effectiveness of a diet was previously developed using the Penn State Particle Separator (PSPS) that had 19-mm and 8-mm sieves to measure the proportion of DM retained on these sieves (Lammers et al. 1996). The approach was later modified to include a third sieve (1.18 mm) since a large proportion of particles passed through the 19 mm and 8 mm sieves (Kononoff et al. 2003). The modified version of the PSPS has since received wide-scale use for measuring the physical effectiveness or physical effective NDF of forages even though others still suggest that the original PSPS with two sieves (19-mm and 8-mm) was a better system for differentiating diets with varying forage particle sizes (Yang and Beauchemin 2006).

Yang and Beauchemin (2006) have compared total and fractional methods of determining peNDF of forages and have concluded that even though calculating the peNDF content of diets based on the fractional NDF content

retained by the individual sieves, increased the values compared to the total NDF content of the sample, it did not change the classification of diets based on their peNDF, particularly if the diets did not vary in proportion of concentrate. They suggested that for diets without varying concentrate level, total peNDF determination was acceptable for ranking diets and the additional laboratory analysis required to estimate fractional NDF was not merited or practical at the farm level.

Other methods of measuring the physical effectiveness of forage fibre have also been suggested. Armentano and Pereira (1997) developed a method to measure the physical effectiveness of fibre based on animal responses such as chewing, ruminal acetic: propionic acid ratio, rumen pH and milk fat concentration. The problem with this method of assessment is that non-forage fibre sources in a diet such as oil may have significant influence on milk fat levels. Consequently, results due to physical effective fibre may be confounded by non-forage fibre nutrients in the diet. The National Research Council (NRC; 1989) guidelines recommends an optimum of 25-28% NDF and 19-21% ADF to meet the physical effective fibre of high producing dairy cows, with at least 75% of the NDF coming from forages.

Improvements in NDF disappearance of silage chopped to a TCL of 0.95 cm or 1.27 cm and incubated in macro *in situ* bags without further grinding (4-6 mm) was found to be inconsistent between long and short cut corn silages (Johnson et al. 2003). However, reducing the TLC of barley silage from 0.47 cm to 1.88 cm reduced the ruminal and total tract retention times, increased DMI of steers by 18%, improved the apparent digestibility of DM and NDF, but had little effect on ruminal VFA concentration (Soita et al. 2002). The increased intake was attributed to faster rate of microbial degradation due to increased surface area for microbial attachment and degradation of silage, resulting in a faster particulate passage rate. However, peNDF of corn silage diets had no effect on passage rate or retention time in the gastrointestinal tract of dairy cows (Yang and Beauchemin 2007). The DMI of dairy cows fed diets that included corn silage cut short (0.48 cm), medium (1.59 cm) or long (2.86 cm) did not differ when the silage accounted

for 46% of the diet (Yang and Beauchemin 2006). These scientists concluded that increasing the TLC of silage from 1.59 cm to 2.86 cm only marginally increased the peNDF of the diet. Increasing the peNDF of a barley silage diet may have negative impact on growth performance especially through lower efficiency of feed use due to poor microbial protein synthesis, a factor that must be balanced with the benefits of reducing the risk of ruminal acidosis when peNDF is increased (Yang and Beauchemin 2006). Total digestibilities of organic matter and NDF were linearly decreased with increasing dietary peNDF and ruminal microbial protein synthesis and microbial efficiency were numerically lower with the high peNDF than with the low or medium peNDF diets. However, this did not affect total starch or N digestibility or milk production (Yang and Beauchemin 2006). In other studies, chopping silage to a TLC of 0.6 cm (lower), 1.3, 2.5 cm (medium) or 3.8 cm (higher) also had no consistent effects on feed intake (Savoie et al. 1992).

While peNDF of the forage component of the diet constitute the main determinant of the physical effectiveness of the diet, the ratio of forage: concentrate greatly influences the peNDF of the diet. Yang and Beauchemin (2007) have clearly demonstrated that the degree of acidosis in cattle fed diets with similar peNDF levels but different forage: concentrate ratios, varies as the ruminal fermentability of feed is not accounted for by the peNDF concept. Furthermore, the processing index, physical effectiveness and fermentability of the grain may also contribute to differences in the level of acidosis in diets of similar peNDF (Zebeli et al. 2010).

In finishing feedlot cattle production systems where fibre in the diet ranges from 10-20%, forage fibre contributes 2.5-7.5% of digestible energy intake (Galyean and Goetsch 1993) and accounts for 69.9% of the variation in DMI. Similarly, the percentage of dietary NDF and eNDF supplied by forage accounts for 92% and 93%, respectively, of the variation in DMI (Galyean and Defoor 2003). However, other factors such as time spent ruminating may also affect DMI. In general, increasing the physical effective fibre of silage increases the time spent chewing and ruminating (Beauchemin and Buchanan-Smith 1989; Sotia et al.

2000) thereby increasing the amount of saliva produced and consequently increasing ruminal pH. However, this relationship has not always been consistent; increasing the physical effective fibre of a diet linearly increased ruminating and total chewing times, but had no effect on ruminal pH (Beauchemin and Yang 2005) or VFA concentrations (Yang and Beachmin 2006). This is because in low-forage diets, increased forage particle length does not completely alleviate subacute acidosis as fermentability of such diets is often greater than the changes in chewing activity and buffering capacity associated with increased forage chop length (Yang and Beauchemin 2007).

1.1.7. Overall Hypothesis, Goal and Objectives

Given that barley and corn silages are the mainstay of the feedlot industry in North America, improvements in their fermentation and nutritive value is expected to greatly impact feedlot operations in this region.

The general null hypothesis of this thesis was that ferulic acid esterase inoculants will not impact fermentation, aerobic stability and nutritive value of barley silage. The overall goal of this thesis was to improve the nutritional value of silages through bacterial inoculation.

The following objectives were formulated to achieve the set goal:

1. To compare the fermentation characteristics, aerobic stability and nutritional value of barley and corn silages ensiled with or without a first-generation homolactic inoculant (Chapter 2).
2. To determine the effects of a third-generation silage inoculant on the fermentation, aerobic stability and nutritional value of barley silage in a growing diet for feedlot cattle (Chapters 3 and 4).
3. To determine the effects of a third-generation silage inoculant and forage chop-length on the fermentation characteristics, aerobic stability and nutritive value of barley silage in a finishing diet for feedlot cattle (Chapter 5).

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CHAPTER 2: COMPARISON OF THE FERMENTATION CHARACTERISTICS, AEROBIC STABILITY AND NUTRITIVE VALUE OF BARLEY AND CORN SILAGES ENSILED WITH OR WITHOUT A MIXED BACTERIAL INOCULANT[1]

2.1. INTRODUCTION

Barley and corn silage are the main forages used in feedlot cattle diets in North America. In western Canada, barley has been the traditional crop for ensiling, however, within the last two decades the seeded area for corn has steadily increased (Statistics Canada 2008). This expansion reflects the development of low-heat unit corn hybrids that can be grown at more Northern latitudes. During this period, the acreage seeded to barley in these regions has declined suggesting a shift from barley to corn silage production. The agronomic costs associated with growing corn are higher than those of barley, but in southern Alberta, DM yield of corn per ha (Beres 2008**)** can be twice that of barley (McKenzie et al. 2004).

Although ensiling technologies have improved considerably, the process is still often unpredictable due to variation in epiphytic bacterial populations and forage composition. Bacterial inoculants have been used to reduce variation in the ensiling process usually by accelerating the post-ensiling decline in pH and improving DM and nutrient retention (Kung and Ranjit 2001). Inoculants can also improve the quality of silage upon feed-out through improved aerobic stability (McAllister et al. 1995; Filya 2003). However, the efficiency of fermentation of inoculated forages still largely depends on interactions of the microbial species in the inoculant with epiphytic microbial populations and chemical components within the forage (Muck 1988; McAllister and Hristov 2000). At optimal maturity for ensiling, barley forage is high in water-soluble carbohydrates (WSC; 100 - 200 g kg^{-1} DM; McAllister et al. 1995; Hargreaves et al. 2009) providing ample

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substrate for the production of lactic acid needed to ensure preservation (McAllister et al. 1995; Zahiroddini et al. 2004; Hargreaves et al. 2009). In comparison, corn harvested at the 2/3 milkline maturity stage has lower levels of WSC (30 - 100 g kg^{-1} DM; Johnson et al. 2003; Filya 2004; Filya et al. 2006), and may have higher populations of epiphytic lactic acid-producing bacteria (LAB; Bolsen et al. 1992; Muck 2004).

Ensiling responses of forages to inoculants can be inconsistent (McAllister et al. 1995; Kung et al. 1993; Muck 2004) and it has been proposed that consistent improvements in fermentation would be more likely if forage-specific inoculants were developed (McAllister et al. 1995; McAllister and Hristov 2000). Others have proposed that a more complex mixture of bacterial species would be efficacious across a range of forages (Muck 1996) and as a result several commercial suppliers have developed inoculants on this premise. The value of inoculants containing mixtures of different strains of *Lactobacillus, Enterococcus* and *Pediococcus* spp. have been assessed on barley (Zahiriddini et al. 2004), corn (Bolsen et al. 1992; Muck 2004), wheat (Sucu and Filya 2005) and alfalfa (Bolsen et al. 1992; Filya et al. 2007) silages. In these studies, the inoculants consistently accelerated pH decline in barley and wheat but the results in alfalfa and corn silages were inconsistent.

Given that the ultimate aim of ensiling is to preserve nutrients and improve their biological availability, the merit of inoculants may be best assessed through improvements in the efficiency of milk (Kung et al. 1993) or meat (Schaefer et al. 1989; Zahiroddini et al. 2004) production. A recent review (1989-2009) across a range of forages showed that homolactic bacterial inoculants had no effect on ADG in 80%, reduced it in 10% and increased it in only 10% of the studies examined (Adesogan et al. 2009). Even though barley and corn are the most widely used silages in Canadian feedlots, a direct comparison of the merit of their inoculation or their feed value in feedlot diets has not been conducted. Therefore, the objectives of this study were to (1) compare the fermentation characteristics and aerobic stability of barley and corn ensiled with or without a

mixed inoculant, and (2) to determine the effect of these two silages on the growth performance of backgrounded feedlot steers.

2.2. MATERIALS AND METHODS

2.2.1. Forages

Corn (*Zea mays*; 39T67; Pioneer Inc., USA) and barley (*Hordeum vulgar*, L.; Chigwell, Field Crop Development Centre, Lacombe, Canada) were planted on 2007 May 24 and 27, respectively at the Lethbridge Research Centre. Approximately 1 ha of a single uniformly irrigated field was divided into equal halves and seeded with each crop. Barley was fertilized with 125 kg N ha^{-1} pre-seeding, and 12.5 kg ha^{-1} N and 57 kg PO_4 ha^{-1} post-seeding. Corn was fertilized in a similar manner post-seeding, but was provided with 170 kg N ha^{-1} at pre-seeding. Soil tests indicated that plant requirements were satisfied and crops were planted in a manner that the water requirements of both could be supplied through a central pivot irrigation system.

Barley was swathed on 2007 Aug. 06 at mid dough stage and wilted (430-460 g kg^{-1} DM) while corn was harvested on 2007 Oct. 01 at 2/3 milkline maturity (320-370 g kg^{-1} DM). The forages were exposed to early winter frost prior to being harvested. The forages were chopped (9.5 mm theoretical length of cut) with a John Deere 6610 forage harvester. To match industry standards, the kernel processor was activated during the harvesting of corn, but not during the harvesting of barley. Both chopped forages were ensiled in laboratory-scale mini silos and Ag-Bags® (10 × 150 feet; Ag-Bag® Warrenton, OR, USA).

Chopped forage was delivered by truck to two Ag-Bag® silage baggers with each truckload being weighed individually. At the bagger, forage was sprayed just prior to compression in the bag either with water (uninoculated) or an inoculant using ATV sprayers (AG Spray Equipment, Hopkinsville, KY). Trucks alternated loads to each of the baggers in an effort to minimize variation in forage quality among treatments. Approximately 150 tonnes of each silage type was generated with the inoculant being applied at a concentration to achieve 1.0 x 10^5 CFU g^{-1} of forage.

2.2.2. Mini Silo Experiment

Samples of each forage type were divided into two lots (24 kg each), spread on separate plastic sheets and sprayed with either water, i.e., uninoculated (barley: B; corn: C) or with an inoculant, SIL-ALL® (Alltech Inc., Canada; inoculated barley: IB; inoculated corn: IC). The inoculant consisted of a mixture of *L. plantarum*, *E. faecium* and *P. acidilactici.* The inoculant solution was prepared by dissolving 0.24 g of the inoculant in 100 mL of water and was sprayed onto 24 kg of pre-weighed forage spread on a plastic sheet yielding 1.0×10^5 CFU of LAB per g of fresh forage. The four corners of the sheet were drawn together and the forage was tumbled inside the sheet for approximately 3 min, followed by hand mixing for an additional min to ensure even inoculation. Uninoculated forages were sprayed with an equal volume of water.

Frseh forages (2.5 - 3 kg) were then packed in mini silos with a hydraulic press to a density of ~ 240 kg m^{-3}. Each silo was weighed with its cap prior to being filled and immediately after sealing and stored at ambient temperature (16-22 ^oC). Triplicate silos for each treatment and sampling date were prepared and opened after 1, 2, 3, 7, 14, 42 days. The final silos for corn were opened after 63 d and after 77 d for barley. Prior to being ensiled (d 0), triplicate samples of each forage were collected for chemical and microbial analyses. Silos were weighed prior to opening to estimate DM loss. The contents of triplicate mini silos were thoroughly mixed after opening and subsampled for laboratory analysis.

2.2.2.1. Aerobic Stability

Triplicate 400 g silage samples obtained from combining subsamples from each triplicate silo opened on the final day of ensiling were placed into separate 4 L insulated containers, covered with two layers of cheesecloth and stored at 20 ^oC for 7 d. Two Dallas Thermochron iButtons (Embedded Data Systems, Lawrenceburg, KY), were embedded in the lower and mid layers of the silage mass in each container and also in the room where the silos were stored, for recording temperature. Ambient temperature and the temperature in each

container were simultaneously monitored at 15 min intervals for 7 d. The contents of each container were thoroughly mixed and sampled after 1, 3, and 7 d of exposure for chemical and microbial measurements.

2.2.3. Feedlot Experiment

2.2.3.1. Animals and Feeding

Ag Bags® were opened after 63 d for corn and 77 d for barley. The ensiled forages were used to formulate four total mixed rations (TMR): uninoculated barley silage-based diet, B; inoculated barley silage-based diet, IB; uninoculated corn silage-based diet C, and an inoculated corn silage-based diet; IC (Table 2.1.). One hundred Angus × Hereford crossed-bred steers (303 ± 2.3 kg) were weighed for two consecutive days, stratified by weight and randomly assigned to one of four treatments within each of three sections (block) of a feedlot barn resulting in 25 steers per treatment. Steers were housed in individual 2.5 m × 3.0 m pens in the Individual Feeding Barn at the Lethbridge Research Centre. A Calan Data Ranger was used to mix silage, barley grain and supplement and the steers were fed once daily starting at 0800 h. The supplement was formulated to meet or exceed the recommended nutrient requirements of growing steers (National Research Council 1996). Offered feed was recorded daily, orts were measured weekly and steers were weighed every 28 d. Feed intake, average daily gain and feed:gain ratio were estimated over an 84 d period. Feed efficiency was expressed as live weight gain per dry matter inatke.

Table 2.1. Dietary and nutrient composition of inoculated or uninoculated barley and corn silage-based TMR fed to feedlot steers

	Barley		Corn			*P* of effects[y]		
	Uninoculated	Inoculated	Uninoculated	Inoculated	SEM[z]	I	F	I x F
Diet composition (g kg^{-1}DM silage)								
Barley silage	599.8	599.8	–	–	–	–	–	–
Corn silage	–	–	599.8	599.8	–	–	–	–
SRB grain[x]	350.1	350.1	350.1	350.1	–	–	–	–
Supplement[w]	50.0	50.0	50.0	50.0	–	–	–	–
Total mixed ration (g kg^{-1}DM silage)								
Dry matter	480.2	474.0	453.4	443.4	3.13	<0.001	0.012	0.542
Organic matter	931.8	932.0	951.8	951.7	2.18	0.975	<0.001	0.946
NDF	312.0	309.2	330.5	307.7	16.77	0.451	0.615	0.557
ADF	172.7	155.4	182.3	182.1	1.97	<0.001	<0.001	<0.001
Starch	324.6	334.5	403.0	399.7	15.35	0.831	<0.001	0.671
Crude protein	146.0	146.2	120.5	119.7	1.75	0.781	<0.001	0.764
ADIN (of total N)[v]	37.9	37.9	57.2	51.5	1.90	0.142	<0.001	0.142
NH_3-N	3.6	4.1	4.1	3.5	0.17	0.857	0.017	0.740

[z]SEM, pooled standard error of least-square means (n = 7).

[y]I, inoculation (inoculated *vs* uninoculated); F, forage type; I x F, inoculation x forage type interaction. Inoculated silages were treated with *Lactobacillus plantarum*, *Enterococcus faecium*, *Pediococcus acidilactici* (Alltech Inc., Canada) at a combined rate of 1.0×10^5 CFU g^{-1} forage.

[x]SRB, steam-rolled barley.

[w]The supplement contained (g kg^{-1} DM) canola meal (1000); urea (20); limestone (250); sodium chloride (3%); ground barley grain (590); feedlot premix (10). The pre-mix contained: calcium carbonate (348), Zinc sulphate (284), manganous sulphate (146), copper sulphate (103), ethylene diaminediiodic acid (20; as an 80% preparation), selenium (50), cobalt sulphate (10), vitamin A (1000 000 IU g^{-1}; 170), vitamin D (500 000 IU g^{-1}; 20) and vitamin E (47).

[v]ADIN, acid detergent insoluble nitrogen.

2.2.4. *In situ* Incubation

Samples of the silage (500 g) used in formulating the diets were collected weekly, pooled monthly and frozen (-40 ^{o}C). Frozen silage samples were lypholized and ground through a 4-mm screen. Monthly samples of each treatment were combined, thoroughly mixed and weighed (5 g per bag) into six monofilament polyester bags (8 cm × 10 cm; 51-μm pore size; Sefar America Inc., Depew, NY). Duplicate polyester bags for each sampling time point were incubated in the rumen of three ruminally-fistulated cows for 1, 2, 4, 8, 16, 24, 48 and 72 h. Bags were placed into large mesh retaining sac (20 cm × 30 cm; 3-5 mm pore size) to ease retrieval.

Cows were fed a TMR similar to that provided to steers with the silage component consisting of an equal mixture of the control barley and corn silage. Upon removal, bags were washed according to the protocol previously described by Zahiroddini et al. (2004). Bags that had not been incubated in the rumen were also included in the washing procedure to estimate soluble losses. Kinetics parameters of *in situ* organic matter disappearance (OMD) were estimated by a non-linear regression procedure of SAS (1991) using the model of Ørskov and McDonald (1979):

$$P = a + b(1 - e^{-c(t-L)})$$

where P is the proportion (%) of OM disappearing at time t; a is the rapidly disappearing fraction (%); b is the slowly disappearing fraction (%); c is the rate at which b is disappearing (%/h); t is the time (h) bags were incubated in the rumen and L is the lag time (h). The effective percentage ruminal OMD (P) in each cow was estimated using the following model:

$$P = a + [bc/(c + k)]\, e^{-(c+k)L}$$

assuming a ruminal particulate outflow rate (k) of 0.05 h^{-1} for silage particles (Agricultural Development and Advisory Service 1986).

The experiment was approved by the Animal Care Committee of Lethbridge Research Centre and the steers were cared for and managed according to the guidelines of the Canadian Council on Animal Care (1993).

2.2.5. Chemical Analysis

Forage and silage samples were processed and analyzed for water-soluble carbohydrates (WSC), ammonia nitrogen (NH_3-N) and starch as described by Zahiroddini et al. (2004). Volatile fatty acids (VFA) and lactic acid were determined by the methods of Kudo et al. (1989) on a Hewlett Packard model 5890A Series Plus II gas-liquid chromatograph (column: 30 m FFAP fused silica capillary, 0.32 mm I.D., 1.0 m film thickness, Phenomenex, Torrance, CA, USA). Total nitrogen (N) was determined by elemental analysis (Dumas Nitrogen) on an NA1500 Nitrogen/Carbon analyzer (Carlo Erba Instruments, Milan, Italy). Crude protein was estimated as nitrogen × 6.25. The DM of the fresh forages, silage samples and TMR was determined by drying at 105^{o}C for 24 h. Organic matter (OM) was estimated by ashing 1 g of sample in a muffle furnace at 550^{o}C for 5 h. Neutral detergent fibre (NDF) was analyzed with the addition of sodium sulfite and α-amylase while acid detergent fibre (ADF) was analyzed with α-amylase omitted from the procedure, using the Ankom 200 system (Ankom Technology Corporation, Fairport, NY). Nitrogen in ADF residues (acid detergent insoluble nitrogen, ADIN) was measured as described above.

Fifteen grams (15 g) of fresh forage or silage samples were mixed with 135 mL of deionized water, blended for 30 sec and filtered through two layers of cheesecloth and the pH of the filtrate was measured with a Symphony pH meter (VWR, Mississauga, ON). The rate of pH decline was estimated for the first 7 d by calculating the difference between the pH of the forage at harvest and the pH of the silage on d 7 of ensiling and dividing it by 7 d. For assessing buffering capacity (BC), 15 g of forage was homogenized in 200 mL of deionized water, pH of the filtrate (~ 206 mL) was lowered to 3.0 with 0.2 N HCl, and then the sample was titrated back to 4.0 with 0.2 N NaOH. The BC was defined as the milliequivalents of NaOH required to raise the pH from 4.0 to 6.0 (Bolsen et al.

1992). The co-efficient of fermentation (FC) of each forage was predicted according to Weissbach and Honig (1996) as follows:

$$FC = DM\% + 8WSC/BC$$

where BC is the buffering capacity of the forage.

2.2.6. Microbial Analysis

For microbiological analyses, forage or silage (10 g) was added to 90 mL of sterile 70 mM potassium phosphate buffer (pH = 7.0) and agitated for 60 s at 260 rpm in a Stomacher 400 Laboratory Blender (Seward Medical Limited, London, UK). The suspension was serially diluted (10^{-2} to 10^{-7}) and 100-µL aliquots of each dilution were spread in triplicate onto semi-selective lactobacilli media (MRS; Oxoid, Basingstoke, Hampshire) for enumeration of LAB (LAB; Hill and Hill 1986), onto nutrient agar (NA; Difco, Detroit, MI) for the enumeration of total culturable bacteria (TB), and onto Sabouraud's dextrose agar (SDA; Difco, Detroit, MI) for the enumeration of yeasts and moulds. Lactobacilli MRS agar and NA were amended with 200 µg mL^{-1} of cycloheximide (Sigma, Mississauga, ON) and SDA with 100 µg mL^{-1} each of tetracycline and chloramphenicol. Lactobacilli MRS agar and NA plates were incubated at 37°C for 24-48 h while SDA plates were incubated at ambient temperature for 72 h. Colonies were counted from plates containing a minimum of 30 and a maximum of 300 colonies.

2.2.7. Statistical Analysis

All data were analyzed by ANOVA using the Mixed procedure of SAS (1991). The data on microbial populations were transformed to $\log_{10}$ CFU g^{-1} of DM of forage or silage prior to statistical analysis. Silage fermentation and aerobic stability data were analyzed for the main effects of inoculation and forage type, and inoculation × forage type, and inoculation × forage type × day interactions in a completely randomized design using the model below:

$$Y_{ijkl} = \mu + T_i + F_j + D_k + TF_{ij} + TFD_{ijk} + e_{ijkl}$$

where Y_{ijkl} is the observation (pH, chemical or microbial data etc); μ is the overall mean effect**;** T_i is the effect of inoculation (uninoculated or inoculated); F_j is the effect of forage type (barley or corn); D_k is the day of ensiling or aerobic exposure; TF_{ij} is the effect of inoculation × forage type interactions; TFD_{ijk} is the effect of inoculation × forage type × day interactions and e_{ijkl} as the residual error. Least-square means of interactions that showed significant differences were separated by a pair-wise Fisher's LSD test at $P \leq 0.05$ unless otherwise stated, using the PDIFF option of LSMEANS. Mean daily temperatures of all four silages and the corresponding ambient daily temperature were analyzed and differences between the ambient temperature and each of the four silages on each day of aerobic exposure were compared using the Dunnett's adjustment option of LSMEANS of SAS.

The parameters (OMD, *a*, *b*, *a*+*b*, and c) generated from the *in situ* data were analyzed for the effects of inoculation and forage type and inoculation × forage type interactions in a completely randomized block design with cow as random factor using the following model:

$$Y_{ijkl} = \mu + T_i + F_j + TF_{ij} + C_k + e_{ijkl}$$

where Y_{ijk} is the observation (OMD, *a*, *b*, *a*+*b*, and *c*); μ is the overall mean effect**;** T_i is the effect of inoculation (uninoculated or inoculated); F_j is the effect of forage type (barley or corn); TF_{ij} is the effect of inoculation × forage type interactions, *k* is the random effect of cow (1-3) and e_{ijkl} is the residual error effect.

All data on DM intake (DMI) and growth performance (weight gain, ADG and feed efficiency) were analyzed including initial body weight as a covariate. Tests of homogeneity of slopes between the covariate (initial weight), and DMI and growth performance parameters were found to be linear and the data were analyzed as a completely randomized block design with section of the feedlot

barn as a random (block) factor and initial weight as the covariate using the following model:

$$Y_{ijklm} = \mu + T_i + F_j + TF_{ij} + B_l + \beta W_k + e_{ijklm}$$

where Y_{ijkl} is the observation or response variable (DMI, ADG, feed efficiency, and total weight gain); μ is the overall mean effect**;** T_i is the main effect of inoculation (uninoculated or inoculated); F_j is the main effect of forage type (barley or corn); TF_{ij} is the effect of inoculation × forage type interaction; β is linear regression coefficient of initial weight on response variable; W_k is initial bodyweight (covariate) of each steer; *l* is the random (block) effect of section of the feedlot barn (1-3) and e_{ijklm} is the residual error effect.

2.3. RESULTS

2.3.1. Forage Characteristics Prior to Ensiling in Mini Silos

The characteristics of the fresh pre-ensiled barley and corn forages are shown in Table 2.2. Dry matter averaged 450 g kg^{-1} DM in barley and 350 g kg^{-1} DM in corn at the time of ensiling. Corn forage had a lower pH, BC and calculated FC than barley but the concentration of CP and WSC in barley was higher than in corn. Starch, OM and ADF contents in corn were also markedly higher than barley. Corn forage similarly had higher epiphytic populations of TB, LAB, moulds and yeasts compared to barley.

2.3.2. Silage Fermentation and Quality

As illustrated in Figure 2.1., inoculation had no effect on pH decline in corn silage throughout the ensiling period. Both IC and C silages had similar ($P > 0.227$) pH and these were lower ($P < 0.001$) than the pH in B and IB. However, inoculation decreased ($P < 0.001$) pH in IB compared to B. The ininoculated and inoculated corn silages also had faster rates ($P < 0.002$) of pH decline compared to B and the estimated rate of decline tended ($P = 0.058$) to be more rapid for IC than IB silage. Dry matter loss during ensiling was not affected ($P = 0.118$) by inoculation

of either silage, but was higher ($P < 0.001$) in barley compared to corn silage (Table 2.3.). Dry matter, OM, ADF, NDF and CP contents were not affected ($P \geq 0.136$) by inoculation, however, OM ($P < 0.001$), ADF ($P < 0.001$) and starch ($P = 0.053$) were higher in corn than in barley while DM ($P < 0.001$), NDF ($P = 0.046$) and CP ($P < 0.001$) were higher in the latter than in the former.

The WSC concentration of IB was reduced ($P < 0.001$) by inoculation compared to B, however, the concentration in both barley silages was higher ($P \leq 0.001$) than that in either IC or C. Concentrations of WSC in B and IB increased by 33% (72.6 g kg^{-1}) and 20% (65.9 g kg^{-1}), respectively compared to the original forage (54.7 g kg^{-1}) during the first 7 d of ensiling (Figure 2.2.), before declining to terminal concentrations of 63.1 and 37.6 g kg^{-1} in B and IB, respectively. In contrast, the concentration of WSC in C (9.7 g kg^{-1}) and IC (11.1 g kg^{-1}) on d 7 was similar to that in the original forage (11 g kg^{-1}), thereafter declining to the low levels measured on d 63. The decline in WSC in IB after d 7 of ensiling coincided with an increase in lactic acid levels up to d 42 of ensiling (Figure 2.3.). This culminated in higher ($P = 0.006$) terminal lactic acid concentration in IB silage as compared to other silages (Table 2.3.).

Table 2.2. Chemical and microbiological composition (mean ± standard deviation) of corn and barley forages prior to treatment and ensilage

	Forage[z]	
Item	Barley	Corn
pH	6.16±0.05	5.84±0.01
Buffering capacity[y]	622.95±29.11	295.62±37.65
Co-efficient of fermentation[x]	45.98±0.85	35.28±0.14
Chemical composition (g kg^{-1}DM silage)		
Dry Matter	452.8±8.4	349.8±0.16
Organic matter	937.6±2.1	957.2±0.9
Crude Protein	136.8±1.9	92.8±1.3
ADF	200.3±2.7	240.6±4.0
NDF	407.8±6.8	404.3±4.9
Starch	232.9±3.2	322.8±6.4
ADIN (of total N)	22.5±1.9	39.5±2.5
Water soluble carbohydrates	546.5±7.6	108.8±9.9
NH_3-N	0.8±0.04	0.7±0.03
Microbial composition (Log_{10} CFU g^{-1} DM)		
Total culturable bacteria	7.4±0.1	8.7±0.1
LAB[w]	6.1±0.2	7.4±0.8
Moulds	4.8 ±0.1	5.8±0.1
Yeasts	5.3±0.3	6.9±0.3

[z]Means (±SEM) are samples (n = 3) collected from each forage prior to treatment and ensiling.

[y]Expressed as mEq. of 0.2 N NaOH required to raise pH of 1 kg (DM) of forage from 4 to 6.

[x]Co-efficient of fermentation: DM% + 8WSC/BC (Weissbach and Honig 1996)

[w]LAB, Lactic acid-producing bacteria.

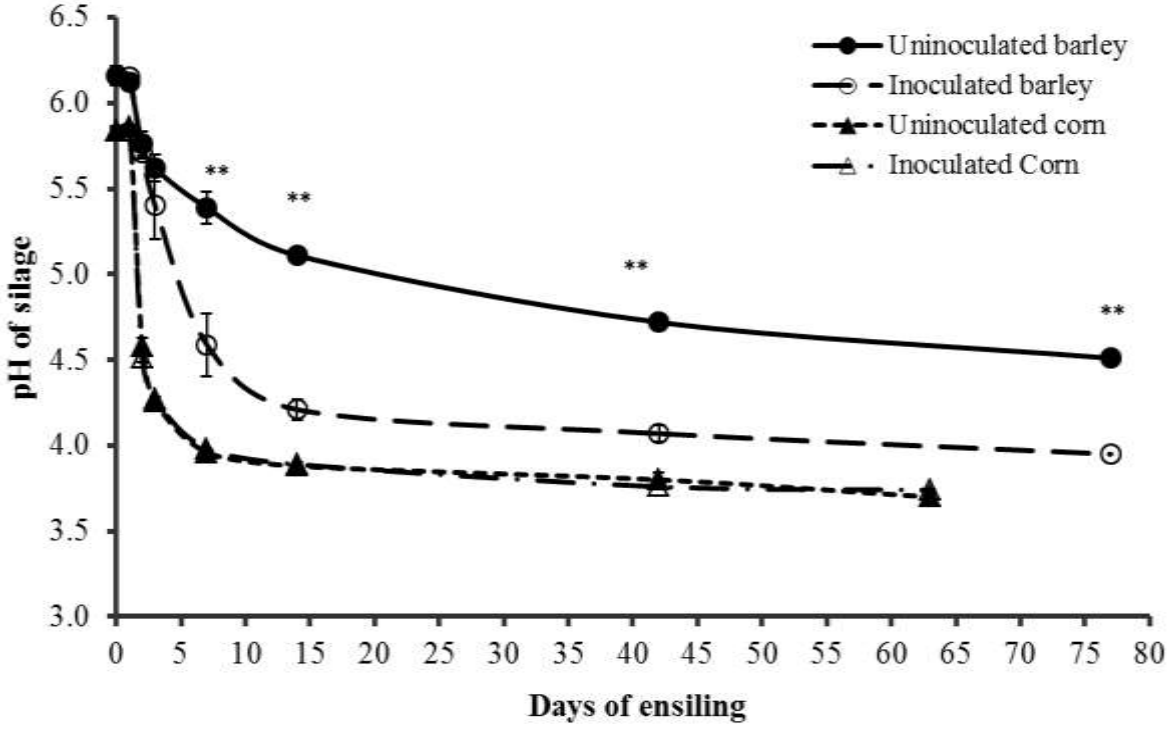

Figure 2.1. Decline in pH of barley and corn silages ensiled with or without inoculant in mini silos. Inoculated silages were treated with *Lactobacillus plantarum, Enterococcus faecium, Pediococcus acidilactici* (Alltech Inc., Canada) at a combined rate of 1.0×10^5 CFU g^{-1} forage. Where visible, bars indicate standard error of the mean. ** indicate days on which inoculation decreased ($P < 0.001$) pH in barley silage but had no effect ($P \geq 0.227$) on corn silage.

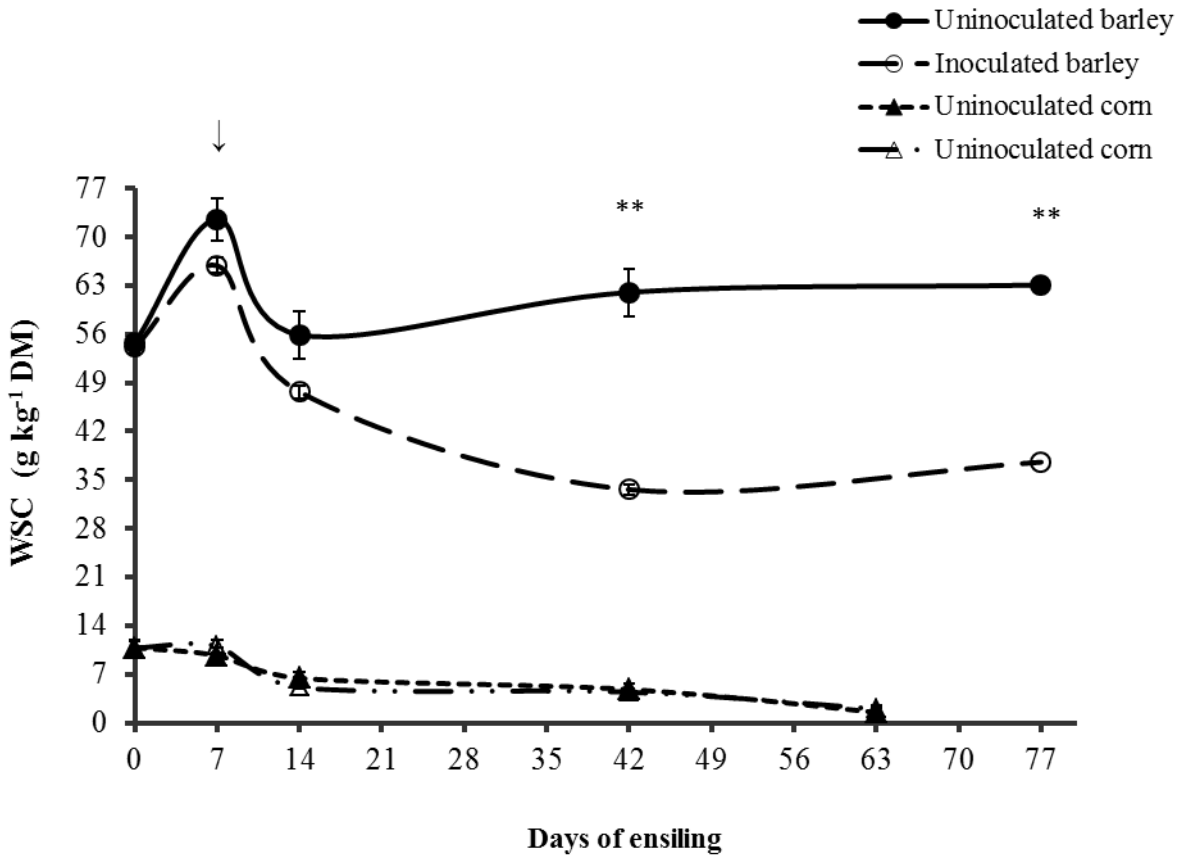

Figure 2.2. Changes in water-soluble carbohydrates (WSC) content of barley and corn silages ensiled with or without an inoculant in mini silos. Inoculated silages were treated with *Lactobacillus plantarum*, *Enterococcus faecium*, *Pediococcus acidilactici* (Alltech Inc., Canada) at a combined rate of 1.0×10^5 CFU g^{-1} forage. Where visible, bars indicate standard error of the mean. Arrow indicates peak in WSC concentration of inoculated and uninoculated barley silages during ensilage; ** indicate days on which inoculation decreased ($P < 0.001$) WSC content in barley silage but had no effect ($P \geq 0.897$) on corn silage.

Lactic acid concentration (Figure 2.3), and thus lactic: acetic acid ratio (8:1) was higher ($P \leq 0.001$) in IB compared to IC and other silages (4:1). Acetic acid concentration in IB was lower ($P = 0.049$) than the concentration in B and IC ($P \leq 0.01$), but tended ($P = 0.085$) to be lower than the level in C (Table 2.3.). Concentration of propionic acid was also lower ($P = 0.006$) in IB silage as compared to B silage, with both being higher ($P \leq 0.001$) than that in C or IC silage. Inoculation did not affect either acetic acid or propionic acid levels in corn silage. Ammonia N concentration, was lower ($P < 0.047$) in C compared to IC but both were higher ($P \leq 0.001$) than either IB or B. Ethanol concentration tended ($P = 0.081$) to be higher in IC compared to other silages and overall was higher ($P = 0.045$) in corn than barley silage.

Yeasts populations were not detected (dilution 10^{-1}) in barley silage throughout the ensiling period, they were immediately detectable in corn silage and remained at 4 $\log_{10}$ CFU g^{-1} DM after 63 d (Table 2.3.). Terminal populations of LAB were similar ($P = 0.529$) between C and IC and bothwere higher ($P \leq 0.044$) than those associated with barley silages (Table 2.3.). Although the terminal LAB population in corn silage was not affected by inoculation, the population in B was higher ($P < 0.001$) than that in IB. The number of total culturable bacteria in IB also was lower ($P \leq 0.001$) than other silages.

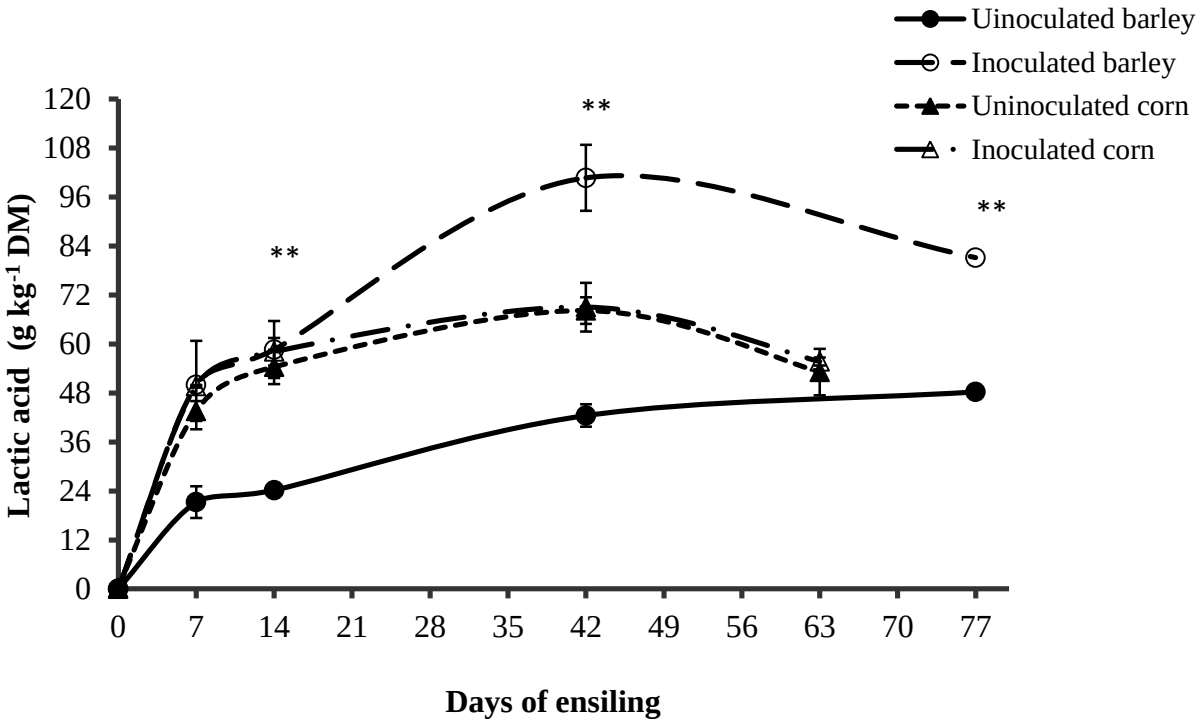

Figure 2.3. Changes in lactic acid concentration in barley and corn silages ensiled with or without inoculant in mini silos. Inoculated silages were treated with *Lactobacillus plantarum, Enterococcus faecium, Pediococcus acidilactici* (Alltech Inc., Canada) at a combined rate of 1.0×10^5 CFU g^{-1} forage.Where visible, bars indicate standard error of the mean. ** indicate days on which inoculation increased ($P < 0.001$) lactic acid concentration in barley silage but had no effect ($P \geq 0.673$) on corn silage.

Table 2.3. Chemical composition, products of fermentation and microbial populations of corn and barley silages ensiled with or without inoculation for 63 d (corn) and 77 d (barley) in mini silos

	Barley		Corn			*P* of effects[z]		
	Uninoculated	Inoculated	Uninoculated	Inoculated	SEM[y]	I	F	I x F
pH	4.51	3.95	3.70	3.74	0.018	<0.001	<0.001	<0.001
pH decline (d^{-1})[y]	0.110	0.225	0.268	0.266	0.013	<0.002	<0.001	0.002
Dry matter loss (g kg^{-1} silage)	78.9	76.4	26.0	41.9	5.14	0.236	<0.001	0.118
Chemical composition (g kg^{-1}DM silage)								
Dry mattter	428.7	431.2	343.0	332.8	6.95	0.598	<0.001	0.390
Organic matter	933.7	932.9	954.1	954.0	1.21	0.690	<0.001	0.795
NDF	409.2	420.8	398.6	402.3	6.81	0.275	0.046	0.569
ADF	222.5	221.4	236.7	249.9	4.63	0.206	<0.001	0.136
Starch	259.4	264.9	305.4	277.2	12.84	0.401	0.053	0.227
Crude protein	142.6	140.7	95.8	92.5	2.00	0.230	<0.001	0.737
Water soluble carbohydrates	63.1	37.6	5.5	6.0	2.48	0.001	<0.001	0.001
Products of fermentation (g kg^{-1}DM silage)								
Lactic acid	48.3	81.2	53.2	55.7	4.15	0.038	0.038	0.006
Acetic acid	13.9	9.8	13.3	15.5	1.24	0.495	0.073	0.033
Propionic acid	2.4	1.9	0.3	0.3	0.11	0.043	<0.001	0.021
Butyric acid	0.1	0. 1	0.0	0.0	0.01	0.878	–	–
NH_3-N[x]	0.2	0. 2	0.4	0.4	0.01	0.275	<0.001	0.064
Ethanol	4.7	4.8	4.9	6.6	0.38	0.05	0.045	0.081
ADIN (of total N)	2.8	3.4	3.2	3.7	0.32	0.083	0.239	0.809
Lactic: acetic acid	3.5	8.3	4.0	3.7	0.39	<0.001	0.001	<0.001
Microbial composition (Log_{10} CFU g^{-1} DM)								
Total culturable bacteria	8.2	7.1	8.7	8.6	0.12	<0.001	<0.001	0.004
Lactic acid-producing bacteria	8.2	7.2	8.6	8.5	0.09	<0.001	<0.001	0.001
Moulds[w]	ND	1.1	0.8	ND	0.48	0.853	0.853	0.199
Yeasts[w]	ND	ND	4.5	4.3	0.13	0.494	–	–

[z]SEM, pooled standard error of least-square means (n = 3); [y]I, inoculation (inoculated *vs* uninoculated); F, forage type; I x F, treatment x forage type interaction. Inoculated silages were treated with *Lactobacillus plantarum*, *Enterococcus faecium*, *Pediococcus acidilactici* (Alltech Inc., Canada) at a combined rate of 1.0×10^5 CFU g^{-1} forage; [x]Estimated for the first 7 d of ensiling as initial pH of forage minus pH at d 7 divided by 7 (pH at d 7 of ensiling: uninoculated barley = 5.39; inoculated barley = 4.59; uninoculated corn = 3.96; inoculated corn = 3.98); [w]significance at P = 0.06.; [u]ND, non detected (dilution 10^{-1}).

2.3.2.1. Aerobic Stability

All four silages were stable for up to 2 d of aerobic exposure, thereafter, the pH of C and IC increased from 4.3 and 4.4 on d 3 to 7.9 and 8.3 on d 7, respectively (Figure 2.4.). Temperatures of C and IC also rose sharply above 10^{o}C on d 2 and peaked at 45^{o}C and 44.6 ^{o}C respectively, by d 6 (Figure 2.5.). In contrast, temperature in B and IB remained below ambient (< 3^{o}C) with pH also remaining near those obtained at the end of the ensiling period. The population of total culturable bacteria in IB was also lower ($P \leq 0.001$) than other silages after 1 and 3 d of aerobic exposure (Table 2.4.).

After 7 d of aerobic exposure, concentration of lactic acid remained relatively high ($P \leq 0.001$) in IB whereas it declined dramatically in corn silage (Table 2.4.). Residual levels of WSC remained fairly constant throughout the aerobic exposure period, remaining very low in both C and IC silage. Residual WSC in B however, increased ($P = 0.010$) from 21 to 26 mg g^{-1} between d 1 and d 3 before declining ($P < 0.001$) to 18.76 mg g^{-1} on d 7.

2.3.3. *In Situ* Ruminal OMD

Inoculation did not affect the kinetics of OMD of either barley or corn silage or the calculated effective OMD (Table 2.5.). However, the size of the slowly disappearing fraction was higher ($P = 0.063$) in barley than corn silage. Inoculation increased ($P < 0.001$) the size of the slowly disappearing (520 *vs* 488 g kg^{-1} DM; $P < 0.001$) and potentially digestible (894 *vs* 871 g kg^{-1} DM; $P < 0.001$) fractions regardless of the forage type.

2.3.4. Feedlot Experiment

The DMI ($P = 0.037$), ADG ($P = 0.002$) were higher and feed efficiency ($P = 0.002$) improved in steers fed barley as compared to corn silage (Table 2.6.). Inoculation of forage did not affect DMI ($P = 0.697$), ADG ($P = 0.999$), or efficiency of gain ($P = 0.912$) of steers.

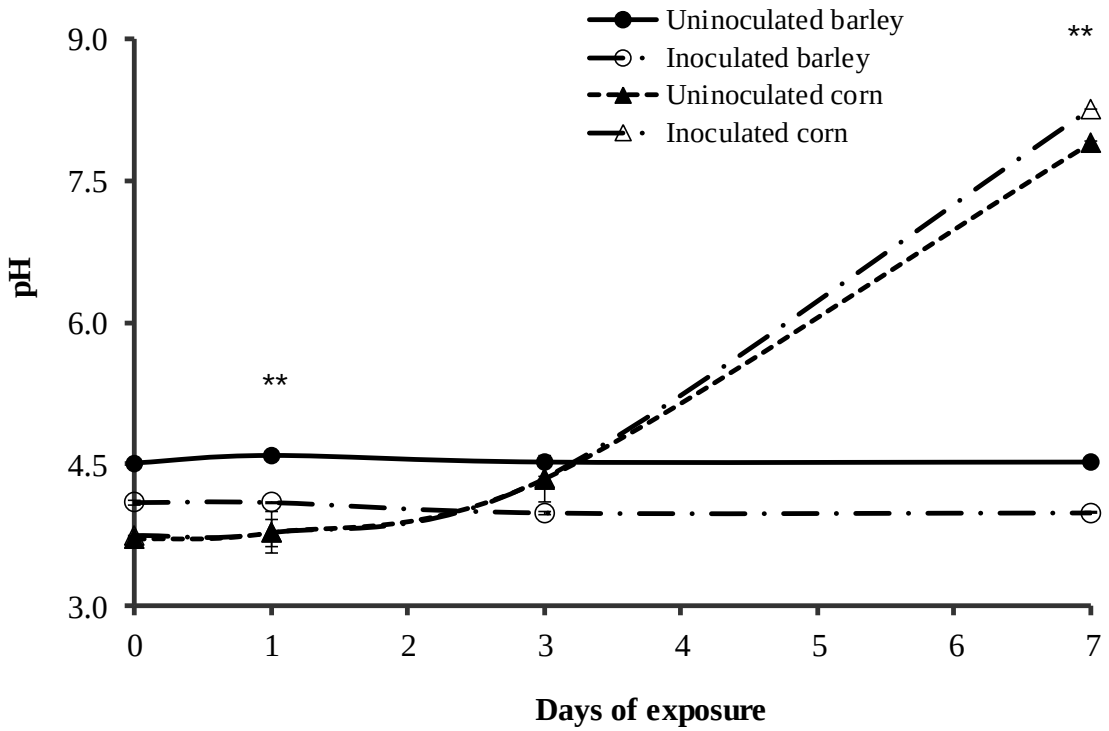

Figure 2.4. Effect of inoculation of barley and corn silages on changes in pH during 7 d of aerobic exposure. Where visible, bars indicate standard error of the mean. ** indicate days on which pH differed ($P < 0.05$) between inoculated and uninoculated barley silages but did not differ ($P \geq 0.117$) between inoculated and uninoculated corn silages. Inoculated silages were treated with *Lactobacillus plantarum*, *Enterococcus faecium*, *Pediococcus acidilactici* (Alltech Inc., Canada) at a combined rate of 1.0×10^5 CFU g^{-1} forage.

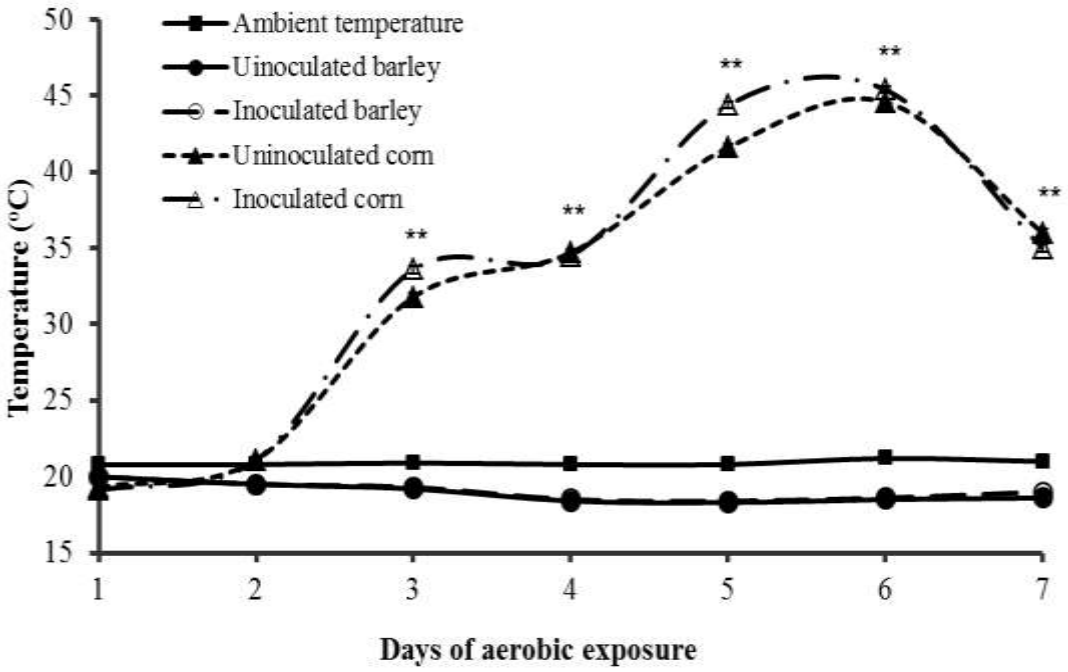

Figure 2.5. Effect of inoculation of barley or corn silage on changes in temperature during 7 days of aerobic exposure. Where visible, bars indicate standard error of least-square means. ** indicate days on which inoculated and uninoculated corn silages had higher ($P < 0.001$) temperatures (>10^{o}C) than ambient temperature while inoculated and uninoculated barley silages were below (< 3^{o}C) ambient temperature. Inoculated silages were treated with *Lactobacillus plantarum*, *Enterococcus faecium*, *Pediococcus acidilactici* (Alltech Inc., Canada) at a combined rate of 1.0×10^5 CFU g^{-1} forage.

Table 2.4. Changes in water-soluble carbohydrates, products of fermentation and microbial composition of inoculated or uninoculated corn and barley silages during a 7-d aerobic exposure

	Barley		Corn			*P* of effects[y]		
	Uninoculated	Inoculated	Uninoculated	Inoculated	SEM[z]	I	F	I x F
Day 1								
Chemical composition (g kg^{-1} fresh weight)								
WSC[y]	21.19	15.06	1.91	2.05	0.968	0.015	<0.001	0.012
Lactic acid	16.83	29.85	19.37	26.46	4.067	0.039	0.919	0.487
Acetic acid	5.86	4.47	5.75	6.33	0.388	0.331	0.054	0.035
Propionic acid	1.11	0.94	0.14	0.17	0.063	0.301	<0.001	0.149
Butyrate	0.09	0.05	0.00	0.00	0.731	0.080	–	–
Ethanol	1.90	1.82	2.37	2.15	0.117	0.234	0.009	0.583
Microbial composition (Log$_{10}$ CFU g^{-1} fresh weight)								
Total bacteria[x]	7.63	6.75	8.20	8.06	0.059	<0.001	<0.001	<0.001
LAB[w]	7.67	6.90	8.18	8.05	0.086	<0.001	<0.001	0.006
Moulds[v]	1.78	1.16	ND	ND	0.731	0.681	–	–
Yeasts[v]	ND	ND	5.31	5.18	0.129	0.504	–	–
Day 3								
Chemical composition (g kg^{-1} fresh weight)								
WSC[y]	26.23	11.87	2.20	2.19	1.018	<0.001	<0.001	<0.001
Lactic acid	13.67	32.59	16.63	16.65	2.468	0.005	0.030	0.005
Acetic acid	6.01	4.50	4.54	3.64	0.491	0.040	0.045	0.553
Propionic acid	1.05	1.01	0.16	0.20	0.048	0.949	<0.001	0.389
Butyrate	0.04	0.04	0.04	0.00	0.022	0.326	0.476	0.395
Ethanol	1.01	1.29	0.35	0.39	0.187	0.423	0.003	0.534
Microbial composition (Log$_{10}$ CFU g^{-1} fresh weight)								
Total bacteria[x]	7.62	6.59	8.00	8.12	0.135	0.009	<0.001	0.003
LAB[w]	7.63	6.49	7.97	7.88	0.128	0.001	<0.001	0.004
Moulds[v]	ND	0.88	ND	ND	–	–	–	–
Yeasts[v]	ND	ND	7.82	7.90	0.076	0.614	–	–
Day 7								
Chemical composition (g kg^{-1} fresh weight)								
WSC[y]	18.76	10.37	1.90	1.01	1.699	0.026	<0.001	0.058
Lactic acid	13.92	23.60	0.44	0.41	1.353	0.007	<0.001	0.007
Acetic acid	6.00	3.71	0.81	0.64	0.440	0.023	<0.001	0.043
Propionic acid	1.15	1.0	0.44	0.46	0.079	0.484	<0.001	0.332
Butyrate	0.04	0.05	0.18	0.13	0.025	0.405	<0.001	0.163
Ethanol	0.54	0.34	0.16	0.25	0.061	0.419	0.005	0.040
Microbial composition(Log$_{10}$ CFU g^{-1} fresh weight)								
Total bacteria[x]	7.43	6.08	9.97	6.63	1.660	0.196	0.379	0.564
LAB[w]	7.49	6.04	9.84	9.91	0.075	<0.001	<0.001	<0.001
Moulds[v]	2.61	2.57	ND	ND	1.144	0.989	–	–
Yeasts[v]	ND	ND	8.84	8.73	0.070	0.468	–	–

[z]SEM, pooled standard error of least-square means (n = 3); [y]I, inoculation (inoculated *vs* uninoculated); F, forage type; I x F, inoculation x forage type interaction. Inoculated silages were treated with *Lactobacillus plantarum*, *Enterococcus faecium*, *Pediococcus acidilactici* (Alltech Inc., Canada) at a combined rate of 1.0×10^5 CFU g^{-1} forage; [y]WSC, Water-soluble carbohydrates; [x] total culaturable bacteria; [w]LAB, Lactic acid-producing bacteria.
[v]ND, non detected (dilution 10^{-1}).

Table 2.5. *In situ* ruminal organic matter disappearance (OMD) and degradation characteristics of barley and corn silages ensiled with or without inoculant

	Barley		Corn			*P* of effects[y]		
(g kg^{-1}DM silage)	Uninoculated	Inoculated	Uninoculated	Inoculated	SEM[z]	I	F	I x F
OMD[x]	573.1	574.0	577.6	574.3	2.69	0.597	0.286	0.351
Rapidly disappearing fraction (*a*)	376.5	371.3	387.4	375.0	6.82	0.205	0.291	0.600
Slowly disappearing fraction (*b*)[w]	495.3	526.4	481.8	514.0	8.22	<0.001	0.063	0.929
Potentially digestible fraction (*a*+*b*)	872.7	898.5	870.1	890.0	4.96	<0.001	0.178	0.466
Rate of disappearance of b (h^{-1}) (*c*)	0.02	0.02	0.02	0.02	0.001	1.00	1.00	1.000
Effective OMD[v]	542.8	513.1	548.8	544.1	21.06	0.903	0.849	0.889

[z]SEM, pooled standard error of least-square means (n = 6).

[y]I, inoculation (inoculated *vs* uninoculated); F, forage type; I x F, inoculation x forage type interaction. Inoculated silages were treated with *Lactobacillus plantarum*, *Enterococcus faecium*, *Pediococcus acidilactici* (Alltech Inc., Canada) at a combined rate of 1.0×10^5 CFU g^{-1} forage.

[x]Organic matter disappearance during 24 h incubation in the rumens of cows (n = 3).

[w]significant effect of forage type (P = 0.06).

[v]Effective OMD was estimated at a particulate passage rate (k) of 0.05 h^{-1} (ADAS 1986).

Table 2.6. Dry mater intake and growth performance of feedlot steers fed inoculated or uninoculated barley and corn silages during 84 d of growing

	Barley		Corn			*P* of effects[y]		
Item	Uninoculated	Inoculated	Uninoculated	Inoculated	SEM[z]	I	F	I x F
Initial body weight (kg)	306.89	298.91	303.75	308.26	5.310	0.709	0.505	0.182
Final body weight (kg)	424.35	422.39	410.17	408.22	4.625	0.655	0.002	0.999
Total body weight gain (kg)	119.97	118.01	105.79	103.85	4.625	0.655	0.002	0.999
Dry matter intake (kg d^{-1})	7.13	7.05	6.88	6.69	0.151	0.359	0.037	0.697
ADG (kg d^{-1})	1.43	1.41	1.26	1.24	0.055	0.655	0.002	0.999
Gain:feed ratio	0.20	0.20	0.18	0.18	0.005	0.653	0.002	0.912

[z]SEM, pooled standard error of least-square means (n = 25).

[y]I, inoculation (inoculation *vs* uninoculation); F, forage type; I x F, inoculation x forage type interaction. Inoculated silages were treated with *Lactobacillus plantarum*, *Enterococcus faecium*, *Pediococcus acidilactici* (Alltech Inc., Canada) at a combined rate of 1.0×10^5 CFU g^{-1} forage.

2.4. DISCUSSION

2.4.1. Forage Characteristics Prior to Ensiling in Mini Silos

The chemical compositions of the forages prior to ensiling were typical of barley (Kung and Ranjit 2001) and corn (Kleinschmit and Kung 2006) previously harvested at similar DM. The CP and WSC concentrations of barley forage ensiled were 1.5 and 5 times, respectively higher than those of corn. Hargreaves et al. (2009) have identified CP and WSC of forages as the two most important compositional factors affecting the nutritional quality of cereal silages.

The concentration of WSC in barley forage was lower than those previously observed in other studies (Zahiroddini et al. 2004; 2006; Hargreaves et al. 2009) but similar to those reported by Kung and Ranjit (2001) and higher than that of Kung et al. (2004). In the present study, barley forage was harvested at a higher DM (45%) than these earlier studies where higher WSC concentrations were reported. Water soluble carbohydrates concentration of whole-crop barley markedly decreased from 12% at the milk or early boot stage of maturity to 6% at the dough stage (Kung et al. 1990; Bergen et al. 1991).

The initial WSC concentration in corn forage has been shown to vary widely with genotype, maturity at harvest and climatic conditions such as crop heat units (CHU) and frost. Johnson et al. (2003) observed a lower WSC concentration of 20-30 g kg^{-1} DM in whole-crop corn exposed to frost prior to being harvested at 2/3 milkline maturity compared to 60-80 g kg^{-1} DM when the same hybrid was harvested at the same location and maturity but without prior exposure to frosty conditions. Shorter photoperiods and lower light intensities, and accompanying lower temperatures as observed during the study period influence the WSC concentration of corn forage (Kruse et al. 2008) and other grass forages (McDonald 1981).

The lower pH in corn compared to barley forage may be due to its lower BC as the higher CP content of barley forage would increase its BC. About 10-20% of the buffering effect of plant constituents on pH is attributed to proteins (McDonald 1981). The higher populations of natural LAB, TB, moulds and yeasts on corn forage are in agreement with Bolsen et al. (1992) who also found higher

numbers of epiphytic organisms on corn forage compared to alfalfa. On the contrary, Filya (2004) harvested whole-crop corn at similar maturity (2/3 milkline; 358 g kg^{-1} DM) and found about half the numbers of LAB, TB, yeasts and moulds observed in our present study. Variations in daily temperature, precipitation and humidity prior to harvesting (Lin et al. 1992) may partly account for the differences between the epiphytic populations observed in the present study and those observed in previous studies.

The BC of barley forage was higher than corn forage prior to ensiling. McAllister and Hristov (2000) reported a BC of between 104 and 411 mEq kg^{-1} DM for barley forage at different stages of maturity. These values are lower than 623 mEq kg^{-1} DM observed in the present study. The BC of 296 mEq kg^{-1} DM observed for corn forage in the present study was however comparable to 225 mEq kg^{-1} DM reported for corn (McDonald 1981). Variations in the organic acid content of soils on which a forage crop is grown, hybrid type and forage maturity at harvest may account for the wide variation in the BC of ensiled barley. Despite a higher BC, the estimated FC which determines the combined effect of WSC, DM and BC on the ensilability of forage was higher in barley than in corn. Well fermented silages are produced from forages whose FC is greater than 35 (Weissbach and Honig 1996), but the outcome is dependent on the nature of the epiphytic LAB population. Ensiling characteristics of forage with an epiphytic LAB population less than 1×10^5 CFU g^{-1} may still be compromised even with a favourable FC value. The FC of corn forage in the present study was 35 compared to 46 in barley and both forages had epiphytic LAB counts greater than 1×10^5 CFU g^{-1} fresh forage. These data suggest that both forages had chemical parameters conducive to the production of high quality silage.

2.4.2. Silage Fermentation and Aerobic Stability

The rate of pH decline during the first 7 d of ensiling was more rapid and the terminal pH was lower in C and IC than in B. Based on pH alone, it appears inoculation favoured corn silage relative to barley silage. However, in silage quality assessments, factors including the concentration of lactic acid relative to

other organic acids and the populations of yeasts are important indices of homolactic fermentation. The terminal lactic acid concentration in IB was 68%, 53% and 46% higher than in B, C, and IC, respectively. In addition to increasing lactic acid concentrations, inoculation of barley reduced both acetic acid and propionic acid levels. This resulted in lactic:acetic acid ratio being 50% higher in IB as compared to other silages. The higher pH in IB compared to C and IC despite its higher lactic acid content may be explained by the higher buffering capacity of barley forage at the time of ensiling. A similar phenomenon has been reported by McAllister and Hristov (2000). These data suggest that the inoculant promoted a homofermentative fermentation in barley more so than in corn. This may reflect differences in the epiphytic LAB populations between the two forages. Previous studies have also indicated that lactic acid accumulation and its final concentration are the most reliable indicators of the ability of an inoculant to improve the fermentation of barley silage (Hristov and McAllister 2002).

There was a simultaneous increase in the concentration of both WSC and lactic acid during the first 7 d of ensiling in both B and IB. This suggests that more WSC was released during ensiling than the rate at which it was being converted to lactic acid. This phenomenon increased the concentration of WSC above the level in the original forage. In general, most commercial inoculants do not have fibrolytic activity however, increased WSC concentration during ensilage is not uncommon. Hydrolysis of plant polysaccharides due to plant enzymatic activity and/or acidic fermentation have caused significant increases in WSC concentration in a number of studies across different silages; grasses (McDonald and Henderson 1974; Heron et al. 1986); cereal grains (Weinberg et al. 1993; Filya et al. 2000), and legumes (Charmley and Veira 1991). These processes may explain the phenomenon of increased WSC concentration observed in our study during ensiling in barley silage. The fact that WSC concentration increased in both uninoculated and inoculated barley silages confirms that the increase was not caused by inoculation. Enzymatic hydrolysis of structural carbohydrates to soluble sugars accounted for an increase in WSC concentration of sterile unwilted alfalfa silage from 55 g kg^{-1} DM in the original forage to 125 g

kg^{-1} DM after 70 d of ensiling before the concentration declined to levels (200 d) similar to the original forage (Charmley and Veira 1991). Similar to our finding, the increase in WSC concentration in the above study started after 1 d of ensiling. Similarly, Heron et al. (1986) also observed an increase in WSC concentration from 124 g kg^{-1} DM in ryegrass forage to 204 g kg^{-1} DM after 153 d of ensilage. Partial acidic hydrolysis of plant structural carbohydrates may also augment the enzymatic process of increasing soluble sugar concentrations during ensiling as evidenced by studies in which silages with either lower pH (Weinberg et al. 1993) or treated with higher molar acid concentrations (McDonald and Henderson 1974) had higher WSC concentrations than the original forages.

It is also worth noting that while initial WSC composition of forages is important in determining the fermentation characteristics of silages, most analytical methods of WSC analysis fail to measure alternative sugars such as sucrose, fructose, fructans, xylose, arabinose and ribose as well as other pentoses. Although these sugars were not measured in our study, xylose and arabinose can be significant substrates for LAB (McDonald 1981). Increases in WSC concentration during ensiling as observed in our study may have lessened the importance of initial levels of WSC, which has been proposed to be the key factor that determines silage quality (Charmley and Veira 1991; Charmley 2001). Satisfactory fermentation of barley has also been achieved with levels of WSC (37- 44 g kg^{-1} DM; Kung et al. 2004) that are lower than that of the current study.

The terminal WSC concentration of corn silages was higher than those reported in other studies (Schaefer et al. 1989; Kleinschmit and Kung 2006), but they fell below those commonly reported in literature from other regions (Filya 2003; 2004; Johnson et al. 2003). The lower terminal concentration of WSC observed in corn reflects its low concentration in the original forage, possibly due to the fact that the corn was subject to frost prior to ensiling as it was grown at the Northern limits of corn production.

As in previous studies, levels of starch, NDF and ADF in barley (McAllister et al. 1995), and NDF and ADF in corn (Kung et al. 1993) silages were unaffected by inoculation. This is probably because most LAB in

commercial inoculants lack the polysaccharidase activity required to hydrolyze plant cell walls (McDonald 1981; McAllister et al. 1995; Filya et al. 2007). The higher ADF in corn compared to barley silage may be attributed to its concentration in corn leaves (Verbic et al. 1995) compared to barley leaves at the dough stage of maturity (Mannerkorpi and Taube 1995).

In the present study, the epiphytic LAB populations on corn were higher than those on barley by a factor of 2 $\log_{10}$ CFU g^{-1} DM of forage. This may partly explain the lack of effects of the inoculant on the fermentation of corn silage. A lack of response of corn forage to inoculants has been previously attributed to high epiphytic LAB populations which negate any response associated with bacteria in the inoculant (Bolsen et al. 1992; Kung et al. 1993). Notwithstanding the lower terminal pH in both corn silages, the terminal yeast population still ranged from 4.3 in IC to 4.5 CFU g^{-1} DM in C. Total culturable bacteria in IC were also 21% higher than in IB. The LAB population in IB was lower than the other silages. Lower terminal counts of LAB in inoculated compared to uninoculated barley silages have been reported and attributed to cell lysis of LAB as a result of the lower terminal pH of the silage (McAllister et al. 1995; Inglis et al. 1999; Zahiroddini et al. 2004). It has similarly been suggested that growth of all microorganisms including LAB is inhibited at pH < 3.8 (McAllister and Hristov 2000). In this study, however, the pH of 4.0 in IB was higher than 3.7 in both C and IC and yet the latter silages had higher counts of LAB than IB. The higher lactic acid content in IB may partly account for this lower population. While pH *per se* can have inhibitory effects on bacteria, the concentration of the undissociated form of lactic acid has been found to be the strongest inhibitor of *L. plantarum* growth (Pieterse et al. 2005).

The principal factor affecting aerobic deterioration of silage is the population of yeasts that initiate the aerobic degradation of residual WSC and/or lactic acid (McAllister et al. 1995; Woolford 1990). In the present study, this relationship was supported by the differences in the aerobic deterioration of corn *vs* barley silage as the higher yeast counts in corn accelerated aerobic spoilage as compared to barley. Yeast populations of 5 $\log_{10}$ CFU g^{-1} DM have been found to

be the threshold population for silage deterioration especially if they are lactic acid- compared to WSC-utilizers (Woolford 1990). The yeasts population in both corn silages ranged from 5 to 9 $\log_{10}$ CFU g^{-1} between d 1 and d 7 of aerobic exposure. The relative decline in lactic acid per unit increase in growth of yeast during the 7-d aerobic exposure in C and IC was 5.4 and 7.2 g kg^{-1} of lactic acid, respectively per 1 $\log_{10}$ increase in yeast CFU. Changes in silage pH within the same period also exhibited a rise from 3.7 to 7.9 in C and from 3.7 to 8.26 in IC. In contrast, pH and lactic acid concentrations in barley silages remained relatively constant throughout the period of aerobic exposure. These data indicate a faster rate of lactic acid degradation relative to residual WSC in corn as compared barley. It seems probable that the low WSC level in corn may have favoured the establishment of lactic acid- as opposed to WSC-utilizing yeast. Unlike WSC-degrading yeast, which can dominate the silage microflora at lower pH, the degradation of lactic acid by lactic acid-utilizing yeasts would account for the accelerated rise in pH observed in aerobically exposed corn silage. The increase in pH predisposes the silage to further deterioration by other spoilage organisms (McAllister et al. 1995).

The present study confirms previous studies which showed that aerobic instability of corn silages is a major problem for farmers using homolactic inoculants containing *L. plantarum* and *E. facium* (Muck 2004). There have been numerous reports of inoculation increasing the susceptibility of corn silage to aerobic deterioration (Woolford 1990; Filya 2003; Muck 2004), possibly because of conserved WSC and synthesized lactic acid serving as a substrate for epiphytic spoilage lactic acid-utilizing microorganisms such as *Candida* and *Saccharomyces* (Woolford 1990). The proliferation of TB in C and IC with increasing days of aerobic exposure also suggests that bacteria played a role in the deterioration of these silages. Other researchers have observed increased temperature of aerated silages as the population of TB increased (McAllister et al. 1995). Higher temperatures and pH, as observed in C and IC also favour some strains of bacilli that can contribute to spoilage of silage (Inglis et al. 1999).

2.4.3. *In Situ* Ruminal OMD and Animal Performance

Other studies involving inoculation of corn (Filya 2003), barley (McAllister et al. 1995) or wheat (Sucu and Filya 2006) silages with additives containing homolactic bacterial inoculants did not improve *in situ* OMD. The increased proportion of potentially digestible fraction ($a + b$) of inoculated silages was mainly due to the higher proportion of the slowly disappearing fraction (b) since the proportion of rapidly disappearing fraction (a) was not affected *in situ*. Inoculation of barley silage with LAB only (Hristov and McAllister 2002) or in combination with fibrolytic enzymes (Zahiroddini et al. 2004) also did not increase the rapidly disappearing fraction (a) of DM.

The higher content of ADF in corn silage-based rations may account for the lower DMI and thus growth performance of steers fed those diets. Cummins (1992) previously evaluated the effect of different dietary ADF concentrations at varying environmental temperatures on DMI of corn silage-based diets and found that at any given temperature, DMI was lower in lactating cows fed higher levels of dietary ADF. The higher CP observed in barley compared to corn silage was similarly reflected in the formulated diets. Even though the difference between the diets was not significant and both diets supplied at least the recommended CP requirements (NRC 1996), this might have also contributed to the superior performance in steers fed the barley silage-based diets compared to corn. In our study, the DM of barley silage based-rations was 3 percentage units greater than those of corn silage. A reduction in voluntary DMI of corn silage diets due to lower DM content, and pH has also been proposed to occur (Shaver et al 1985) and may have been a factor in our study. A positive effect of higher WSC content of barley silage on DMI has also been postulated (McAllister et al. 1995).

Increased ADG and feed efficiency of steers fed barley compared to corn silage-based diets could be explained by increased DMI of the barley-based diets compared to corn. The ADG for steers fed barley silage-based diets was about 14% higher than those fed corn silage-based diets. Based on the average mature weights and DMI of the steers, this corresponds to a 10.6% higher net energy of gain in barley silage diets compared to corn (NRC 1996). Using corn silage diets

similar in composition to those fed in the current study, Woody et al. (1983) found that ADF content of the diet accounted for 78%, 91% and 33% reduction in ADG, feed efficiency and net energy of gain (NE_g), respectively of feedlot steers. Though there were no substantial differences in OMD, differences in the efficiency of energy utilization between the diets may account for the superior performance of steers fed barley silage-based diets. The lack of difference in OMD between barley and corn also suggest that rumen degradable protein was not limiting OMD. Even though there were significant favourable shifts in the fermentation characteristics of IB silage, this did not affect DMI, ADG and feed efficiency of steers. Contrary to these results, McAllister et al. (1995) found increased ADG in lambs fed inoculated silages compared to control although the inoculant failed to increase digestibility. A review of homolactic inoculant use in silages by Adesogan et al. (2009) found that inoculants had no effect on ADG in 80%, negative effects in 10% and positive effects in only 10% of the studies examined.

2.5. CONCLUSION

Inoculation of silage with a mixture of *L. plantarum*, *E. faecium* and *P. acidilactici* decreased the terminal WSC, acetic acid and propionic acid concentrations, and increased lactic acid concentration in barley but not corn silage. However, corn silage had a lower terminal pH than barley silage. Barley silage was also more stable than corn after 2 d of aerobic exposure. The DMI, ADG and feed efficiency of steers fed either barley or corn silage-based diets were not improved by inoculation. Regardless of forage type, however, the inoculant increased the proportion of slowly disappearing and potentially digestible fraction of OM. Barley silage was superior to corn silage at improving the DMI, ADG and feed efficiency of steers. This study confirms the difficulty of equating good fermentation characteristics of silage with improvements in growth performance of animals. It also suggests the need to have forage-specific inoculants based on the relative ease of ensiling of forage or inoculant-forage

synergy since the inoculant appeared to be more efficient at improving the fermentation characteristics and aerobic stability of barley than corn.

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CHAPTER 3: USE OF THERMAL IMAGING AND THE IN SITU TECHNIQUE TO ASSESS THE IMPACT OF AN INOCULANT WITH FERULOYL ESTERASE ACTIVITY ON THE AEROBIC STABILITY AND DIGESTIBILITY OF BARLEY SILAGE[2]

3.1. INTRODUCTION

Lactic acid is the primary end product of fermentation in silages treated with homolactic silage inoculants (first-generation inoculants). Lactic acid contributes to the low pH that preserves the silage during storage, but may be used as an energy source by spoilage microorganisms upon aerobic exposure. Addition of the heterolactic bacterium, *Lactobacillus buchneri* to homolactic silage inoculants (second-generation inoculants) has consistently improved aerobic stability of cereal silages as this species produces acetic acid that inhibits growth of spoilage microorganisms (Taylor et al. 2002; Reich and Kung 2010).

Apart from acetic acid production, some selected strains of *L. buchneri* may also have potential to improve fibre digestibility as they possess ferulic acid esterase (FAE) activity that hydrolyses feruloyl esters during fermentation (third-generation inoculants). However, there are currently only a few studies on the effects of FAE-producing silage inoculants containing *L. buchneri* on aerobic stability (Nsereko et al. 2008; Kang et al. 2009; Addah et al. 2011a). In these latter studies, assessments of aerobic stability were limited to silages stored in mini silos as opposed to farm silos. Although these lab-based studies provide excellent information on the aerobic stability of silage, they do not fully represent ensiling practices used at the farm level (Cherney et al. 2004; Borreani and Tabacco 2010). Therefore, there is a need for a rapid and simple method to assess aerobic stability directly from large farm silos during feed-out.

Infrared (IR) cameras are non-contact devices that detect and convert IR radiation emitted from the surface of a body into a thermal image in real-time

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(Vadivambal and Jayas 2011). Thermal imaging has been used to monitor food safety, and quality of agricultural products such as meat, fruits and vegetables (Gowen et al. 2010), and in grading wheat (Manickavasagan et al. 2010) and detecting spoilage in grain silos (Manickavasagan et al. 2006). The technology may have application in assessing heating in silages, but there is no published literature on its application for this purpose.

Feruloyl esters are the most abundant hydroxycinnamic acids in plant cell walls that form linkages between lignin and hemicellulose and limit ruminal fibre digestion by inhibiting attachment (Akin et al. 1988) and growth (Varel and Jung 1986) of ruminal fibrolytic bacteria. Hydrolysis of these ester linkages can remove these barriers and increase ruminal fibre digestion (Yu et al. 2005). Current biological strategies to increase ruminal silage digestibility include treatment of forages at ensiling with bacterial inoculants (Rizk et al. 2005; Addah et al. 2011b) and fibrolytic enzymes (Sheperd and Jung 1996) or a combination of both (Zahiroddini et al. 2004). Plant breeding has also attempted to improve digestibility by selecting mutant corn hybrids that possess fewer feruloyl linkages (Jung et al. 2010ab). Treatment of both ryegrass and corn silage with a third generation FAE-producing inoculant improved *in situ* ruminal fibre digestibility (Nsereko et al. 2008), but results with corn silage may be hybrid-dependent (Kang et al. 2009).

The objectives of this study were firstly to assess the effects of a FAE-producing silage inoculant on aerobic stability of barley silage stored in cylindrical Ag-Bags® silos using thermal imaging analysis and secondly to determine the effects of the inoculant on ruminal function and *in situ* digestibility of barley silage.

3.2. MATERIALS AND METHODS

3.2.1. Forage Production and Processing

A uniformly irrigated field was seeded with barley (*Hordeum vulgare*, L.; Chigwell, Field Crop Development Centre, Lacombe, Canada) at the Lethbridge Research Centre, AB, Canada. The crop was fertilized with 125 kg N ha^{-1} pre-

seeding, and 12.5 kg N ha^{-1} and 57 kg PO_4 ha^{-1} post-seeding. Soil tests indicated that the nutrient requirements for barley were satisfied. The crop was swathed at the mid-dough stage, wilted (330-354 g kg^{-1} DM) and chopped to a 10-mm theoretical length using a forage harvester (John Deere 6610; Moline, IL, USA).

3.2.2. Silage Preparation and Sampling

Chopped forage was delivered by two trucks to two Ag-Bag baggers (Ag-Bag, Miller-St. Nazianz, Inc. Co., St. Nazianz, WI, USA) concurrently. Trucks delivered alternate loads to each of the baggers to minimize treatment differences in forage quality due to harvest location and time. Sprayers (AG Spray Equipment, Hopkinsville, KY, US) were used to apply either water (UN) or a FAE-producing silage inoculant (11GFT®; Pioneer Hi-Bred Ltd., Chathan, ON, Canada) solution (IN) at a rate of 1 L per tonne of forage in two separate Ag-Bags® (3.0 × 45.7 m; Ag-Bag Int. Ltd., Warrenton, OR, USA) just prior to compression. The inoculant solution was prepared by suspending 100 g of the inoculant in 100 L of deionized water. The inoculant contained a mixed bacterial culture of 1.0×10^{11} CFU g^{-1} of FAE-producing *Lactobacillus buchneri* LN4017 (ATCC no. PTA-6138), 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 (ATCC no. PTA-6139) and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (ATCC no. PTA-6135). The application of the inoculant yielded a combined rate of 1.3×10^5 CFU of lactic acid producing bacteria (LAB) per g of forage.

Approximately 150 tonnes of each silage type was generated and stored in the bags. Triplicate composite forage samples were taken from trucks delivering the chopped forage to the Ag-baggers at equal intervals over the duration of filling of the Ag-Bags®. Samples were immediately transferred to the laboratory for chemical and microbial analysis as described below.

The bags were opened after 90 d of ensiling and the silages used to formulate two diets containing (DM basis) either 763 g kg^{-1} of the UN silage (UN diet) or 767 g kg^{-1} of the IN silage (IN diet) as shown in Table 3.1. Silage was sampled from each bag after 95, 123 and 175 d of ensiling for chemical and

microbial analyses. At each sampling, samples were collected from five different locations on the feed-out face of each bag immediately after silage was removed from the face for feeding. Collected samples were immediately stored at -20^{o}C until analyzed.

3.2.3. Aerobic Stability

After 226 d of feed-out of the silage for a separate growth performance experiment (Addah et al. 2011a), aerobic stability of the silages was assessed by thermal imaging on 3 consecutive day using a T200 IR thermal camera with 240 × 180 pixels, a thermal sensitivity of 0.08°C at 30°C and a spectral range of 7.5 to 13.0 μm (FLIR Systems Inc., Wilsonville, OR, US). Silage (~ 1 m) was removed from the face of each bag on the 1st d (d 0) of assessment. Thermal images of the silages were then immediately taken at a distance of 10 m from the face of each bag and thereafter for 3 consecutive days at 0900 h each day. The time interval among capturing the images was ≤ 18 s. In addition, ambient temperature at the feed-out face of each bag was recorded continuously with three Dallas Thermochron iButtons (Embedded Data Systems, Lawrenceburg, KY, USA) that were programmed to record temperature every 15 min over the 3 d of measurement. The Thermochron iButtons were placed at the base of the face of each bag. Hourly wind speed, solar radiation and relative humidity data corresponding to the times at which the thermal images were taken on each day of assessment were recorded at the Lethbridge Research Center weather station located ~100 m from the silos.

Three thermal images of each Ag-Bag® silo were taken on d 0, 1, 2 and 3 of assessment and used to estimate the mean, minimum and maximum temperatures over the feed-out face of each bag using ThermaCAM software (ThermaCAM Quick Report 1.1, 2007; FLIR Systems Inc., AB, Sweden). Visual appraisal of silage spoilage was also made during the period of assessment.

3.2.4. *In Situ* Ruminal Silage Disappearance, Ruminal Fluid Sampling and pH Measurement

The experimental protocol was approved by the Animal Care Committee of Lethbridge Research Centre and cattle were cared for and managed according to the guidelines of the Canadian Council on Animal Care (1993).

In situ ruminal dry matter disappearance (DMD) and α-amylase-treated neutral detergent fibre disappearance (NDFD), and fermentation pattern of each silage diet were assessed using a total of six ruminally-cannulated Angus-Charolais crossbred heifers (~450 kg of BW) housed in individual tie stalls in the Metabolism Barn of the Lethbridge Research Centre. Heifers were randomly assigned to either an UN or IN silage-based diet (Table 3.1). The two diets were prepared daily as a totally mixed ration (TMR) to meet or exceed the nutrient requirements (National Research Council 1996) with heifers provided with free access to feed (09:00 h daily) and water. The TMR was prepared using a Calan Data Ranger (American Calan, Northwood, NH, USA) to mix the silage with the steamed-rolled barley grain and supplement. The ingredient composition of the TMR for both diets was identical and only differed with regard to if it contained UN or IN silage (Table 3.1). The heifers were adapted to either the UN or IN diet for 14 d followed by 5 d of data collection. All heifers were fed 10.5 kg DM of diet per day based on estimated intakes in a companion experiment (Addah et al. 2011a). Intake at this level resulted in all of the offered diet being consumed and no orts throughout the experiment. The animals were allowed a minimum 1 h of outdoor daily exercise prior to feeding in the morning.

Table 3.1. Ingredient and chemical compositions (mean ± standard deviation) of total mixed ration (TMR) containing uninoculated and inoculated whole-crop barley silages fed to ruminally cannulated heifers

Item (DM basis)	Uninoculated	Inoculated[y]
Ingredient composition of diets (g kg^{-1}DM silage)[z]		
Barley silage	763	767
Steamed-rolled barley grain	179	177
Supplement[x]	58	56
Chemical composition of TMR (g kg^{-1}DM silage)[z]		
Dry matter	411±0.2	435±0.4
Organic matter	924±0.3	922±0.3
NDF	367±0.2	363±0.8
ADF	253±1.9	238±0.5
Starch	277±1.4	286±1.5
Crude protein	131±0.3	131±0.5
ADIN[w] (of total N)	159±3.6	119±1.5

[z]Samples were collected weekly and composited monthly (n = 4) for analyses.

[y]The whole-crop barley silage component of the diet was treated at ensiling with a feruloyl esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^{5} CFU g^{-1} of fresh forage.

[x]The supplement contained (g kg^{-1} DM): canola meal (100); urea (20); limestone (250); sodium chloride (30); ground barley grain (590); feedlot premix (10). The pre-mix contained (g kg^{-1}): calcium carbonate (348), Zinc sulphate (284), manganous sulphate (146), copper sulphate (103), ethylene diaminediiodic acid (2; as an 800 g kg^{-1} preparation), selenium (50), cobalt sulphate (1), vitamin A (1000 000 IU per g; 17 g kg^{-1}), vitamin D (500 000 IU per g; 2 g kg^{-1}) and vitamin E (500 IU per mg; 47 g kg^{-1}).

[w]ADIN, Acid detergent insoluble nitrogen.

In situ ruminal DMD and NDFD of silage were determined in heifers fed the UN diet only. No *in situ* digestibility was assessed using heifers fed IN silage. Silages were sampled (~500 g) weekly from the Ag-Bags® during feed-out and pooled every 2 wk, and frozen at -40°C. Samples were lyophilized and ground in a Wiley mill (standard model 4, Arthur H. Thomas, Philadelphia, PA, USA) through a 4-mm screen. Ground samples were mixed thoroughly and weighed (5 g per bag) into monofilament polyester bags (5 cm × 10 cm; 50 μm pore size; Ankom Technology Corp., Fairport, NY, USA). The bags containing dried ground silage samples were placed into two large mesh retaining sacs (20 cm × 30 cm; 3-5 mm pore size) that allowed ruminal fluid to percolate freely and were placed in the rumen of each heifer. Bags containing ground UN or IN silages were incubated in the rumens of each heifer fed the UN diet for 1, 2, 4, 8, 24, 48, 72, 96 and 120 h for determination of *in situ* DMD and NDFD. Three bags per treatment for incubation time points 1-72 h and five bags per treatment for incubation time points 96-120 h were inserted into the rumens of each heifer at 0800 h prior to feeding in the morning on the first day of a 5-d data collection period. Upon retrieval from the rumen, bags were rinsed under running tap water, and washed in a domestic washing machine through 5 cycles of 5 min each without soap or a spin cycle. Bags were subsequently dried in a forced air oven at 55°C for 48 h and weighed. The residues from replicate bags for each treatment and incubation time point were pooled within heifer and ground through a 1-mm screen using an ultracentrifugal mill (ZM-100 Retsch Inc., Newtown, PA, USA). Ground samples of the pooled residues and of silage samples, not subjected to ruminal incubation (0 h), were analyzed for DM and NDF concentrations as described below and used to estimate *in situ* DMD and NDFD.

Ruminal contents were collected from the reticulum, ventral, caudal and dorso-ventral sections of the reticulo-rumen of heifers immediately after retrieving bags at 48, 72 and 120 h. Ruminal contents were similarly collected from those on the IN diet at approximately the same time as for those fed the UN diet. The combined contents from each section of the rumen of each heifer were thoroughly mixed and strained through two layers of PECAP nylon (Sefar Canada

Inc., Ville St. Laurent, QC, Canada) for determination of VFA and ammonia-N concentrations. For VFA determination, 3.0 mL of the filtrate was immediately deproteinized with 0.6 mL of 25% (w vol^{-1}) metaphosphoric acid. For ammonia-N determination, 3.2 mL of the filtrate was combined with 0.30 mL of 65% (w vol^{-1}) trichloroacetic acid. The samples were stored at -20^{o}C until analyzed for VFA and ammonia-N as described below.

An indwelling LRCpH electrode connected to a data logger (Dascor, Inc., Escondido, CA, USA) as described by Penner et al. (2006) was inserted into the rumens of heifers just prior to feeding and rumen sampling at 08:00 h. For heifers on the UN diet, the loggers were inserted immediately prior to placing the *in situ* bags into the rumen. Heifers on the IN diet were inserted with the loggers concurrently with those on the UN diet. The pH logger was held in the ventral sac with 0.5-kg sealed stainless steel weights and suspended approximately 60 cm into the rumen by a cable anchored to the ruminal cannula plug. Prior to inserting the loggers, the electrodes were standardized in pH 4 and 7 buffers and the loggers programmed to record ruminal pH every min for 5 d (1440 records per day) after which data were downloaded.

3.2.5. Chemical Analyses

A 15-g sample of fresh forage or silage was combined with 135 mL of distilled water and blended in a Waring blender (Waring Commercial, Torrington, CT, USA**)** for 30 s at full speed. The suspension was filtered through two layers of cheesecloth and the pH of the filtrate was immediately measured with a Symphony pH meter (VWR, Mississauga, ON, Canada). The filtrate was then divided into two portions. One portion was immediately boiled for 10 min to halt fermentation and stored at -20^{o}C for subsequent determination of water-soluble carbohydrates (WSC; glucose equivalent) by the Nelson-Somogyi (1944) method using a Dynatech MRX micro-plate reader (Dynatech Laboratories Inc., Chantilli, VA, USA) at 630 nm.

The second portion was stored on ice until centrifuged (10,000 × *g*; 4^{o}C) for 15 min. The supernatant was collected for analysis of VFA, lactic acid and

ammonia-N. For determination of VFA, 1.5 mL of the supernatant was deproteinized with 0.3 mL of 25% (w vol^{-1}) metaphosphoric acid, combined with 0.2 mL of 0.1 M crotonic acid as internal standard, and analyzed on a Hewlett Packard model 5890A Series Plus II gas-liquid chromatograph (Hewlett Packard Co., Palo Alto, CA, USA) with 30 FFAP fused silica capillary, 0.32 mm I.D. and 1.0 m film thickness (Phenomenex, Torrance, CA, USA). For lactic acid determination, 400 uL of the deproteinized sample was combined with 50 uL of 3 mM malonic acid (5 mg mL^{-1}) as an internal standard. Lactic acid was methylated and then quantified using the method of Kudo et al. (1987) on the same chromatograph and column used for VFA analysis. To determine ammonia-N concentration, 1.6 mL of the supernatant was combined with 0.15 mL of 65% (w vol^{-1}) trichloroacetic acid and analyzed by the phenol-hypochlorite method as described by Broderick and Kang (1980).

For starch determination, fresh forage or silage samples first were lypholized and ball-ground using a mixer mill (MM 400, Retsch Inc. Newtown, PA, USA). Starch was determined after hydrolysis to α-glucose polymers using amyloglucosidase (Megazyme Int. Ltd., Wicklow, Ireland, UK) and 1,4 α-D-glucan glucano-hydrolase (Brennfag Canada Inc., Toronto, ON Canada) as previously described by Herrera-Saldana et al. (1990). Samples were read on a micro-plate reader at a wavelength of 490 nm.

Crude protein content of fresh or lyophilized silage samples was determined by ball-grinding samples (5 mg) for combustion analysis for total N (Dumas Nitrogen) using an NA1500 Nitrogen/Carbon analyzer (Carlo Erba Instruments, Milan, Italy). Crude protein was calculated as total N × 6·25.

Oven dry matter of the fresh forage, silage samples and TMR was determined by drying at 105^{o}C for 24 h in a forced air oven. Silage DM was corrected for volatilization of VFA, lactic acid and ammonia-N after oven-drying by the equation of Porter et al. (1995). Organic matter was determined by ashing samples (1 g) in a muffle furnace at 550^{o}C for 5 h. Subsamples of the fresh forage, silage and TMR were stored at -20^{o}C prior to lyophilizing, and ground through a 1-mm screen for analysis of NDF and acid detergent fibre (ADF) using

an Ankom 200 system (Ankom Technology Corporation, Fairport, NY, USA). Neutral detergent fibre was analyzed with the addition of sodium sulfite and heat-stable α-amylase and expressed inclusive of residual ash whereas ADF was analyzed without α-amylase. Nitrogen in ADF residues (ADIN) was measured as described above for N analysis.

3.2.6. Microbial Analyses

For microbiological analyses, 10 g of forage or silage was added to 90 mL of sterile 70 mM potassium phosphate buffer (pH=7.0) and agitated (260 rpm) for 60 s in a Stomacher 400 Laboratory Blender (Seward Medical Limited, London, UK). The suspension was serially diluted (10^{-2} to 10^{-7}) and 100-µL aliquots of each dilution were spread in triplicate onto semi-selective lactobacilli media (de Man-Rogosa-Sharpe, MRS; Oxoid, Basingstoke, Hampshire, UK) for enumeration of LAB (Hill and Hill 1986), onto nutrient agar (NA; Difco, Detroit, MI, USA) for the enumeration of total culturable bacteria, and onto Sabouraud's dextrose agar (SDA; Difco, Detroit, MI, USA) for the enumeration of yeasts and moulds. Lactobacilli MRS agar and NA were amended with 200 µg mL^{-1} of cycloheximide (Sigma-Aldrich, St. Louis, MO, USA) and SDA with 100 µg mL^{-1} each of tetracycline and chloramphenicol. Lactobacilli MRS agar plates were incubated at 37°C for 24-48 h and SDA plates were incubated at ambient temperature for 72 h. Colonies were counted from plates containing a minimum of 30 and a maximum of 300 colonies.

The FAE activity of the inoculant was confirmed by a procedure previously described by Donaghy et al. (1998) with modification as described by Addah et al. (2011a) using ferulic acid (ethyl 4-hydroxy-3-methoxycinamate; Sigma-Aldrich, St. Louis, MO, USA) as substrate. The extent of the de-esterification of the substrate (clearing zone) was assumed to be proportional to the extent of FAE activity.

3.2.7. Statistical Analyses

All analyses were conducted using SAS (Statistical Analysis System Institute Inc., Cary, NC). Data on microbial counts were transformed to log-normal count $\log_{10}$ and expressed on DM basis prior to statistical analysis.

In situ silage DMD and NDFD was calculated as the difference in DM and NDF concentration before and after ruminal incubation. The disappearance data were fitted to a non-linear regression equation using NLIN procedure of SAS for estimating the kinetics of *in situ* DMD and NDFD using the following model (McDonald 1981a):

$$P = a + b\,(1 - e^{-c(t-L)}) \text{ for } t > L$$

where P is the proportion (g kg^{-1}) of DMD or NDFD at time t, a is the rapidly disappearing fraction (g kg^{-1}), b is the slowly disappearing fraction (g kg^{-1}), c is the rate at which b is disappearing (g kg^{-1} h^{-1}), t is the time (h) bags were incubated in the rumen, and L is the lag time (h).

The effective ruminal DMD or NDFD in each cow was estimated assuming a ruminal particulate outflow rate (k) of 0.028 h^{-1} for barley silage (Eun et al. 2004) using the following model (McDonald 1981a):

Effective disappearance $= a + [bc/(c + k)]e^{-(c + k)L}]$

All data on predicted *in situ* DM and NDF disappearance and kinetics of degradation of DM and NDF generated from the NLIN procedure were analyzed by the MIXED procedure of SAS for the fixed effects of inoculation in a completely randomized block design with heifer as a random variable in the following model:

$$Y_{ij} = \mu + T_i + A_j + e_{ij}$$

where Y_{ij} = response variable (DMD or NDFD, kinetics of degradation), μ = overall mean, T_i = effect of inoculation (UN or IN; i = 1-3 bags for 1-72 h of incubation and 1-5 bags for 96-12 h of incubation), A_j = random effect of heifer (j

= 1-3) and e_{ij} = random error associated with each bag, assumed to be normally distributed.

Prior to insertion and after each removal of the pH electrode from the rumen, the millivolt reading of each pH electrode was recorded in pH 4 and 7 buffer solutions at 39^{o}C. The relationship between millivolts and pH was considered to be linear (Penner et al. 2006) and pH was predicted from the corresponding millivolts reading by linear regression procedure of SAS. The predicted pH data of each heifer was then summarized into 24-h minimum, maximum and mean pH, and duration (time, h) and area (pH × time) below pH 6.2, 6.0 and 5.8 using PROC MEANS of SAS. Only days (24-h period) in which pH was ≥ 6.2 and ≥ 6.0 for more than 4 h, and ≥5.8 for more than 1.5 h were included in the estimates of duration and areas under pH thresholds. For graphical illustrations, pH values were first averaged for 30-min interval for each heifer and then averaged over the three heifers for each period.

Differences in least-square means due to inoculation were declared significant at $P \leq 0.05$, and tendencies toward significance were discussed at $0.05 < P < 0.10$.

3.3. RESULTS

3.3.1. Silage Fermentation Characteristics

The FAE-producing capability of the inoculant was confirmed by de-esterification of ferulic acid-modified MRS agar plates indicated by the formation of distinct clearing zones around wells inoculated with the unautoclaved inoculant solution and the IN silage filtrate as opposed to their absence in the autoclaved solution and UN silage, respectively.

The chemical compositions of whole-crop barley at harvest and after ensiling are shown in Table 3.2 and 3.3, respectively. The DM content of the UN silage was 36.9 g kg^{-1} greater than the IN silage. The concentration of acetic acid was approximately three times higher in the IN than UN silage (48.8 *vs* 18.4 g kg^{-1} DM) whereas lactic acid concentration differed by a similar margin, but was higher in the UN (69.7 g kg^{-1} DM) than IN (22.0 g kg^{-1} DM) silage. The ratio of

lactic-to-acetic acid concentration was thus greater in UN (3.8) than IN (0.4) silage.

Yeasts and moulds were not detected (silage dilution = 10^{-1}), but the populations of LAB and total cultuarble bacteria were relatively greater in the IN compared to UN silage.

3.3.2. Aerobic Stability

Examples of thermal IR images of the feed-out face of the silages taken at the beginning (d 0) and end (d 3) of aerobic stability assessment are shown in Figure 3.1. The average ambient temperature, wind speed and solar radiation corresponding to the times at which the images were taken were 21-27 ^{o}C, 2.2-8.7 m s^{-1} and 0.5-1.0 h over the 3 d that images were taken (Table 3.4). The average temperature of the thermal images showed greater heating of the UN silage at the beginning (d 0) of assessment, through to the final day (d 3) compared to that of IN silage (Figs. 3.1 and 3.2). Compared to ambient temperature, the IN silage was at least 2^{o}C below ambient on d 1 and 3 whereas the UN silage was 2^{o}C above ambient after d 0 of assessment (Figure 3.2). Similarly, the average daily minimum and maximum temperatures for the UN silage thermograms were 19.5 ^{o}C and 39.2^{o}C compared to 16.2 ^{o}C and 37.0 ^{o}C for the IN silage, respectively. Spoilage was also visually more evident on the face of UN as compared to IN silage.

Table 3.2. Chemical and microbial composition (mean ± standard deviation) of forages prior to treatment and ensiling

Item (DM basis)[z]	
pH	7.12 ± 0.1
Dry matter (g kg^{-1} silage)	350.5 ± 5.0
Chemical composition (g kg^{-1}DM silage)	
Organic matter	930.9 ± 4.5
NDF	458.2 ± 2.3
ADF	242.8 ± 0.4
ADIN (of total N)[y]	52.0 ± 1.4
Starch	247.0 ± 29.3
Water soluble carbohydrates	10.3 ± 5.0
Crude protein	126.0 ± 1.2
Microbial composition (log_{10} CFU g^{-1} DM silage)	
Total culturable bacteria	8.0 ± 0.04
Lactic acid-producing bacteria	5.2 ± 0.09
Moulds	6.8 ± 0.01
Yeasts	7.3 ± 0.02

[z]Composite forage samples (n = 3) were collected from trucks at equal intervals over the duration of ensiling the forage in Ag-Bag® silos.
[y]ADIN, acid detergent insoluble nitrogen.

Table 3.3. Fermentation characteristics (mean ± standard deviation) of whole-crop barley silages stored in Ag-Bag® silos

Item[z]	Uninoculated	Inoculated[y]
pH	4.17 ± 0.13	4.17 ± 0.13
Dry matter (g kg^{-1})	416.9 ± 6.36	374.6 ± 3.05
Chemical composition (g kg^{-1} DM silage)		
Organic matter	934.4 ± 3.00	924.4 ± 2.18
NDF	415.9 ± 30.97	452.7 ± 6.04
ADF	263.9 ± 22.9	304.6 ± 15.69
ADIN (of total N)[x]	100.9 ± 3.66	116.8 ± 7.70
Starch	235.7 ± 45.7	196.9 ± 10.61
Water soluble carbohydrates	146.1 ± 27.2	42.1 ± 0.10
Crude protein	122.7 ± 5.25	125.9 ± 4.33
End products of fermentation (g kg^{-1} DM silage)		
Acetic acid	18.4 ± 1.25	48.8 ± 6.82
Propionic acid	25.3 ± 0.36	5.3 ± 3.06
Butyric acid[w]	ND	ND
Lactic acid	69.7 ± 0.01	32.2 ± 0.18
Succinic acid	34.5 ± 0.04	37.7 ± 4.3
Ammonia-N (of total N)	12.6 ± 4.36	10.1 ± 1. 05
Lactic: acetic ratio	3.8 ± 0.16	0.7 ± 0. 09
Microbial composition (log_{10} CFU g^{-1} DM silage)		
Total culturable bacteria	6.7 ± 0.58	8.8 ± 0.68
Lactic acid-producing bacteria	6.6 ± 0.39	9.3 ± 0.40
Moulds[w]	ND	ND
Yeasts[w]	ND	ND

[z]Means (± standard deviation) are samples (n = 3) collected from different sections of Ag-Bag® silos over time (d 95, 123 and 175) during feed-out.
[y]Inoculated whole-crop barley silage was treated with a feruloyl esterase-producing inoculant containing a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^{5} CFU g^{-1} of fresh forage.
[x]ADIN, acid detergent insoluble nitrogen.
[w]ND, not detected (silage diluted to 10^{-1}).

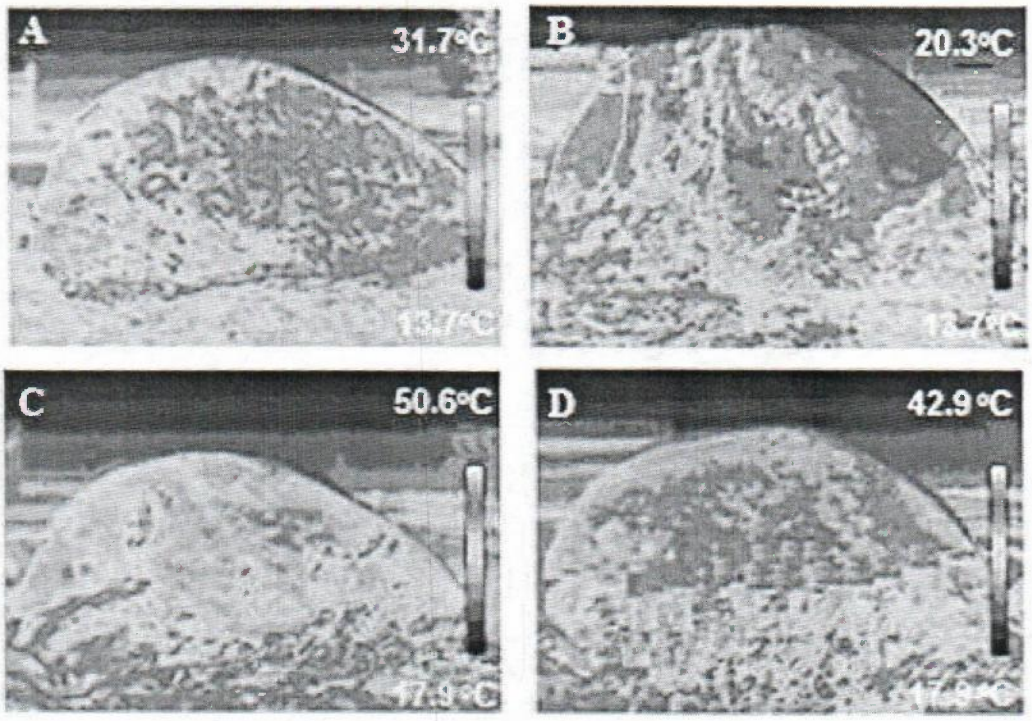

Figure 3.1. Infrared thermal images of uninoculated and inoculated whole-crop barley silages ensiled in Ag-Bag® silos and exposed to air during feed-out.
Day 0: A = uninoculated silage; B = inoculated silage
Day 3: C = uninoculated silage; D = inoculated silage
Inoculated whole-crop barley silage was treated at ensiling with a feruloyl esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^{5} CFU g^{-1} of fresh forage.
Temperature values indicated on each image represent the minimum and maximum temperatures of the feed-out face of each bag.

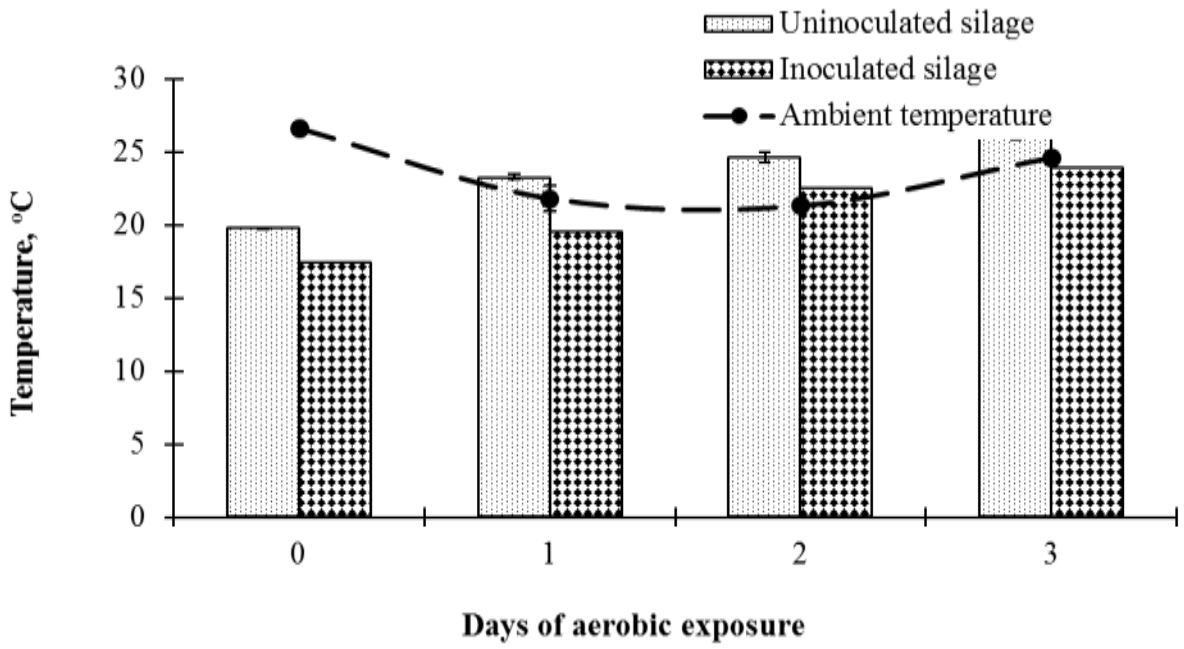

Figure 3.2. The effect of inoculation on aerobic stability of whole-crop barley silages ensiled in Ag-Bag® silos and exposed to air during feed-out. Inoculated whole-crop barley silage was treated at ensiling with a feruloyl esterase-producing inoculant containing 1.0 x 10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0 x 10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0 x 10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 at (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) a combined rate of 1.3 × 10^5 CFU g^{-1} of fresh forage. Where visible, vertical bars indicate standard error of the mean.

Table 3.4. Environmental conditions (mean ±standard deviation) corresponding to times (0900 - 1100 AM) at which aerobic stability of Ag-Bag® silages was assessed by thermal image analysis

Day of assessment	Temperature (^{o}C)[z]	Relative humidity (%)[y]	Short wave radiation (kJm^{-2})[y]	Wind (m s^{-1})[y]
0	26.6	44.0 ± 2.40	1565.8 ± 356.7	2.2 ± 0.06
1	21.8	36.5 ± 3.39	3036.6 ± 180.7	2.6 ± 0.12
2	21.3	81.1 ± 3.96	1344.3 ± 533.1	8.7 ± 0.55
3	24.6	51.0 ± 5.80	2838.6 ± 216.4	3.9 ± 1.05

[z]Recorded with Thermochron iButtons thermocouples (Embedded Data Systems, Lawrenceburg, KY, USA) placed at the face of the silage.

[y]Recorded hourly from the Lethbridge Research Centre Weather Station located at ~ 100 m from the silos.

3.3.3. Ruminal *In Situ* Disappearance and Fermentation Pattern

The effects of inoculation on *in situ* silage DMD and NDFD are shown in Table 3. 5. Dry matter disappearance at both 24 and 48 h of incubation did not differ (P = 0.06) between treatments. The rapidly disappearing DM fraction (P = 0.01) and effective DMD (P = 0.01) for UN were greater than IN silage. Inoculation increased (P = 0.01) NDFD both at 24 and 48 h of incubation compared to UN silage. The proportion of rapidly disappering silage NDF was also greater (P = 0.04) for UN than IN silage, however the proportion of slowly disappearing (P = 0.02) and potentially degradable (P = 0.03) NDF fractions were greater for IN than UN silage.

Daily ruminal fermentation patterns, and pH parameters including duration and areas under pH thresholds 6.2, 6.0 and 5.8 for heifers fed the UN and IN diets are presented in Table 3.6. Diurnal variations in ruminal pH for heifers fed the UN and IN diets are illustrated in Figure 3.3. Ruminal pH were similar up to 12 h post-feeding, but were noticeably lower in cattle fed IN as compared to UN silage for the remainder of the day. Duration and area under pH 5.8 were 2.1 and 0.4 for heifers fed IN silage as compared to 1.8 and 0.1, respectively for those fed UN silage.

The molar proportion of propionic acid was higher when heifers were fed IN silage than when fed UN silage. The ratio of non-glucogenic-to-glucogenic ruminal VFA (NGR) was also on the average, lower for heifers fed IN silage than for those fed UN silage (4.9 *vs* 5.6).

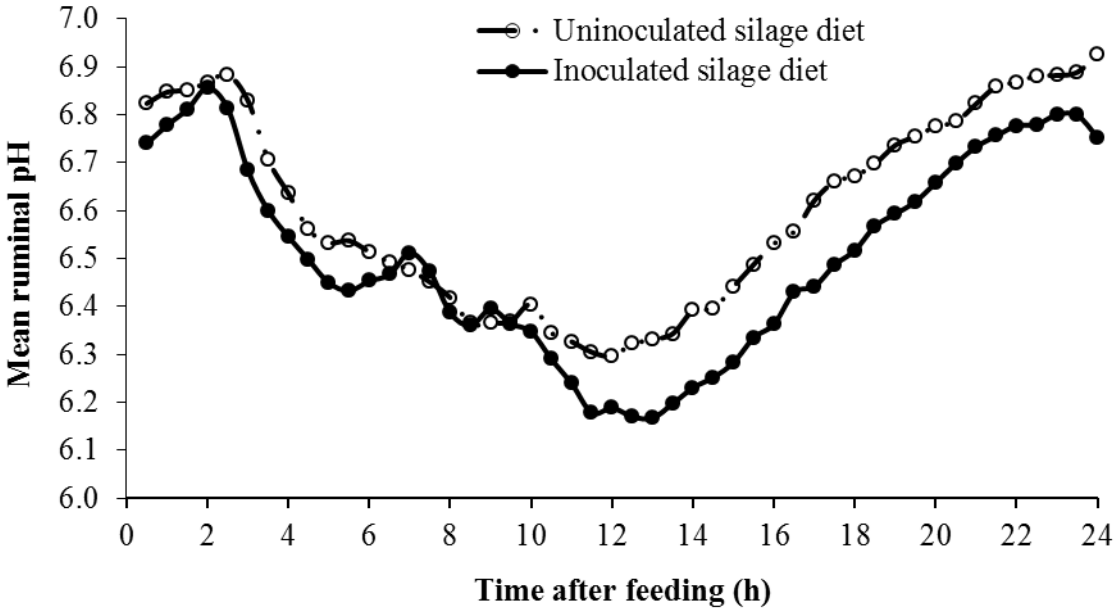

Figure 3.3. Mean daily ruminal pH profiles of heifers fed uninoculated and inoculated whole-crop barley silage diets. The whole-crop barley silage component of the inoculated diet was treated at ensiling with a feruloyl esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^{5} CFU g^{-1} of fresh forage.

Table 3.5. Effects of inoculation on *in situ* ruminal disappearance of dry matter and neutral detergent fibre of whole-crop barley silage

Item (DM basis)	Uninoculated	Inoculated[z]	SEM[y]	*P*-value
DM disappearance (g kg^{-1})				
24 h	569.9	558.0	4.13	0.06
48 h	657.5	646.7	5.70	0.13
Kinetics of DM disappearance (g kg^{-1})[x]				
Rapidly disappearing fraction (*a*)	432.9	411.4	3.74	0.01
Slowly disappearing fraction (*b*)	395.4	390.6	11.44	0.77
Rate of disappearance of b (c; per h)	0.20	0.20	0.002	0.30
Potentially degradable fraction (*a*+*b*)	828.3	802.0	12.6	0.16
Effective DM disappearance[w]	523.1	507.5	2.53	0.01
Lag (h)	0.0	0.0	-	-
NDF disappearance (g kg)				
24 h	200.1	214.8	19.28	0.01
48 h	321.6	351.2	20.49	0.01
Kinetics of NDF disappearance (g kg^{-1})				
Rapidly disappearing fraction (*a*)	87.87	74.35	8.37	0.04
Slowly disappearing fraction (*b*)	506.63	593.03	20.62	0.02
Rate of disappearance of b (*c*; per h)	0.19	0.22	0.01	0.80
Potentially degradable fraction (*a*+*b*)	594.50	673.60	21.05	0.03
Effective NDF disappearance[w]	235.1	249.7	5.19	0.11
Lag (h)	2.87	4.04	1.90	0.68

[z]Inoculated whole-crop barley silage was treated at ensiling with a feruloyl esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 at (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) a combined rate of 1.3×10^{5} CFU g^{-1} of fresh forage.

[y]SEM, pooled standard error of least-square means (n = 9).

[x]Kinetics of disappearance estimated from the model of McDonald (1981a).

[w]Effective disappearance was calculated at a ruminal particulate outflow rate of at 0.028 h^{-1} for barley silage (Eun et al. 2004).

Table 3.6. Ruminal fermentation and pH profile (mean ± standard deviation) of Angus-Charolais heifers fed uninoculated or inoculated whole-crop barley silage diets

Item	Uninoculated	Inoculated[z]
Feed intake (kg d^{-1})[y]	10.5	10.5
Total volatile fatty acids (m*M*)[x]	99.7 ± 12.4	115.2 ± 18.67
Molar proportions (mol 100 mol^{-1})[x]		
Acetic acid	70.1 ± 1.69	70.1 ± 1.98
Propionic acid	14.7 ± 0.86	16.8 ± 1.49
Butyric acid	11.1 ± 1.24	9.3 ± 1.98
Isobutyric acid	1.1 ± 0.17	1.0 ± 0.14
Valeric acid	0.9 ± 0.08	1.0 ± 0.16
Isovaleric acid	1.3 ± 0.16	1.4 ± 0.22
Acetic:propionic	4.7 ± 0.56	4.2 ± 0.48
Non-glucogenic ratio[w]	5.6 ± 0.60	4.9 ± 0.58
Ammonia-N (m*M*)	5.0 ± 1.34	4.6 ± 1.22
Daily ruminal pH[v]		
Mean	6.6±0.23	6.5±.023
Minimum	6.1±0.26	6.0±0.25
Maximum	7.1±0.09	7.0±0.11
Duration under pH threshold (h d^{-1})[v]		
6.2	8.0±0.02	8.4±0.07
6.0	5.2±0.04	4.5±0.03
5.8	1.8±0.02	2.1±0.01
Area under pH threshold (pH x h d^{-1})[v]		
6.2	1.5±0.63	1.6±0.80
6.0	0.8±0.18	1.0±0.12
5.8	0.1±0.01	0.4±0.13

[z]Inoculated whole-crop barley silage component of the diet was treated at ensiling with a feruloyl esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^{5} CFU g^{-1} of fresh forage.

[y]Heifers were fed to appetite based on intakes reported in Addah et al. 2011a.

[x]Values are means (± standard deviation) of rumen samples collected on day 1, 3 and 5 of data collection from each of three ruminally cannulated heifers (n = 6).

[w]None-glucogenic ratio = [Acetic + 2(isobutyric+butyric) + isovaleric + valeric]/[propionic + isovaleric + valeric].

[v]Ruminal pH recorded every minute with an indwelling probe and the data for each of three cannulated heifers were summarized into 24-h for a 5-d data collection period (n = 15).

[u]Only days (24-h period) in which pH was ≥ 6.2 and ≥ 6.0 for more than 4 h, and ≥5.8 for more than 1.5 h were included in these estimates.

3.4. DISCUSSION

3.4.1 Silage Fermentation Characteristics

The chemical and microbiological characteristics of the unensiled forage were similar to whole-crop barley forage previously harvested at the mid-dough stage of grain maturity (Addah et al. 2011b). Feruloyl esterase activity of the inoculant was confirmed by the formation of clearing zones on the modified MRS agar plates and the extent of clearing zones was assumed to be proportional to FAE activity (Addah et al. 2011a). Very few LAB possess phenolic acid esterase activity. Most microorganisms that possess phenolic esterase-producing capabilities are aerobic fungi and are therefore unsuitable for use as silage inoculants. This may explain the current scarcity of third generation fibrolytic silage inoculants on the market.

The absence of butyric acid, and yeasts and moulds in both silages suggest that they were well ensiled. The common characteristic of silage inoculated with *L. buchneri* is a higher acetic acid concentration and terminal pH due to the degradation of lactic acid to acetic acid at the later stages of fermentation. This was evident in IN silage as its lactic-to-acetic acid ratio was only 0.43 compared to 4.81 in the UN silage. Even though it was not possible to effectively monitor the fermentation profile during ensiling in the Ag-Bag® silos, the fermentation characteristics were generally suggestive of heterolactic fermentation typical of whole-crop barley silage inoculated with a FAE-producing inoculant in mini silos (Addah et al. 2011a). Similar findings have been reported for corn and ryegrass silages (Nsereko et al. 2008; Kang et al. 2009).

The effect of lower pH on the degradation of lactic acid into acetic by *L. buchneri* has been shown in time-course studies of silages ensiled in mini silos (Addah et al. 2011a) and in batch cultures simulating silage fermentation (Oude Elferink et al. 2001). In these studies, acetic acid was largely formed from the anaerobic microbial degradation of lactic acid. Some strains of *L. plantarum* isolated from commercial silage inoculants also degraded lactic acid into acetic acid, but this response was also accompanied by increased concentrations of succinic acid (Lindgren et al. 1990). The inoculant used in the current study was a

mixed culture of *L. buchneri* plus *L. plantarum* and *L. casei*. However, the greater acetic acid concentration observed for IN (48.8 g kg^{-1} DM) compared to UN (18.4 g kg^{-1} DM) silage in the present study was not accompanied by a similar increase in succinic acid or its metabolite, propionic acid. This suggests that the greater acetic acid concentration is likely more attributable to the degradation of lactic acid by *L. buchneri.* Higher acetic acid concentration preserves the silage by inhibiting the growth of yeasts, both during storage and silage feed-out. This characteristic is the basis for the inclusion of *L. buchneri* in silage inoculants, but the heterolactic fermentation can be associated with increased DM losses (Driehuis et al. 2001).

3.4.2. Aerobic Stability

The intensity of IR radiation emitted by a body is a function of it surface temperature. Complete microbial oxidation of WSC and lactic acid results in the evolution of about 15 kJ g^{-1} and 16 kJ g^{-1} of heat, respectively (McDonald 1981b). Heat emission from the feed-out face of silages as indicated by a rise in temperature can therefore be used as a measure of the extent of aerobic deterioration. The higher temperature of UN as compared to IN silage exposed to air (Figure 3.1) suggests that this technique has potential for documenting heating in silage ensiled in farm-scale systems. Higher concentrations of acetic acid in IN silage prior to the aerobic stability test and its associated antimicrobial properties may have reduced the activity of spoilage microorganisms, contributing to the lower temperature in IN as compared to UN silage. Differences in surface temperature were noted immediately upon exposure, with UN silage being notably above ambient temperature with prolonged periods of aerobic exposure (Figure 3.2). Whereas the lower heating observed in IN silage is most likely attributable to reduced microbial activity, other factors such as differences in DM content or packing density could also have influenced the stability of the silages upon exposure to air. High DM silages generally have a lower packing density and greater air penetration into the mass as compared to silages with lower DM content. However, it is worth noting that the specific heat capacity of plant

materials increases with increasing moisture (low DM) content (McDonald 1981b; Ruppel et al. 1995) and therefore the IN silage whose DM content was 36.9 g kg^{-1} lower than the UN silage would be expected to have reduced stability if packing density was a factor. The UN silage had more residual WSC and lactic acid than the IN and these could have served as substrates that promoted the growth of spoilage microorganisms. Unfortunately, we were unable to conduct microbial analysis of the silage during the period of image capture. However, we did visibly observe more mould in UN compared to IN silage. Even though moulds *per se* may have a secondary role in silage deterioration, the growth of yeasts often precedes that of moulds in the microbial succession that occurs during the aerobic spoilage of silage (McDonald 1981b; Inglis et al. 1999).

The rise in temperature of aerobically exposed silages via oxidation of residual sugars and fermentation acids represent a potential loss of nutrients that otherwise would have been utilized directly by the host or rumen microbes. The depletion of these potentially digestible nutrients during feed-out could decrease the nutritive value of the silage by as much as 16% compared to its nutritive value at time opening the silo (Tabacco et al. 2011).

The technique of using IR thermal image analysis to assess heating of silages stored in large farm silos could serve as a simple and rapid method of directly measuring and visualizing heat distribution over the feed-out face of silos in real-time. Thermal images showed differential heating between UN and IN silage immediately upon aerobic exposure, whereas in laboratory aerobic stability studies uninoculated silage did not begin to show signs of spoilage until after 5 d of exposure (Addah et al. 2011a). This difference suggests that stability of silages assessed under laboratory conditions may not be fully representative of the stability of silages stored in large silos on-farm. Greater aerated surface area of the feed-out face of the Ag-Bag® silages along with increases in silage mass may have resulted in the accelerated rate of deterioration of face silage ensiled in Ag-Bags®.

In a previous study, the aerobic stability of silages stored in bunker silos were assessed by constructing virtual thermograms of the feed-out face after

measuring the temperature of the face with thermometers (Borreani and Tabacco 2010). This method may be suitable for outdoor farm silos but it was based on contact measurements with the possibility of microbial cross-contamination. Also, with this method, it was not possible to directly measure the temperature distribution over the entire surface area of the feed-out face and so the temperatures of regions on the face that were not measured directly may not have been properly interpolated from the spatial grid. As shown in Figure 3.1, with IR imagery, the images delineated the temperature profile of the full feed-out face in a two-dimensional manner indicating regions of higher temperature that were associated with deterioration. Application of the technology may also reduce costs associated with personnel and chemical reagents used for conventional assessment of silage aerobic stability. Atmospheric conditions that are likely to interfere with signal acquisition by thermal cameras include ambient temperature and wind speed (Gowen et al. 2010; Manickavasagan et al. 2006; 2010). Ambient temperature was 21-27^{o}C during the time the images were taken. Wind speed was 2.6-3.9 m s^{-1} on d 0, 1 and 3, and 8.7 m s^{-1} on d 2 (Table 3.4). Manickavasagan et al. (2006) found effects of wind speed on thermal image acquisition of grain silos stored in a room (20-30^{o}C) at wind speeds of 1.0-2.0 m s^{-1}, however, Madding (2002) suggested that a wind speed of $\leq$ 4.5 m s^{-1} did not interfere with outdoor IR thermography. In the present study, wind speeds only exceeded this recommended cut off on day 2. Even under these conditions, relative comparisons between silages may be still meaningful provided that comparisons are made on the same day at similar wind speeds. It is worth noting also that the IR camera used in the current study had built-in atmospheric correction filters to provide automatic compensation for the effects ambient temperature, and environmental influence on image acquisition was likely negligible in terms of its impact on comparisons between treatments.

3.4.3. Ruminal *In Situ* DMD and NDFD

The higher concentration of lactic acid and residual WSC in the UN may explain its higher proportion of rapidly disappearing DM fraction (*a* fraction) given that

WSC and lactic acid are readily soluble in the rumen. The lower residual WSC and lactic acid concentration in the IN silage is likely an indication of greater utilization of WSC by LAB in IN compared to UN silage during ensiling. The *a* fraction for the IN silage DM was however greater than those reported for barley silages treated with homolactic inoculants without any proven fibrolytic activity (Hristov and McAllister 2002; Baah et al. 2011), but lower than those observed by Zahiroddini et al. (2004) when barley silages were treated with LAB alone or in combination with exogenous enzymes. At an assumed ruminal particulate passage rate of 0.028 h^{-1} (Eun et al. 2004), the effective disappearance of silage NDF did not differ between treatments but was 15 g kg^{-1} greater for IN than UN silage.

Compared to UN, inoculation increased ruminal NDFD disappearance at both 24 and 48 h of incubation. The NDFD disappearance (215 g kg^{-1}) of IN silage after 24 h of incubation was greater than the range (117-135 g kg^{-1}) reported for untreated barley silage harvested at similar maturity (Eun et al. 2004). Using a similar FAE-producing inoculant, Kang et al. (2009) did not find an improvement in *in situ* NDFD disappearance of two corn silage hybrids after 24 h, but did report an 11% increase relative to the control after 48 h of incubation. In the present study, inoculation improved ruminal NDFD by 7% and 9% after 24 and 48 h, respectively. This was comparable to the improvement (6.8%) observed for corn silage inoculated with a FAE-producing inoculant (Nsereko et al. 2006). The improvements in NDFD of perennial ryegrass and corn silages treated with a FAE-producing inoculant were 9-11% and 16 % respectively after 48 h (Nsereko et al. 2008).

Arabinoxylans and glucuronoarabinoxylans, the major digestible components of forage NDF may be ester-linked to ferulic acid or cross-linked to each other by other hydroxycinnamic acids. Ferulic acid also forms ether linkages with lignin further reducing NDF digestibility. The processes that enhance ruminal silage fibre digestibility due to inoculation occur mainly during ensiling. De-esterification of fibre fractions during ensiling increases the susceptibility of the fibre to greater *in situ* degradation as observed for IN silage. These findings concur with those of Nsereko et al. (2008) who found greater *in situ* NDF

degradation in ryegrass silages inoculated with a FAE-producing inoculant even though the NDF concentrations were either not altered or even higher for the treated than for the control silage after ensiling. Kang et al. (2009) also did not observe reductions in NDF concentrations after ensiling, but reported improvements *in situ* ruminal NDF digestibility in one of two hybrids inoculated with a FAE-producing silage inoculant after 48 h of incubation. Inoculation of silage with FAE-producing inoculants can increase ruminal NDF degradation if feruloyl linkages are completely or partially cleaved from feruloylated arabinoxylans rendering the fibre more susceptible to hydrolysis by ruminal microorganisms (Yu et al. 2005; Nsereko et al. 2008). It has been suggested that partial hydrolysis of forage fibre, without release of ferulic acid, may be responsible for increased ruminal fibre digestibility of silage inoculated with FAE-producing inoculants (Nsereko et al. 2008). However, it is worth noting that the opposite effect is observed if silage undergoes extensive fermentation in the silo prior to being fed. This is because partial hydrolysis of hemicellulose and utilization of rapidly fermentable end products increases the concentration of the indigestible fibre fraction in the residue, thereby decreasing ruminal NDF digestibility (Van Vuuren et al. 1995; Sheperd and Kung 1996). Treatment of corn silage with a cocktail of fibrolytic enzymes at ensiling decreased NDF concentration of the silage, but increased the indigestible fraction and reduced *in vitro* NDF digestibility (Sheperd and Kung 1996). Feruloyl esterase activity in the IN silage might have increased the concentration of de-esterified fibre fractions leading to higher concentration of potentially digestible NDF in the silage. It has also been proposed that the ability of FAE-producing LAB to multiply and produce unknown metabolites that reduce toxin production during fermentation may also contribute to improved fibre digestibility of silage (Nsereko et al. 2008).

Occasionally, silage inoculants have improved digestibility and animal performance without any significant shifts in silage fermentation (Steen et al. 1989; Adesogan et al. 2009). A series of experiments by Weinberg (Weinberg et al. 2003, 2004) that examined the effect of silage inoculants on ruminal fibre digestibility and fermentation when in-silo fermentation was unaltered, have

suggested that LAB exert their influence in the rumen by causing favourable shifts in rumen microbial ecology and fermentation (Weinberg et al. 2003; 2004) as well as improvements in DM and NDF digestibility (Weinberg et al. 2007).

3.4.4. Ruminal Fermentation Pattern and pH Profile

The ruminal fermentation patterns in heifers fed both diets were generally comparable with those reported by Khorasani and Kennelly (1997) for high forage diets (800 g kg^{-1} DM barley silage). The molar proportion of propionic acid for heifers fed the IN diet was 14% greater than those fed the UN diet. This is consistent with the greater *in situ* NDFD observed for the IN silage and may reflect an increased release of pentoses such as xylose, arabinose and ribose from ruminal NDF degradation. The major potentially digestible fractions of hemicellulose include xylose and arabinose. Comparatively large amounts of xylose relative to arabinose are produced from hydrolysis of hemicellulose during ensiling of barley (MacGregor and Ewards 1968). Even though we did not measure the concentration of xylose and arabinose in the silages fed to heifers, increased partial de-esterification due to inoculation of the IN silage during ensiling may have supported increased *in situ* NDF digestibility and the release of xylose. Fermentation of this sugar may account for the increased propionic acid levels observed in the rumen of heifers fed IN silage. Previous studies support this hypothesis as mixing perennial ryegrass silage with xylose reduced the molar proportion of acetic acid and increased the molar proportion of propionic acid in the rumen (Chamberlain et al. 1993). Efficiency of utilization of metabolizable energy for growth in beef cattle vary with the proportions of energy derived from glucogenic compared to non-glucogenic VFA produced in the rumen with efficiency for growth being greater when NGR is optimum (between 3.0 and 4.0; Ørskov 1977).

Ruminal pH thresholds 5.8, 6.0 and 6.2 were chosen because ruminal fibre digestion has been shown to be inhibited at these thresholds (Russell and Wilson 1996; Plaizer et al. 2001; Zebeli et al. 2008). Ruminal pH profile for heifers fed the IN silage diet was consistent with the concentration of ruminal fermentation

acids. In both groups of heifers, pH was generally lowest 12 h after feeding and highest in the morning following rumination during the night (Figure 3.3). In a previous study, a duration of 6.6 h and area of 1.4 under pH threshold 5.6 reduced the 24-h *in situ* ruminal NDFD and the potential degradable NDF fraction of alfalfa-grass hay by 34% and 25%, respectively in cows fed a TMR containing 700 g kg^{-1} of forage (Plaizer et al. 2001). In the current study, duration and area under pH threshold 5.8 were 2.1 h and 0.4 for heifers fed the IN silage diet (767 g kg^{-1} DM silage), a difference that did not reduce fibre digestion. Ruminal fibre digestibility is impeded at pH below 6.0, because growth of most fibrolytic rumen bacteria is inhibited (Russell and Wilson 1996) and fibre attachment mechanisms are impeded (Sung et al. 2007). Even though some individual heifers attained a ruminal pH below 5.8 (Table 3.6), this duration was short-lived and unlikely to negatively impact digestion as optimal ruminal conditions for fibre digestion have been defined as when daily mean pH is < 5.8 for a duration not longer than 5.2 h d^{-1} (Zebeli et al. 2008). Others suggest that subclinical acidosis is considered to exist when the pH falls below 5.8 for more than 12 h d^{-1} (Schwartzkopf-Genswein et al. 2003), conditions that were not observed in the present study.

3.5. CONCLUSIONS

The fermentation characteristics of whole-crop barley silage inoculated with this FAE-producing silage inoculant at ensiling were generally indicative of a heterolactic fermentation, a characteristic of silages treated with *L. buchneri*-containing inoculants. Thermal IR assessment of aerobic stability of silages stored in large bag silos during feed-out suggest greater heating of UN silage during feed-out as compared to IN silage. Thermal imaging offers prospects as a practical method for assessing the aerobic stability of silages on-farm. Treatment of whole-crop barley silage with this third-generation FAE-producing inoculant also increased NDFD after 24 and 48 h of incubation. Treatment of barley silage with this FAE-producing inoculant could improve the aerobic stability and fibre digestibility of barley silage. Further studies will be required to determine the

ability of IR imaging to assess the aerobic stability of a variety of silage types in different ensiling systems throughout the year.

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CHAPTER 4: EFFECTS OF A THIRD-GENERATION ESTERASE INOCULANT ON FERMENTATION PATTERN AND AEROBIC STABILITY OF BARLEY SILAGE, AND ON THE EFFICIENCY OF WEIGHT GAIN OF GROWING FEEDLOT CATTLE [3]

4.1. INTRODUCTION

Homolactic lactobacilli produce lactic acid as the end product from the fermentation of hexose sugar. This characteristic was the primary basis for selection of the first-generation of silage inoculants in the 1970's (Lesins and Schulz 1968). In 2001, the US FDA approved *Lactobacillus buchneri* as a second-generation heterolactic inoculant for use as a silage additive, because its production of acetic acid was shown to extend the aerobic stability of silage by inhibiting growth of yeasts and moulds (Muck 2004).

Occasionally, inoculation of silage with *L. buchneri* has increased silage DM loss during fermentation, possibly due to a prolonged rate of pH decline following ensiling (Driehuis et al. 1999; 2001). In addition, silage with a high acetic acid concentration can reduce palatability (Buchanan-Smith 1990). Consequently, most of the commercial silage inoculants marketed today contain a mixture of both heterolactic and homolactic lactobacilli.

Ferulic acid (FA) is the most abundant and most inhibitory phenolic acid that limits ruminal fibre digestion of cereal forages (Yu et al. 2005), an impediment that was reduced through selection for low ferulate mutant corn hybrids (Jung et al. 2010). *In situ* digestibility studies have shown that complete or partial hydrolysis of FA linkages in normal forage hybrids may therefore directly improve ruminal digestion or increase the susceptibility of cell walls to ruminal digestion (Nsereko et al. 2008; Kang et al. 2009). This property has become the target for the development of a third-generation of silage inoculants.

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However, to date, no studies on the effects of FAE-producing inoculants on growth performance of feedlot cattle have been published.

This study therefore determined the effects of a FA esterase (FAE)-producing silage inoculant containing *L. buchneri* and homolactic lactobacilli on fermentation characteristics and aerobic stability of whole-crop barley, and on growth performance of growing feedlot steers.

4.2. MATERIALS AND METHODS

4.2.1. Forage Agronomy and Processing

A uniformly irrigated field was seeded with barley (*Hordeum vulgare*, L.; Chigwell, Field Crop Development Centre, Lacombe, Canada) on May 4, 2009 at the Lethbridge Research Centre, AB Canada. The crop was fertilized with 125 kg N ha^{-1} pre-seeding, and 12.5 kg N ha^{-1} and 57 kg PO_4 ha^{-1} post-seeding. Soil tests indicated that nutrient requirements for the crop were satisfied. The crop was swathed on Aug. 13, 2009 at the mid-dough stage of grain maturity (330 - 354 g kg^{-1} DM) and chopped to a 10-mm theoretical length using a forage harvester (John Deere 6610; Moline, IL, USA).

4.2.2. Mini Silo Experiment

4.2.2.1. Silage Preparation

A portion of the chopped forage was divided into six 25-kg lots (3 lots per treatment), and spread out on separate clean plastic sheets. Each lot was hand-sprayed with either 75 mL (3 mL kg^{-1}) deionized water (UN) or with an equal volume of deionized water containing a commercially marketed inoculant (11GFT; Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^5 CFU of lactic acid-producing bacteria (LAB) g^{-1} of fresh forage (IN). The inoculant contained a mixed bacterial culture of 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017 (ATCC no. PTA-6138) that produced FAE, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 (ATCC no. PTA-6139) and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (ATCC no. PTA-6135). The four

corners of the sheet were drawn together and the forage was tumbled inside the sheet for approximately 3 min and hand mixed for an additional min to ensure thorough mixing.

Approximately 2.5 to 3.0 kg of the UN or IN forage was then packed into each labelled mini silo (10.4 cm in diameter × 35.6 cm in height) with a hydraulic press to achieve a density of ~ 240 kg m^{-3}. Each labelled silo was weighed with its cap prior to being filled and immediately after sealing. The silos were stored at ambient temperature (20^{o}C) and opened after 1, 2, 3, 4, 7, 14 and 95 d of ensiling. Triplicate silos were prepared and opened for each treatment and sampling d except for d 95 for which six silos were prepared and opened for each treatment. The procedure for filling the silos involved randomly selecting one of three sets of triplicate silos (1, 2 or 3) of each treatment across all sampling days and filling them with each 25-kg lot of forage. Prior to being ensiled (d 0), triplicate forage samples were also collected from each lot for chemical and microbial analyses. Silos were weighed prior to opening to calculate DM loss. The six mini silos opened on d 95 were subsampled for assessment of aerobic stability and for laboratory analysis.

4.2.2.2. Aerobic Stability

The six mini silos opened on d 95 for each treatment were composited into three samples by bulking the contents of two replicates into one sample. This resulted in three composited silage samples per treatment. Subsamples of silage (400 g) obtained from composited silage samples were placed into separate triplicate 4-L insulated containers (13.5 cm in diameter × 30.9 cm in height) per treatment, covered with two layers of cheesecloth and stored at ambient temperature (18 - 20°C) for 21 d. Two Dallas Thermochron iButtons (Embedded Data Systems, Lawrenceburg, KY, USA), were embedded in the silages at ~9.0 cm and ~18.0 cm from the bottom of each container containing the silages. Two additional Thermochron iButtons were placed in the room where the silos were stored for recording ambient temperature. The Thermochron iButtons were programmed to record temperature every 15 min for 21 d. The contents of each container were

mixed thoroughly and sub-sampled on 1, 2, 3, 5, 7, 14 and 21 d of exposure for determination of pH, chemical and microbial parameters. The Thermochron iButtons were repositioned at a similar depth in the container after each subsampling.

4.2.3. Animal Experiment

The experimental protocol was approved by the Animal Care Committee of Lethbridge Research Centre and cattle were cared for and managed according to the guidelines of the Canadian Council on Animal Care (1993).

4.2.3.1. Ag-Bag® Silage Preparation, Animals and Feeding

Chopped forage was delivered by two trucks to two Ag-Bag baggers (Ag-Bag, a Miller-St. Nazianz, Inc. Co., St. Nazianz, WI, USA). Forage placed in one bag was sprayed with water (UN) whereas forage placed in the second bag was sprayed with an inoculant solution at a rate of 1 L $tonne^{-1}$ of forage (IN) using an all-terrain vehicle (ATV) sprayers (AG Spray Equipment, Hopkinsville, KY, US) just prior to being compressed into Ag-Bags® (3.0 × 45.7 m; Ag-Bag Int. Ltd., Warrenton, OR, USA). Trucks delivered alternate loads to each of the baggers to minimize treatment differences in forage quality due to harvest location and time. Approximately 150 tonnes each of UN and IN silage were generated with the inoculant being applied at the same concentration (1.3×10^5 CFU of LAB g^{-1} of forage) as in the mini silo experiment. The bags were opened after 90 d of ensiling and the silages were used to formulate 2 growing feedlot cattle diets containing either the uninoculated silage (referred to as the UN diet) or inoculated silage (referred to as the IN diet). The ingredients and assayed chemical compositions of the total mixed ration (TMR) are shown in Table 4.1. Feed ingredients and TMR were sampled weekly and composited every 28-d period for chemical analysis. Five silage samples were collected from each Ag-Bag® silo and combined into a composite sample on each day of sampling. Silage was sampled on d 95, 123 and 175 of ensiling which corresponded to d 5, 33 and 85 of

feeding out the silages. The samples were thoroughly mixed and immediately sent to the laboratory for microbial and chemical analyses as per min silos.

Forty Angus × Hereford crossed-bred steers with an initial average body weight of 242 ± 2.7 kg (mean ± SEM) were stratified by weight and allocated randomly to each of two treatments. Steers were housed in individual 2.5 m × 3.0 m pens at the Lethbridge Research Centre. Steers were weighed on two consecutive days at the beginning (Nov. 9 and 10, 2009) and end (Mar. 2 and 3, 2010) of the study with the average of consecutive weights used as the initial and final weights, respectively. Steers were implanted with Component E-S (Ivy Animal Health, Inc., Overland Park, KS, USA) at the start of the experiment. A Calan Data Ranger (American Calan, Northwood, NH, USA) was used to mix the silage with steamed-rolled barley grain and supplement (Table 4.1). The supplement contained (g kg^{-1} DM) canola meal (100); urea (20); limestone (250); sodium chloride (30); ground barley grain (590) and a feedlot premix (10). The feedlot premix consisted of a mineral supplement containing: calcium carbonate (348), Zinc sulphate (284), manganous sulphate (146), copper sulphate (103), ethylene diaminediiodic acid (20; as an 80% preparation), selenium (50), cobalt sulphate (10), vitamin A (1000 000 IU g^{-1}; 17 g kg^{-1} DM), vitamin D (500 000 IU g^{-1}; 2 g kg^{-1}) and vitamin E (500 mg^{-1}; 47 g kg^{-1}). Diets were formulated to meet or exceed the nutrient requirements of growing steers (National Research Council 1996). Steers had free choice access to their feed and water with fresh feed provided once daily (0900 h). The amount of feed offered was recorded daily, orts were collected daily, and weighed, sampled and discarded weekly and steers were weighed every 28 d. Silage and orts DM were also monitored weekly throughout the experiment, but DM contents were sufficiently uniform that adjustments in diet composition were not necessary. Feed intake, ADG and feed efficiency (expressed as gain $feed^{-1}$) were estimated for the 112-d feeding period.

Table 4.1. Ingredient and assayed chemical compositions (mean ± standard deviation) of the total mixed ration (TMR) containing uninoculated or inoculated whole-crop barley silages fed to growing feedlot cattle (DM basis)[z]

Item	Uninoculated	Inoculated
Ingredient composition of diets (g kg^{-1} DM)		
Barley silage[x]	763	767
Steamed-rolled barley grain	179	177
Supplement[w]	58	56
Chemical composition of TMR (g kg^{-1} DM)		
Dry matter	411±10.2	435±12.0
Organic matter	924±1.7	922± 2.4
NDF	367±16.8	363±12.4
ADF	253±18.9	238±10.4
Starch	275±9.3	287±4.9
Crude protein	131±3.8	131±2.7
ADIN[v] (total N)	154±38.9	119±3.8

[z]The whole-crop barley silage component of the TMR was either not inoculated or inoculated at ensiling with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^{5} CFU g^{-1} of fresh forage.

[y]Four monthly samples were collected and analyzed (n = 4) for each component.

[x]Corrected for volatilization of VFA, lactic acid and ammonia-N after oven-drying at 105°C using the equation of Porter et al. (1995).

[w]The supplement contained (g kg^{-1} DM) canola meal (100); urea (20); limestone (250); sodium chloride (30); ground barley grain (590); feedlot premix (10). The pre-mix contained: calcium carbonate (348), Zinc sulphate (284), manganous sulphate (146), copper sulphate (103), ethylene diaminediiodic acid (2; as an 80% preparation), selenium (50), cobalt sulphate (1), vitamin A (1000 000 IU g^{-1}; 17), vitamin D (500 000 IU g^{-1}; 2) and vitamin E (500 mg^{-1}; 47).

[v]ADIN, Acid detergent insoluble nitrogen.

4.2.4. Chemical Analyses

For chemical analyses of silage samples, 15 g of fresh forage or silage was mixed with 135 mL of distilled water, blended in a Waring blender (Waring Commercial, Torrington, CT, USA**)** for 30 sec at full speed, and filtered through two layers of cheesecloth. The pH of the filtrate was immediately measured with a Symphony pH meter (VWR, Mississauga, ON, Canada). The filtrate was then divided into two portions. One portion immediately was boiled for 10 min to halt fermentation and was stored at -20^{o}C for subsequent analysis of water-soluble carbohydrates (WSC; glucose equivalent) by the Nelson-Somogyi (1944) method on a Dynatech MRX micro-plate reader (Dynatech Laboratories Inc., Chantilli, VA, USA) at 630 nm.

The second portion of the filtrate was stored on ice until it was centrifuged for 15 min at 10,000 × *g* (4^{o}C); the supernatant was collected for analysis of VFA, lactic acid and NH_3-N. For determination of VFA, 1.5 mL of the supernatant was deproteinized with 0.3 mL of 25% (wt vol^{-1}) metaphosphoric acid, combined with 0.2 mL of 0.1 M crotonic acid as internal standard, and analyzed on a Hewlett Packard model 5890A Series Plus II gas-liquid chromatograph (Hewlett Packard Co., Palo Alto, CA, USA) with a 30-m Zebron free fatty acid phase fused silica capillary, 0.32-mm i.d., and 1.0-µm film thickness column (Phenomenex, Torrance, CA). For lactic acid determination, 400 uL of the deproteinized sample was combined with 50 uL of 3 mM malonic acid (5 mg mL^{-1}) as an internal standard. Lactic acid was methylated and then quantified using the method of Kudo et al. (1987) on the same column and chromatograph as was used for VFA analysis. To determine NH_3-N, 1.6 mL of the supernatant was combined with 0.15 mL of 65% (wt vol^{-1}) trichloroacetic acid and analyzed by the phenol-hypochlorite method as described by Broderick and Kang (1980).

For starch determination, forage or silage samples first were freeze-dried and ball-ground using a mixer mill (MM 400, Retsch Inc. Newtown, PA, USA). Starch was determined after hydrolysis to α-glucose polymers using amyloglucosidase (Megazyme Int. Ltd., Wicklow, Ireland, UK) and 1,4 α-D-glucan glucano-hydrolase (Brennfag Canada Inc., Toronto, ON Canada) as

described by Herrera-Saldana et al. (1990). Samples were read on a micro-plate reader at a wavelength of 490 nm.

For determination of CP, fresh or lypholized ball-ground samples (5 mg) were subjected to combustion analysis for total N (Dumas Nitrogen) using an NA1500 Nitrogen/Carbon analyzer (Carlo Erba Instruments, Milan, Italy). Crude protein was calculated as N × 6·25.

Dry matter of the fresh forage, silage samples and TMR was determined by drying at 105^{o}C for 24 h in a forced air oven. Ag-Bag® silages were corrected for volatile losses using the equation of Porter et al. (1995). Organic matter was determined by ashing samples (1g) in a muffle furnace at 550^{o}C for 5 h. Subsamples of the fresh forage, silage and TMR were stored at -20^{o}C until being lyophilized, and ground through a 1-mm screen for analysis of NDF and ADF using an Ankom 200 system (Ankom Technology Corporation, Fairport, NY, USA). Neutral detergent fibre was analyzed with the addition of sodium sulfite and α–amylase whereas ADF was analyzed without α–amylase. Nitrogen in ADF residues (ADIN) was measured as described above for N analysis.

4.2.5. Microbial Analyses

For microbiological analyses, forage or silage samples (10 g) were added to 90 mL of sterile 70 mM potassium phosphate buffer (pH = 7.0) and agitated for 60 s at 260 rpm in a Stomacher 400 Laboratory Blender (Seward Medical Limited, London, UK). The suspension was serially diluted (10^{-2} to 10^{-7}) and 100-µL aliquots of each dilution were spread in triplicate onto semi-selective lactobacilli media (de Man-Rogosa-Sharpe, MRS; Oxoid, Basingstoke, Hampshire, UK) for enumeration of LAB (Hill and Hill 1986), onto nutrient agar (NA; Difco, Detroit, MI, USA) for the enumeration of total culturable bacteria, and onto Sabouraud's dextrose agar (SDA; Difco, Detroit, MI, USA) for the enumeration of yeasts and moulds. Lactobacilli MRS agar and NA were amended with 200 µg mL^{-1} of cycloheximide (Sigma-Aldrich, St. Louis, MO, USA) and SDA with 100 µg mL^{-1} each of tetracycline and chloramphenicol. Lactobacilli MRS agar plates were incubated at 37°C for 24 - 48 h and SDA plates were incubated at ambient

temperature for 72 h. Colonies were counted from plates containing a minimum of 30 and a maximum of 300 colonies.

The FAE activity of the inoculant was confirmed by a procedure previously described by Donaghy et al. (1998) using ethyl 4-hydroxy-3-methoxycinamate (10.3% wt vol^{-1}; Sigma-Aldrich, St. Louis, MO, USA) at a final concentration of 5% vol vol^{-1} (10 μL mL^{-1} of MRS media) in each plate. The inoculant was suspended in deionized water (111.1 mg mL^{-1}). Six wells (6.94 mm in diameter) were made on triplicate media plates. Solutions were either autoclaved or not autoclaved prior to each well being inoculated (3 wells $solution^{-1}$; 35 μL $well^{-1}$). A similar procedure was used for determination of FAE in silage samples. Subsample (15 g) of the IN or UN silage from d 3, 7, 14 and 95 of ensiling was added to 10 mL of 70 mM potassium phosphate buffer (pH = 7.0) and agitated (60 s at 260 rpm) as for microbial detachment above. The suspension was then filtered through four layers of cheesecloth and used to inoculate each well as per the inoculant solution. Hydrolysis of ester linkages of the substrate resulted in the formation of a clearing zone that was assumed to be proportional to FAE activity (Donaghy et al. 1998).

4.2.6. Statistical Analyses

Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Data on microbial populations were transformed to $\log_{10}$ CFU g^{-1} of DM of forage or silage prior to statistical analysis. Silage fermentation characteristics and aerobic stability data measured over time in mini silos were assessed using a repeated measures analysis with each mini silo (n = 3) as the experimental unit for the ensiling data and each 4-L insulated container (n = 3) as the experimental unit for the aerobic stability data. Least square means of daily temperatures of both silages and the corresponding ambient daily temperature were computed and difference among the ambient temperature and silage temperature on each day of aerobic exposure were separated using the Dunnett's adjustment option of LSMEANS procedure of. For repeated measures analysis, six covariance structures [compound symmetry, heterogeneous compound

symmetry, autoregressive order one, heterogeneous autoregressive order one, unstructured and unstructured order one] were evaluated for each variable with the covariant structure yielding the smallest value for Akaike's information criteria and Bayesian information criteria being selected for presentation and discussion of results. Silage fermentation characteristics and aerobic stability were analyzed for the fixed effects of inoculation and inoculation × day interactions.

All data on DMI and growth performance of steers (weight gain, ADG and gain-to-feed ratio) were analyzed for the fixed effects of inoculation as a completely randomized design with initial weight as a covariate in the model and pen as the experimental unit.

Differences in least-square means of all fixed effects were declared statistically significant at $P \leq 0.05$.

4.3. RESULTS

4.3.1. Mini Silo Experiment

4.3.1.1. Forage Characteristics and Effect of Inoculation on NDF Concentration during Ensiling

The composition of samples of barley forage taken prior to ensiling and 95 d after ensiling is presented in Table 4.2. The chemical and microbiological composition of the forage prior to ensiling was comparable with those of Zahiroddini et al. (2004) for barley forage harvested at a similar DM content. The FAE-producing capability of the inoculant was confirmed by the formation of distinct clearing zones around wells inoculated with the unautoclaved inoculant solution as opposed to their absence in the autoclaved solution, a response indicative of de-esterification of ethyl 4-hydroxy-3-methoxycinamate. For silage samples, only wells inoculated with silage filtrate obtained from the IN silage on d 3 of ensiling showed clearing zones, indicative of FAE activity. Thereafter, no FAE activity was detected in silages ensiled for 7, 14 and 95 d.

Ensiling did not alter ($P \geq 0.12$) starch, ADF and ADIN concentration, and the total culturable bacteria population. However, the concentrations of NDF and ADF were higher ($P < 0.01$) in IN than in UN silage after 95 d of ensiling.

4.3.1.2. Silage Fermentation Characteristics

The IN silage exhibited a homolactic fermentation during the initial stages of ensiling as indicated by greater ($P \leq 0.01$) lactic acid (Figure 4.1a.) concentrations, and a more rapid ($P < 0.01$) decrease in pH (Figure 4.2.), particularly during the first 7 d of ensiling. The concentrations of acetic (Figure 4.1b.) and propionic (data not shown) acids between d 3 and 14 d after ensiling were not altered by inoculation. However, fermentation products in the d 95 IN silage were indicative of a heterolactic fermentation with pH and acetic acid concentration being greater ($P < 0.001$) and the lactic acid concentration being lower ($P < 0.001$) for IN than UN silage (Table 4.2.). Inoculation decreased ($P < 0.01$) the concentration of residual WSC compared to that in the UN silage, an indication of greater utilization of WSC by LAB in the IN silage compared to the UN silage during ensiling (Reich and Kung 2010). Butyric acid concentrations were below detectable limits for IN silages, but present at 6.0 g kg^{-1} DM for UN silage. Ammonia-N concentration and DM loss did not differ between IN and UN silage.

Table 4.2. Effects of inoculation on pH, ferulic acid esterase activity, chemical composition and products of fermentation of whole-crop barley silage after 95 d of ensiling in mini silos[z]

Item	Unensiled forage (± SD)[y]	Silage			
		Uninoculated	Inoculated	SEM[x]	*P*-value[x]
pH	7.12 ± 0.064	3.99	4.43	0.056	<0.001
pH decline[w] (units d^{-1})	–	0.36	0.40	0.002	<0.001
Dry matter[v]	342.9 ± 5.0	314.6	314.7	5.07	0.981
Chemical composition (g kg^{-1} DM silage)					
Organic matter	930.9 ± 4.5	924.0	923.4	0.92	0.108
NDF	458.2 ± 2.3	375.7	414.0	7.60	0.001
ADF	242.8 ± 0.4	238.5	257.3	4.61	0.004
ADIN (of total N)[u]	52.0 ± 1.45	59.8	70.2	4.72	0.103
Starch	246.9 ± 29.3	238.9	228.8	4.56	0.186
WSC (glucose equiv.)[t]	10.3 ± 5.0	19.42	39.7	0.809	0.001
Crude protein	126.0 ± 1.2	128.8	13.37	1.32	0.010
Dry matter loss	–	100.1	108.0	12.2	0.524
End products of fermentation (g kg^{-1} DM silage)					
Acetic acid	15.5 ± 0.16	17.3	42.4	0.88	<0.001
Propionic acid	2.5 ± 1.11	2.83	2.89	0.128	0.648
Butyric acid	5.3 ± 1.47	5.59	0.00	0.924	<0.001
Lactic acid	0.1 ± 0.18	74.03	38.51	2.248	<0.001
Succinic acid	1.1 ± 0.09	2.44	2.42	0.054	0.737
Ammonia-N (of total N)	10.0 ± 0.66	93.5	95.4	3.500	0.220
Lactic: acetic ratio	0.04 ± 0.012	4.29	0.91	0.192	<0.001
Microbial composition (log_{10} CFU g^{-1} DM silage)					
Total culturable bacteria	8.0 ± 0.04	8.0	8.5	0.49	0.457
Lactic acid-producing bacteria	5.2 ± 0.10	7.9	9.7	0.05	<0.001
Moulds[s]	6.8 ± 0.01	ND	ND	-	-
Yeasts[s]	7.3 ± 0.02	ND	ND	-	-

[z]Whole-crop barley silage was either not inoculated or inoculated at ensiling with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^5 CFU g^{-1} of fresh forage.

[y]Means ± SD (n = 3) for forage samples collected before treatment and ensiling.

[x]Standard errors of least-square means (n = 3) and *P*-values represent statistical comparison between inoculated and uninoculated silage only and do not include comparison with the unensiled crop.

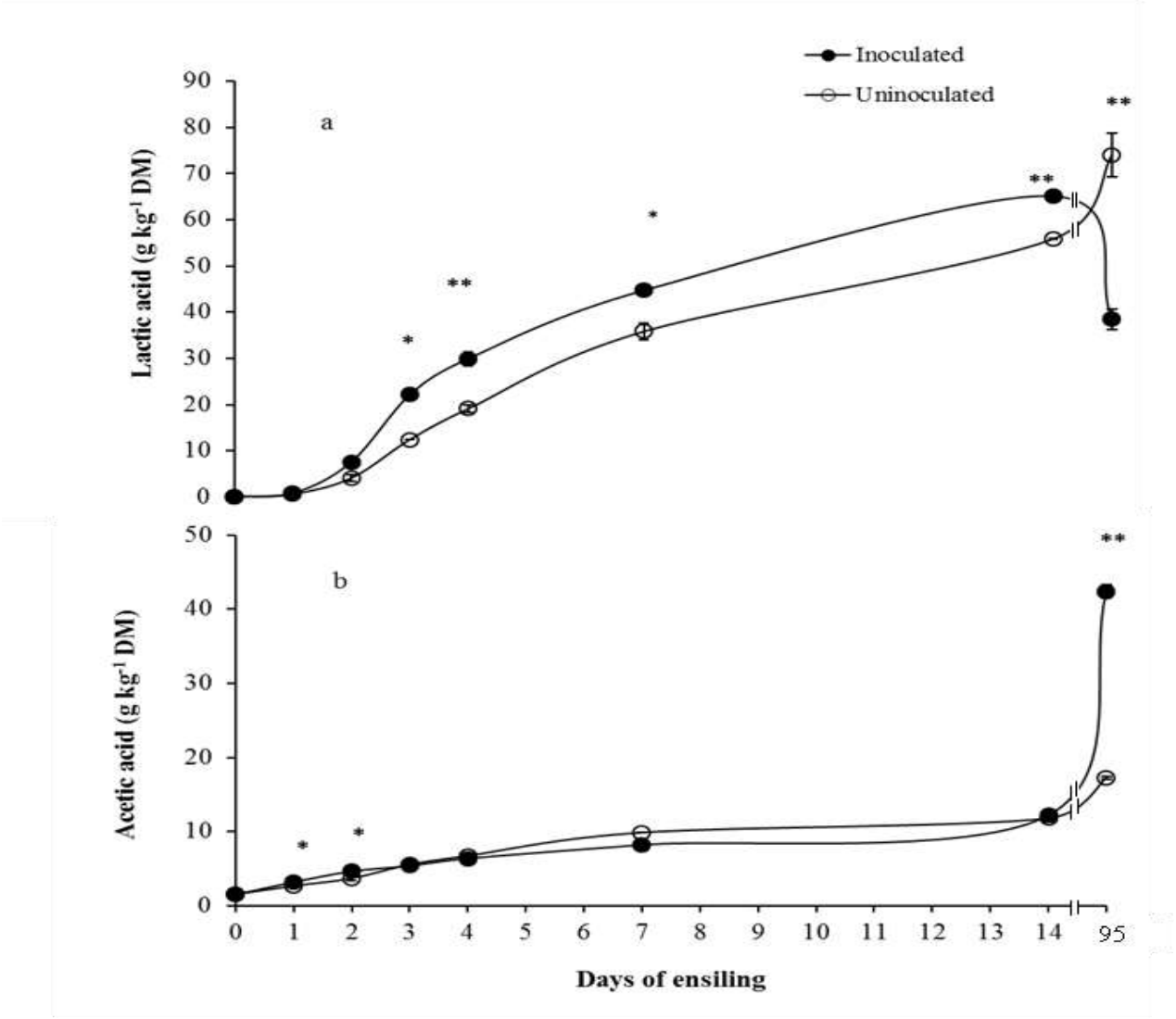
[w]Estimated for the first 7 of ensiling as initial pH of forage minus pH at d 7 (pH at d 7 of ensiling; inoculated = 4.33; uninoculated = 4.61) divided by 7 d.

[v]Corrected for volatilization of VFA, lactic acid and ammonia-N after oven-drying at 105°C using the equation of Porter et al. (1995).

[u]ADIN, Acid detergent insoluble nitrogen.

[t]WSC, water-soluble carbohydrates (glucose equivalent).

[s]ND, not detected (silage diluted to 10^{-1}).



Figue 4.1. Effects of inoculation on lactic (a) and acetic (b) acid concentrations during 95 d of fermentation of whole-crop barley in mini silos. Where visible, vertical bars indicate standard errors for least-square means. Asterisks indicate days when concentrations of lactic or acetic acid differed (* = $P < 0.05$; ** = $P < 0.01$) between inoculated and uninoculated silage. Inoculated silage was treated at ensiling with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^{5} CFU g^{-1} of fresh forage.

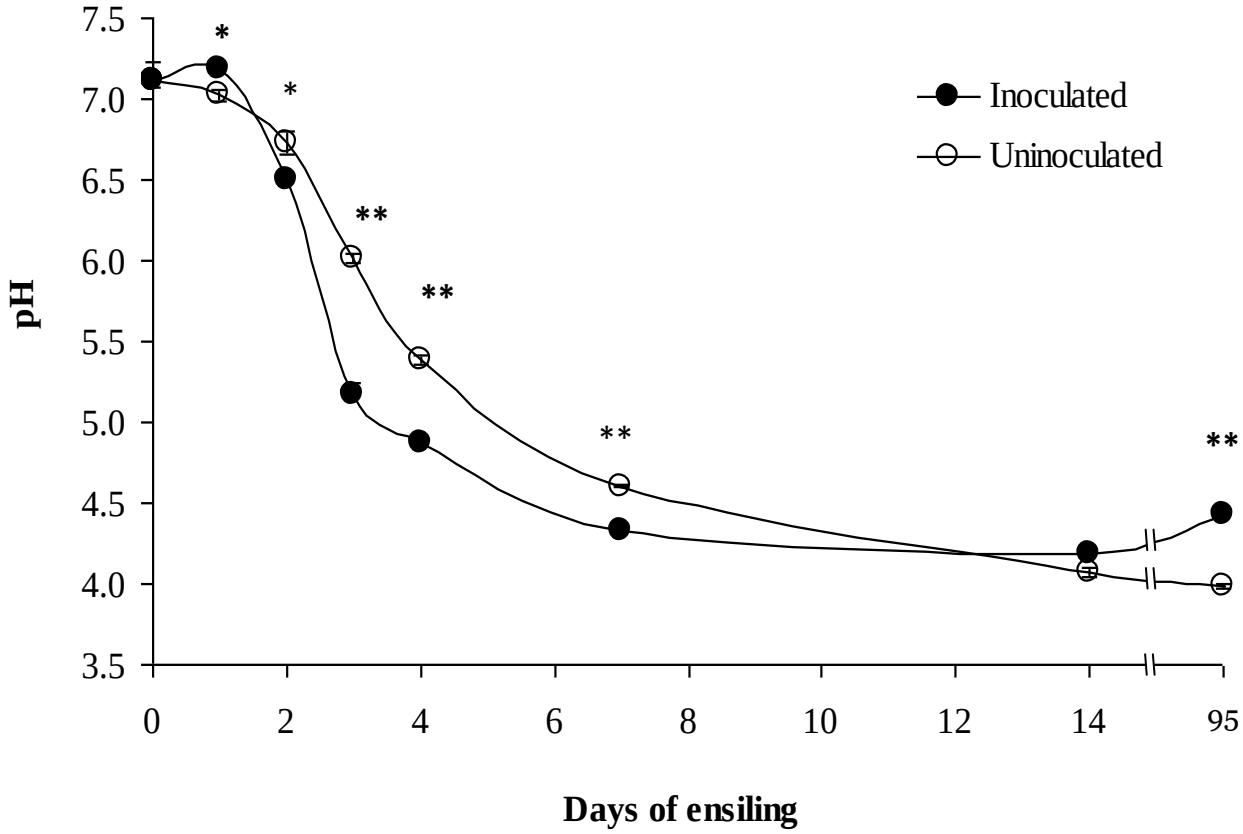


Figure 4.2. Effect of inoculation on pH during 95 d of fermentation of whole-crop barley in mini silos. Where visible, vertical bars indicate standard errors for least-square means. Asterisks indicate days when pH differed (* = $P < 0.05$; ** = $P < 0.01$) between uninoculated and inoculated silage. Inoculated silage was treated at ensiling with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^{5} CFU g^{-1} of fresh forage.

4.3.1.3. Aerobic Stability

The UN silages remained stable only until d 5 after which they began to deteriorate as reflected by a greater ($P < 0.001$) yeast population on d 21 of exposure (Table 4.3.), and increases in pH (Figure 4.3a.) and temperature (Figure 4.3b.). The pH of UN silage at 95 d persisted for the first 5 d of aerobic exposure, but rose ($P \leq 0.01$) sharply thereafter reaching 8.8 on d 7. In contrast, the pH of IN silage remained below 4.7 for the entire 21 d of aerobic exposure. The temperature of the UN silage was more than 5^{o}C above ($P < 0.001$) ambient temperature after 7 d of aerobic exposure, reaching 31^{o}C on d 18, but the temperature of IN silage never exceeded ambient temperature by more than 1^{o}C during the experiment (Figure 3b). Yeasts reached 5 $\log_{10}$ CFU kg^{-1} DM in UN silage after 5 d of aerobic exposure and were up to 9 $\log_{10}$ CFU g^{-1} DM of silage by 14 d; yeast populations of the IN silage never exceeded 1 $\log_{10}$ CFU g^{-1} DM of silage throughout the 21 d of exposure. Yeast populations for the UN paralleled the increases in pH and temperature shown in Figure 4.3. Acetic acid and lactic acid concentrations in silages sampled on d 21 of aerobic exposure were higher ($P < 0.001$) for the IN compared to the UN silage (Table 4.3.).

The IN silage exhibited reduced susceptibility to spoilage upon exposure to air. Higher ($P < 0.001$) residual WSC concentration in UN silage (Table 4.2.) may have served as a readily available source of energy for growth of yeasts leading to the deterioration of the UN silage upon exposure to air as indicated by its elevated pH and temperature (Figure 4.3.). However, the concentration of acetic acid in IN silage even after 5 and 14 (data not shown), and 21 d of air exposure was 3, 42 and 44 times higher respectively, than for UN silage.

Table 4.3. Effect of inoculation on the chemical and microbiological composition of whole-crop barley silage exposed to air for 21 d[z]

Item	Uninoculated	Inoculated	SEM[y]	*P*-value
Dry matter (g kg^{-1})	405.6	410.1	4.58	0.464
Chemical composition (g kg^{-1} DM silage)				
WSC (glucose equivalent)[x]	7.74	2.76	0.143	<0.001
Acetic acid	1.39	61.21	1.433	<0.001
Propionic acid	20.81	2.27	0.102	0.231
Butyric acid[w]	ND	ND	–	–
Lactic acid	0.79	11.36	0.363	<0.001
Microbial composition (log_{10} CFU g^{-1} DM silage)				
Lactic acid-producing bacteria	9.54	8.93	0.098	0.011
Mould	7.71	2.68	0.307	<0.001
Yeast	6.53	0.80	0.863	<0.001

[z]Whole-crop barley silage was either not treated or inoculated at ensiling with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^5 CFU g^{-1} of fresh forage.

[y]Three samples were collected and analyzed (n = 3) for each component.

[x]WSC, water-soluble carbohydrates (glucose equivalent).

[w]ND, not detected (silage diluted to 10^{-1}).

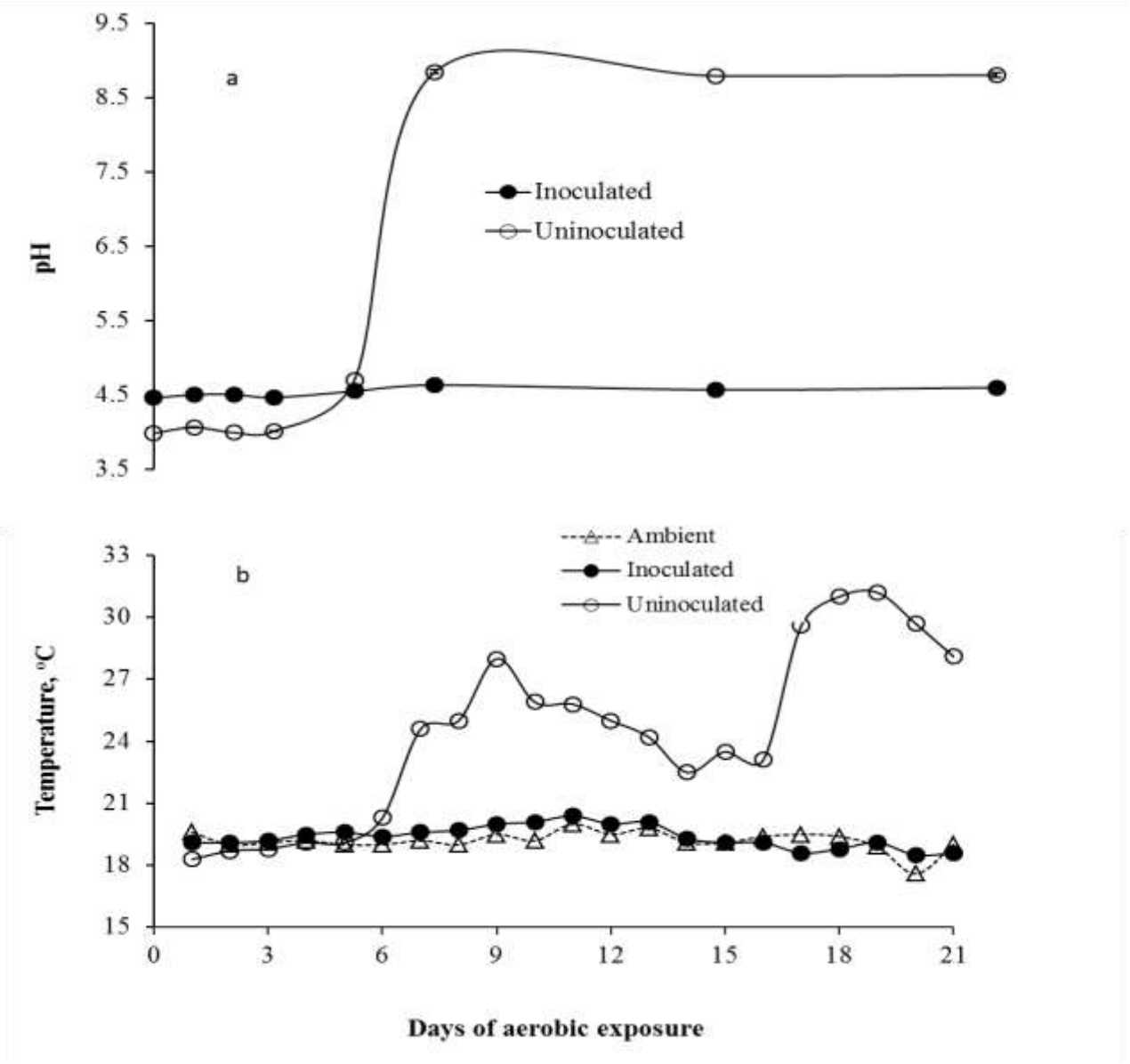


Figure 4.3. The effect of inoculation on changes in pH (a) and temperature (b) of whole-crop barley silages exposed to air for 21 d after 95 d of ensiling in mini silos. Where visible, vertical bars indicate standard errors for least-square means. Inoculated silage was treated at ensiling with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^{5} CFU g^{-1} of fresh forage.

4.3.2. Feedlot Experiment

The compositions of the total mixed ration TMR containing uninoculated or inoculated whole-crop barley silages that were offered to the steers are shown in Table 4.1. Using net energy equations from the NRC (1996), the estimated ME for the IN silage diet was 7.7% greater than that of the UN silage diet (2.8 *vs* 2.6 Mcal kg^{-1} DM). The composition of silages from Ag-Bags® used for formulating the TMR that was offered to the steers in the feedlot experiment is presented in Table 4.4. Results for DMI and growth performance of steers fed UN or IN silage diets are reported in Table 4.5. Dry matter intake was lower ($P = 0.019$) for steers fed the IN than for those fed the UN diet but average daily gain did not differ ($P = 0.65$) between the two groups of steers. The efficiency of feed conversion was greater ($P = 0.03$) for steers fed IN (0.19) as compared to those fed UN silage (0.17).

4.4. DISCUSSION

4.4.1. Mini Silo Experiment

4.4.1.1. Forage Characteristics and Effect of Inoculation on NDF Concentration during Ensiling

Wells inoculated with silage filtrate obtained from the IN silage on d 3 of ensiling showed clearing zones, indicative of FAE activity but this was not observed in silages ensiled for 7, 14 and 95 d .This is consistent with the optimum range of pH for expression of FAE activity; 5.6 (Topakas et al. 2007) to 6.5 (Donaghy et al. 1998) after 3 d of incubation. The pH of the silage was within this optimal range at 3 d of ensiling but declined thereafter (Figure 4.1.). Nsereko et al. (2008) previously used the formation of cleared zones on the same substrate to confirm the FAE activity of several strains of *L. buchneri*.

Hemicellulose and cellulose constitute the potentially digestible fractions of forage NDF. Hemicellulose contains arabinoxylans and glucuronoarabinoxylans that may be linked to FA or coumaric acid or cross-linked to each other by these hydroxycinnamic acids. Ferulic acid in turn may be linked directly to lignin or serve as an initiation site for lignification as the plant

matures. Various microorganisms produce classes of FAE that differ in their affinity towards ester linkages and the type of linkage formed between FA and arabinose units (*O*-5 or *O*-2). Based on these properties, FAE have been classified into four groups; I, II, II and IV (Topakas et al. 2007). Group I are able to hydrolyze only the *O*-5 but not *O*-2 FA-carbohydrate-linkages, whereas group II can hydrolyze both linkages. Additionally, groups I and IV in contrast to II and III, also can sparingly hydrolyze the 5-5′ diferulic acid (Topakas et al. 2007). The type and specificity of the FAE produced by *L. buchneri* LN4017 contained in the inoculant used in the current study was not determined, but most gramineceous plants have FA linked to the *O-5* position of the arabinose side chains of arabinoxylans (Chesson 1988; Yu et al. 2005). Incomplete hydrolysis of these esters can render the feruloylated polysaccharides more fragile and susceptible to further ruminal degradation, but may not cause release of either free FA, xylose or arabinose leaving NDF levels unaltered. Therefore an increased esterase activity in silage may not decrease NDF concentration per se even though it can increase the extent of ruminal NDF digestion through an increase in the concentration of potentially digestible NDF fractions in the silage (Nsereko et al. 2008; Kang et al. 2009). The NDF contents of FAE–inoculated silages have been either similar to (Kang et al. 2009) or higher (Nsereko et al. 2008) than that of the uninoculated silage.

The relative importance of NDF content versus NDF digestibility in altering forage intake and ruminal digestion remains uncertain. A reduction in NDF content or an increase in ruminal NDF digestibility should reduce ruminal bulk fill allowing intake of diets rich in forage to increase. However, extensive degradation of ruminally fermented substances during silage fermentation reduces the amount of energy available for growth of ruminal microbes (Charmley 2001). By degrading the more readily fermented NDF, extensive fermentation of forage prior to feeding should decrease the digestibility of the remaining NDF (van Vuuren et al. 1995).

4.4.1.2. Silage Fermentation Characteristics

Degradation of acidic products into less acidic metabolites appears to be a primary metabolic pathway employed by *L. buchneri* under low pH conditions during fermentation (Nishino et al. 2004; Driehuis et al. 2001). Oude Elferink et al. (2001) reported that as pH declines below 4.3, a wider transmembrane pH gradient causes this microbe to convert lactic acid with a pKa of 3.9, to products with greater pKa including acetic acid (pKa = 4.8), 1, 2-propanediol (pKa = 14.9) and ethanol (pKa = 15.9). In the present study, a pH below 4.3 was attained for IN silage after 14 d of ensiling (Figure 4.2.) with indications of the conversion of lactic acid to acetic acid occurring at some point between d 14 and d 95 (Figure 4.1.).

In addition to *L. buchneri* LN4017, the inoculant used also contained *L. plantarum* LP7109 and *L. casei* LC3200. Some strains of *L. plantarum* can in the absence of WSC, or even when WSC are present together with citrate as an electron acceptor, metabolize lactic acid to acetic, succinic and formic acids (Lindgren et al. 1990). However, the increases in acetic concentration observed with the IN silage likely are attributable to the degradation of lactic acid by *L. buchneri* as concentrations of succinic acid or its metabolite; propionic acid did not differ between IN and UN silage (Table 4.2.). Changes in pH between d 14 and d 95 presumably reflected changes in the end products of fermentation. The relatively higher pH of the IN silage also may account for its higher population of viable LAB in IN silage (9.7 $\log_{10}$ CFU g^{-1} DM of silage) than for UN silage (7.9 $\log_{10}$ CFU g^{-1} DM of silage) at 95 d after ensiling.

Concerns have been expressed in the past about commercial silage inoculants containing *L. buchneri* resulting in increased loss of DM and energy due to the anaerobic conversion of lactic acid to acetic acid resulting in a higher terminal pH (Driehuis et al. 1999; 2001; Nishino et al. 2004). In the present study, the initial rapid acidification for IN silage as compared to UN silage may reflect the homofermentative activity of *L. plantarum* LP7109 and *L. casei* LC3200 in this inoculant. Co-inoculation of *L. buchneri* LN4017 with *L. plantarum* LP7109 and *L. casei* LC3200 in this study therefore probably had a synergistic

effect on fermentation by inducing a rapid decline in pH that should reduce DM loss. These results are consistent with previous reports where silages were co-inoculated with *L. buchneri* and homolactic lactobacilli (Kleinschmit and Kung 2006; Kang et al. 2009; Reich and Kung 2010). Strategies to reduce DM losses associated with inoculating silages with heterolactic *L. buchneri* therefore should include co-inoculation with homolactic lactobacilli (Driehuis et al. 2001; Filya 2003) and an optimization of the inoculation rate (Driehuis et al. 1999; 2001).

The loss of DM (100 g kg^{-1} DM) observed with IN silage was lower than previously reported for barley silage inoculated with *L. buchneri* plus *L. pentosaceus* (Zahiroddini et al. 2006), but higher than losses typically reported for corn silages treated with similar inoculants (Kang et al. 2009; Reich and Kung 2010). An increase in DM loss associated with anaerobic degradation of lactic acid to acetic acid must be balanced against the reduced aerobic deterioration of acetic acid-rich silages during feeding. In most cases, the increased loss of DM associated with anaerobic metabolism of lactic acid by *L. buchneri* is far less than the loss of DM arising from the oxidation of lactic acid and WSC during exposure of fermented silages to air (Driehuis et al. 2001).

4.4.1.3. Aerobic Stability

Increased stability of the IN silage therefore could be attributed to the strong anti-mycotic effect of acetic acid on aerobic spoilage organisms. The deterioration of the UN silage despite the absence (dilution 10^{-1}) of moulds until after 21 d of aerobic exposure, confirms the secondary role of moulds in silage deterioration. Yeasts populations often dictate the pace of barley silage spoilage and their predominance precedes that of moulds in barley silage (McAllister et al. 1995).

Consistent improvements in the aerobic stability of forage crops such as barley (Taylor et al. 2002; Zahiroddini et al. 2006), corn (Nishino et al. 2004; Kang et al. 2009; Reich and Kung 2010), grass (Driehuis et al. 2001), wheat and sorghum (Weinberg et al. 1999) and alfalfa (Kung et al. 2003; Zhang et al. 2009) inoculated with microbial additives containing *L. buchneri* at ensiling have been reported. Typically, whole-crop barley silage inoculated in this manner remains

stable for 4 to 14 d (Taylor et al. 2002; Zahiroddini et al. 2006). In another study where aerobic stability of barley silage inoculated with *L. buchneri* was extended for 31 d, an inoculation rate five times higher than that used in the current study was required (Kung and Ranjit 2001) even though a higher rate of inoculation does not necessarily result in increased aerobic stability.

4.4.2. Feedlot Experiment

The fibre and fermentation characteristics of these silages were similar to those observed for silages ensiled in the mini silos, however, the DM concentration of the UN silage was greater than in the IN when the silages were ensiled in the Ag-Bag® silos, possibly due to the higher concentration of starch and WSC in the former. Similar to the mini silo experiment, the lower residual WSC concentration in the IN silage (Table 4.4.) may be a reflection of greater utilization of WSC by LAB in the IN silage (Reich and Kung 2010).

In this study, the DM of silages were determined by oven-drying at 105^{o}C for 24 h. Earlier studies by Clancy et al. (1977) have shown that this method of drying silage tends to underestimate DM concentration and lead to underestimation of silage DM and digestible energy intake. Therefore, the equation of Porter et al. (1995) was used to correct for volatilization of VFA, lactic acid and ammonia in the silages and the DMI of the diets adjusted accordingly. Dry matter intake was lower for steers fed the IN than for those fed the UN diet. However, expressed as percent of body weight, the DMI for both groups of steers (UN = 2.0%; IN = 1.8%) were not markedly different from the DMI (1.8%) previously reported for steers fed a diet with similar levels of inoculated barley silage (Zahiroddini et al. 2004).

Silage fermentation characteristics can influence DMI mainly through their effects on palatability (Shaver et al. 1985; Buchanan-Smith 1990). In the current study, the primary differences in the fermentation characteristics of the terminal silages were in pH, lactic acid and VFA concentrations (Table 4.4.); the pH and acetic acid concentration were higher whereas lactic acid concentration was lower in IN than in UN silage. Silage treated with an inoculant containing *L.*

buchneri resulted in an acetic acid concentration as high as 59 g kg^{-1} DM compared to 40 g kg^{-1} DM for the control, failed to depress DMI by dairy cows fed a TMR that included 350 g kg^{-1} DM of inoculated barley silage plus 150 g kg^{-1} DM of regular corn silage (Taylor et al. 2002). Furthermore, in a study involving 24 different silages, Krizsan and Randby (2007) found that even though acetic acid, propionic acid, butyric and lactic acid negatively affected DMI, only lactic, propionic and butyric acids were sufficiently associated with reduced DMI as to merit inclusion in their two best fit three-variable models developed for predicted intake. However, in a two-variable model for predicting silage intake, the depressive effect of acetic acid on DMI for dairy cows was found to be 54% greater than that of lactic acid (Huhtanen et al. 2002). Hydrogen ion concentration rather than any specific fermentation acid(s) has been reported to reduce intake of growing steers (Shaver et al. 1985). Similarly, a weak relationship exists between silage pH and silage DMI (Huhtanen et al. 2002). It is therefore most likely that in the present study, the 7% lower DMI of the IN silage diet compared to UN silage diet may be due to the fact that steers on the former were receiving enough of their nutrient requirements for growth as a result of improved digestibility of the IN silage diet and did not have to increase their intake to the level of those on the latter. This assertion is supported by the fact that the lower DMI by steers on the IN diet did not reduce ADG as compared to those on the UN diet.

Table 4.4. Chemical and microbial composition (mean ± standard deviation) of whole-crop barley silages ensiled in Ag-Bag® silos that were used to formulate TMR and offered to feedlot steers[z]

Item	Uninoculated[y]	Inoculated[y]
pH	4.17±0.13	4.59±0.04
Dry matter (g kg^{-1})	418.6±15.19	353.2±7.80
Chemical composition (g kg^{-1} DM silage)		
Organic matter	934.4±3.00	924.4±0.22
NDF	415.9±30.97	452.7±6.04
ADF	263.9±2.29	304.7±15.69
ADIN (of total N)	100.9±3.66	116.7± 7.70
Starch	235.6±45.75	196.7±10.61
WSC	146.1±27.25	43.0±0.01
Crude protein	122.7±5.25	125.9±4.33
End products of fermentation (g kg^{-1} DM silage)		
Acetic acid	18.4±1.35	48.8±6.82
Propionic acid	2.5±0.36	5.3±3.07
Butyric acid[3]	ND	ND
Lactic acid	69.7±1.82	32.3±1.80
Succinic acid	3.5±0.39	3.8±0.43
Ammonia-N (of total N)	126.1±43.57	101.2±10.54
Lactic: acetic ratio	3.81±0.16	0.67±0.09
Microbial composition (Log_{10} CFU g^{-1} DM)		
Total culturable bacteria	6.72±0.58	8.80±0.68
Lactic acid-producing bacteria	6.64±0.39	9.30±0.40
Moulds[w]	ND	ND
Yeasts[w]	ND	ND

[z]Whole-crop barley silage was either not inoculated or inoculated at ensiling with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^5 CFU g^{-1} of fresh forage.

[y]Three samples were collected and analyzed (n = 3) for each component.

[x]ADIN, Acid detergent insoluble nitrogen.

[w]ND, not detected (silage diluted to 10^{-1}).

Table 4.5. Effect of inoculation of whole-crop barley silage on dry matter intake and growth performance of growing feedlot steers fed for 112 d[z]

Item	Uninoculated	Inoculated	SEM[y]	*P-value*
Initial body weight (kg)	243	242	3.84	0.919
Final body weight (kg)	387	390	4.15	0.648
Total body weight gain (kg)	145	148	4.15	0.633
Dry matter intake[x] (kg d^{-1})	7.6	7.1	0.17	0.019
ADG (kg)	1.29	1.31	0.04	0.650
Gain: feed ratio	0.17	0.19	0.01	0.027

[z]Whole-crop barley silage was either not inoculated or inoculated at ensiling with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chathan, ON, Canada) at a combined rate of 1.3×10^{5} CFU g^{-1} of fresh forage.

[y]Number of steers: $n = 20$.

[x]Corrected for volatilization of VFA, lactic acid and ammonia-N after oven-drying at 105°C using the equation of Porter et al. (1995).

Average daily gain did not differ between the two groups of steers with both being comparable to the rate of gain (1.10 kg d^{-1}) reported by Zahiroddini et al. (2004) for growing feedlot cattle fed a diet based on barley silage for a similar period of time. The efficiency of feed conversion was superior for steers fed IN (0.19) as compared to those fed UN silage (0.17; Table 4.5.). Although there are currently no studies examining the effects of FA esterase-producing inoculants on growth performance of steers, some comparisons can be made with homolactic inoculants. Zahiroddini et al. (2004) reported an efficiency of 0.15 for Angus x Hereford growing steers fed barley silage treated with an inoculant containing Pediococcus and Lactobacillus strains of lactic acid producing bacteria whereas studies by Schaefer et al. (1989) indicated an efficiency of 0.17 for steers fed corn silage treated with an inoculant containing similar species.

Feruloyl esters limit ruminal fibre digestion by inhibiting attachment (Akin et al. 1988) and growth (Varel and Jung 1986) of major fibrolytic bacteria on feruloylated polysaccharides. Inoculation of both ryegrass and corn silages (Nsereko et al. 2008) and one of two corn silages (Kang et al. 2009) with a FAE-producing inoculant that hydrolyzes these linkages during ensiling has therefore previously increased *in situ* NDF digestibility without reducing NDF concentration after ensiling. Feeding corn silage from a mutant corn hybrid, with lower concentration of FA linkages similarly increased fibre digestibility by lambs and milk yield of dairy cows (Jung et al. 2010). The effects of the same inoculant on *in situ* NDF disappearance of barley silages ensiled in Ag-Bag® silos that were offered to the steers in this study were determined and reported separately (Addah et al. 2011). Inoculation increased *in situ* NDF disappearance by 40.5% and 14.5% after 24 and 48 h of incubation, respectively. Even though we did not determine the relationships among ruminal clearance rate, apparent digestibility and DMI, other studies have shown that slower ruminal clearance rate increases ruminal feed residency time resulting in greater ruminal digestibility for low-intake compared to high-intake cattle offered forage-based diets (Shaver et al. 1988; Merchen and Bourquin 1994). This relationship, coupled with the higher dietary ME of the IN silage diet, could have accounted for the

lower DMI and superior feed efficiency of steers offered the IN silage diet compared to those offered the UN silage diet that had a greater DMI.

4.5. CONCLUSIONS

Inoculation of whole-crop barley silage with a mixture of homolactic LAB and *L. buchneri* that produces FAE significantly increased lactic acid concentration and decreased pH during the first 7 d of ensiling. Yet by d 95 after ensiling, pH and acetic acid concentration were higher and lactic concentration was lower for IN than for UN silage. In addition to improving ensiling and aerobic stability of silages, growing steers fed a diet containing the IN silage had superior feed utilization efficiency. Feed intake was lower for steers fed the IN silage diet, but this did not affect live weight gains. This study indicates that inoculation of whole-crop barley silage with a mixture of homolactic LAB, and *L. buchneri* that produces FAE may have the capacity to reduce feed cost in feedlot operations through extension of aerobic stability and improvement in efficiency of feed use for gain.

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CHAPTER 5: EFFECTS OF CHOP-LENGTH AND A THIRD-GENERATION ESTERASE INOCULANT ON FERMENTATION AND AEROBIC STABILITY OF BARLEY SILAGE, AND GROWTH PERFORMANCE OF FINISHING FEEDLOT STEERS [4]

5.1. INTRODUCTION

Forage is included in finishing feedlot diets to provide "effective" fiber and a longer theoretical chop length (TLC) is one approach to increase the effective fiber content of the diet. However a longer TLC may impede compaction and oxygen removal from the silage during packing, delaying the transition to anaerobiosis, slowing the rate of acidification and negatively affecting silage quality. A longer TLC also may increase DM loss during feed-out by enhancing the ingress of air into the silage and its susceptibility to aerobic deterioration (Savoie et al. 1992; Ruppel et al. 1995). Barley silage is the primary dietary forage used by the feedlot industry in Western Canada. It is usually chopped to a TLC of about 0.95 cm prior to being ensiled (Mills and Kung, 2002; Addah et al. 2012a, 2012b). Effects of TLC on the fermentation process should be minimal when TLC is 1.9 cm or less (Hara and Tanigawa, 2010) or when silages are packed to the same density (Marsh, 1978), but effects of TLC on aerobic stability of barley silage have not been studied.

Even though a shorter TLC may increase digestibility of fiber in forage diets (Soita et al. 2002), in high concentrate diets, silage with shorter TLC are less physically effective and may predispose cattle to a higher risk of acute or sub-acute ruminal acidosis. In finishing feedlot diets, grain accounts for the majority of the diet and forages normally comprise less than 10% of the diet DM. Shorter TLC also reduces rumination and the production of saliva that contains the sodium bicarbonate that serves to buffer rumen pH.

In contrast, a longer TLC can contribute to rumen fill and a reduction in dry matter intake (DMI). Reducing the TLC of barley silage from 1.88 cm to 0.47 cm

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increased DMI of steers fed silage-only diets by 18% (Soita et al. 2002). In contrast, DMI of dairy cows was not altered by chop length of corn silage if it only comprised 460 g kg^{-1} of the DM of the diet (Yang and Beauchemin, 2006). Similarly, within a TLC range of 0.6 - 3.8 cm for grass silage, Savoie et al. (1992) also found no consistent effect of TLC on silage quality, DMI or growth performance of dairy cattle when silage constituted 560 g kg^{-1} (DM basis) of the diet. Responses in feed intake and growth performance of beef or dairy cattle to chop length of forage have not been consistent. It is clear that increasing the TLC of silage reduces fuel consumption during chopping, a response that lowers the cost and time of harvest (Tremblay et al. 1990), as well as the emission of greenhouse gases from the combustion of fossil fuels.

Treatment of forages with an exogenous fungal ferulic acid esterase (FAE) hydrolyzes feruloylated polysaccharides and releases sugars (Krueger et al. 2008). The inoculation of whole-crop barley silage chopped at 0.95 cm TLC with a FAE-producing bacterial inoculant resulted in favourable shifts in silage fermentation, improved silage digestibility (Addah et al. 2012b) and the growth efficiency of feedlot cattle fed high silage (~760 g kg^{-1}) diets (Addah et al. 2012a). The application of an FAE-producing inoculant on barley silage chopped to a greater TLC in finishing feedlot diets could prove desirable with coarser TLC reducing the risk of acidosis and inoculation with a mixed FAE inoculant enhancing silage preservation while potentially improving silage digestibility and animal performance.

This study determined whether increasing the TLC of barley silage at harvest from 0.95 cm to 1.9 cm and its inoculation with a FAE-producing inoculant improves silage quality and its utilization by finishing feedlot steers.

5.2. MATERIALS AND METHODS

5.2.1. Silage Preparation and Sampling

Whole-crop barley (*Hordeum vulgare*, L.; Chigwell, Field Crop Development Centre, Lacombe, Canada) from a single field was harvested at the mid-dough stage of kernel maturity (220 - 350 g kg^{-1} DM) on August 23, 2010 and chopped at either 0.95 cm (short; SC) or 1.9 cm (long; LC) TLC on a single day. To minimize treatment differences in forage characteristics due to harvest location and time of harvest,

alternate truckloads were harvested and delivered by two trucks concurrently to two Ag-Bag baggers® (Ag-Bag, Miller-St. Nazianz, Inc. Co., St. Nazianz, WI, USA) and compressed into the Ag-Bag® silos (3.0 m × 45.7 m; Ag-Bag Int. Ltd., Warrenton, OR, USA). Prior to delivery to the baggers, forage from each truck load was sampled. These samples were compiled, thoroughly mixed, and used in a mini silo experiment to produce silage so fermentation characteristics and aerobic stability could be measured.

5.2.2. Mini Silo Experiment

5.2.2.1. Silage Fermentation Characteristics

Subsamples of each type of chopped forage (0.95 cm or 1.95 cm) was divided into six 25-kg lots (3 lots/treatment) and spread out on separate clean plastic sheets. Each lot was then sprayed with either 75 mL (3 mL/kg) of deionized water (uninoculated) or an equal volume of deionized water containing a commercially marketed inoculant (11GFT, Pioneer Hi-Bred Ltd., Chatham, Ontario, Canada) at a rate of 2.8×10^5 CFU of lactic acid-producing bacteria (LAB) per g of fresh forage (inoculated). This resulted in four treatments: short chop forage (0.95 cm; SC) without inoculation, short chop forage (0.95 cm; SC) inoculated with 11GFT inoculant, long chop forage (1.95 cm; LC) without inoculation and long chop forage (1.95 cm; LC) inoculated with 11GFT inoculant.

The inoculant contained a mixed bacterial culture of 1.0×10^{11} CFU/g of *Lactobacillus buchneri* LN4017 (ATCC PTA-6138) that produces FAE, 2.0×10^{10} CFU/g of *Lactobacillus plantarum* LP7109 (ATCC PTA-6139), and 1.0×10^{10} CFU/g of *Lactobacillus casei* LC3200 (ATCC PTA-6135). Viability and label levels of the bacteria in the inoculant were confirmed prior to use.

The four corners of the sheet containing sprayed forage were drawn together and the forage was thoroughly mixed by tumbling it inside the sheet for approximately 3 min and hand-mixing it for an additional min. Approximately 3.0 kg of each of the treated forages were then placed into each triplicate labelled polystyrene mini silo (10.4 cm diameter × 35.6 cm height) and compressed (~240 kg/m^3) using a hydraulic press. Each silo was weighed with its cap prior to filling and again immediately after sealing. The silos were stored at room temperature (~20°C) and opened 1, 2, 3, 7, 14, 42, and 64

d after ensiling. Triplicate silos were prepared and opened for each treatment and sampling day except for d 64 for which six silos per treatment were prepared to have sufficient silage for the aerobic stability study described below. These silos were subsequently compiled into three replicates for chemical analysis. The procedure for filling the silos involved randomly selecting one of three sets of triplicate silos (1, 2, or 3) from each treatment across all sampling days and filling them with a 25-kg lot of forage. Prior to ensiling, triplicate subsamples also were collected from each lot for chemical and microbial analyses (Table 5.1). Silos were weighed before opening to calculate DM loss.

5.2.2.2. Aerobic Stability

Subsamples of silage (~1.2 kg) obtained from each of the pooled silage samples were placed into separate triplicate 4-L insulated containers (13.5 cm diameter × 30.9 cm height) per treatment, covered with two layers of cheesecloth, and stored at ambient temperature (18-20°C) for 8 d. Two Dallas Thermochron iButtons (Embedded Data Systems, Lawrenceburg, KY) were embedded in the silages at ~9.0 cm and ~18.0 cm from the bottom of each container containing the silages. Two additional Thermochron iButtons were placed in the room where the silos were stored for recording ambient temperature. The Thermochron iButtons were programmed to record temperature every 15 min. Data from buttons embedded in the silage and those placed in the room were downloaded after 8 d of aerobic exposure.

5.2.3. Feedlot Experiment

5.2.3.1. Silage Production, Animal Management and Feeding

The experimental protocol for the feedlot experiment was approved by the Animal Care Committee of Lethbridge Research Centre with care and management of steers following the guidelines of the Canadian Council on Animal Care (1993).
Prior to being compressed into Ag-Bag® silos, forages were sprayed with either deionized water (uninoculated) or an inoculant solution at a rate of 1 L per tonne of forage (inoculated) using ATV sprayers (AG Spray Equipment, Hopkinsville, KY, US). The inoculated and uninoculated SC forages were ensiled first into different ends of one

Ag-Bag® silo followed by the LC forage in a second Ag-Bag silo. The inoculant was applied at the same rate (2.8×10^5 CFU of LAB g^{-1} fresh forage) as in the mini silo experiment. One Ag-Bag® silo was used to ensile the uninoculated and inoculated SC; the other was used to ensile the uninoculated and inoculated LC.

Each Ag-Bag® silo was opened at both ends 311 d after ensiling. Silages were used to formulate four total mixed rations (TMR) daily. The TMR contained (DM basis) 850 g kg^{-1} dry-rolled barley grain, 100 g kg^{-1} of one of the experimental silages (uninoculated SC, inoculated SC, uninoculated LC or inoculated LC), and 50 g kg^{-1} beef supplement. Whole and processed barley grains were sampled weekly to determine the extent of processing (processing index). Processing index was measured as the weight of 500 mL of grain after processing expressed as a percentage of the weight before processing (Beauchemin et al. 2001). The processing index of the barley grain was monitored weekly and maintained at 82.2 ± 1.59%.

The ingredient composition of the diets thus differed only in silage type (Table 5.2). Diets were formulated to meet or exceed the nutrient requirements of finishing steers (National Research Council, 1996).

Feed ingredients (silage, dry-rolled barley grain and supplement) and TMR were sampled weekly for DM determination and chemical analysis. Silage was obtained by sampling from five locations from the feeding face of each silo (two each from the lower and upper areas and one from center area) and bulked into a single sample on each sampling day. Samples for chemical analysis were composited across each 28-d period and stored (-40^{o}C) for subsequent chemical analysis. Subsamples of the TMR composited across each 28-d period were also used for measurement of particle size distribution using the Penn State Particle Separator (PSPS, Lammers et al. 1996) with modifications by Kononoff et al. (2003). Physical effective fibre of the TMR was determined using the PSPS as the sum of the proportions of NDF retained on the 19 and 8 mm screens by multiplying NDF content of the TMR by the proportions of DM retained on the respective screens (Yang and Beauchemin, 2006). The DM of ingredients measured weekly was used to adjust the proportion of ingredients in the TMR. The supplement contained (g kg^{-1} DM): ground barley grain (563), canola meal (100), calcium carbonate (250), cane molasses (25), salt (30), urea (20), feedlot vitamin-

mineral premix (10), vitamin E (0.66) and monensin-sodium (Rumensin-80®; 2.24; Rumensin-80®, Elanco Animal Health, Indianapolis, IN). The ingredient and assayed chemical compositions of the TMR are shown in Table 5.2. In addition, silage from each silo was sampled monthly (days 322, 371 and 406 after ensiling) for chemical and microbial analysis.

One hundred and sixty Angus × Hereford crossed-bred steers with an initial mean body weight of 495 ± 4.62 kg (mean ± SD) were assigned randomly to one of the four treatments and allocated randomly to sixteen feedlot pens (17 × 12.7 m) at the Lethbridge Research Centre. This resulted in ten steers per pen with four replicate pens per treatment. Steers were weighed on two consecutive days at the beginning (June 27 and 28, 2011) and end (October 13 and 14, 2011) of the 108-day feeding study with the mean of consecutive weights being used as initial and final weights, respectively. Steers were implanted with component T-ES (Elanco Division of Eli Lilly Canada Inc. ON, Canada). Each implant contained: 120 mg trenbolone acetate, UPS 24 mg estradiol and 29 mg tylosin tartrate.

The TMR was prepared daily and delivered to pens using a Beck 220 TMR Mixer (Beck Implement, Inc., Elgin, MN, USA). Steers were fed once daily at 0900 until August 10, 2011 and thereafter twice daily in two equal portions at 0900 and 1200. The amount of feed offered was recorded daily; orts were collected daily and weighed, and sampled and discarded weekly. Steers were weighed full every 28 d. By the end of the feeding trial (108 d), a buffer zone about 7 m long remained in the center of each Ag-Bag® silo at the interface of each inoculated and uninoculated silage. Feed intake, ADG and feed efficiency (expressed as gain per feed) were estimated for the 108-d feeding period. The DM of the TMR offered and the orts collected daily were used to calculate daily dry DMI for each pen. Pen DMI was divided by the number of steers within a pen to calculate mean DMI for each steer in each pen.

After 108 d of feeding, 8 steers from each pen were transported to a commercial abattoir (Cargill Foods, High River, AB). Steers were slaughtered and hot carcass weight (kidneys removed) measured and recorded. The hot carcass weights were recorded with kidney, heart and pelvic fats removed. Dressing percentage was estimated as warm carcass weight divided by final live weight before shipping adjusted for a 3%

shrinkage multiplied by 100. The carcass was chilled for 48 h at 1^{o} C and the interface between the 12th and 13th ribs was assessed by a single trained grader for quality grade (Prime = slightly abundant; choice = small; select = slightly) and marbling score (0-1000) within each grade according to the grading standards of the USA Department of Agriculture (USDA, 1997). The *longissmus* muscle (rib eye) area, the cross-sectional area between the 12th and 13th ribs, and fat covering were also measured and recorded. Liver conditions were scored at slaughter according to the Elanco scoring system as described by Brink et al. (1990). Briefly, a score of 0 was given for each liver with no abscess, 1 for a liver with one or two small abscesses with a diameter < 2.5 cm, 2 for a liver with one or several large abscesses, and 3 for a liver with open abscesses or abscesses with a diameter (s) > 2.5 cm. Saleable meat, the proportion of carcass weight that could be sold commercially, was calculated as the weight of bone-in cuts trimmed to a desired fat level and bone content and expressed as a percentage of the warm carcass weight.

5.2.4. Chemical Analyses

For chemical analyses of silage samples, 15 g of fresh forage or silage was mixed with 135 mL of distilled water. This mixture was blended in a Waring blender (Waring Commercial, Torrington, CT, USA**)** for 30 sec at the highest setting, and filtered through two layers of cheesecloth. The pH of the filtrate was measured immediately with a Symphony pH meter (VWR, Mississauga, ON, Canada). The filtrate was then divided into two portions. One portion was immediately boiled for 10 min to halt fermentation and stored at –20^{o}C for subsequent analysis of WSC (glucose equivalent) by the Nelson-Somogyi (1944) method on a Appliskan® 1.437 (SkanIt Software 2.3 RE) micro-plate reader (Thermo Scientific, Hudson, NH, USA) at 620 nm.

The second portion of the filtrate was stored on ice until it was centrifuged for 15 min at 10,000 × *g* (4^{o}C); the supernatant fluid was collected for analysis of volatile fatty acids (VFA), lactic acid, and NH_3-N. For determination of VFA, 1.5 mL of the supernatant fluid was deproteinized with 0.3 mL of 25% (wt/vol) metaphosphoric acid, combined with 0.2 mL of 0.1 M crotonic acid as internal standard, and analyzed with a Hewlett Packard model 5890A Series Plus II gas-liquid chromatograph (Hewlett

Packard Co., Palo Alto, CA, USA) with a 30-m Zebron free fatty acid phase fused silica capillary, 0.32-mm i.d., and 1.0-μm film thickness column (Phenomenex, Torrance, CA, USA). For lactic acid determination, 400 μL of the deproteinized sample was combined with 50 μL of 3 mM malonic acid (5 mg mL^{-1}) as an internal standard. Lactic acid was methylated and then quantified using the method of Kudo et al. (1987) on the same column and chromatograph used for VFA analysis. To determine NH_3-N, 1.6 mL of the supernatant was combined with 0.15 mL of 65% (wt/vol) trichloroacetic acid and analyzed by the phenol-hypochlorite method described by Broderick and Kang (1980).

For starch determination, forage or silage samples were first freeze-dried and ball-ground using a mixer mill (MM 400, Retsch Inc. Newtown, PA, USA). Starch was determined by hydrolyzing to α-glucose polymers using amyloglucosidase (Megazyme Int. Ltd., Wicklow, Ireland) plus 1,4 α-D-glucan glucano-hydrolase (Brennfag Canada Inc., Toronto, ON Canada) as described by Herrera-Saldana et al. (1990). Samples were read on a Thermo Scientific Appliskan® 1.437 (SkanIt Software 2.3 RE) micro-plate reader (Thermo Scientific, Hudson, NH, USA) at a wavelength of 490 nm.

Table 5.1. Chemical and microbial composition (mean ± standard deviation) of whole-crop barley forage chopped at two theoretical lengths of chop prior to ensiling in mini silos

Item	Short Chop (TLC = 0.95 cm; *n* = 3)	Long chop (TLC = 1.95 cm; *n* = 3)
pH	6.70 ± 0.06	6.68 ± 0.07
Dry matter (g kg^{-1})	302.7 ± 0.0	282.1 ± 3.2
Chemical composition (g kg^{-1} DM basis)		
Neutral detergent fiber[z]	519.3 ± 2.73	496.8 ± 7.18
Acid detergent fiber	298.7 ± 2.3	280.4 ± 8.2
Acid detergent insoluble N	8.4 ± 1.5	8.5 ± 1.0
Water-soluble carbohydrates[y]	29.1 ± 2.9	32.9 ± 2.3
Starch	178.9 ± 1.69	153.1 ± 0.1
Crude protein	135.6 ± 2.1	137.8 ± 0.4
Microbial counts (Log_{10} CFU g^{-1} DM)		
Lactic acid bacteria	4.5 ± 0.2	4.7 ± 0.4
Yeasts	6.9 ± 0.2	6.9 ± 0.1
Moulds	6.5 ± 0.1	6.3 ± 0.1

TLC, theoretical chop length.

[z]Neutral detergent fiber assayed with heat-stable amylase and expressed inclusive of residual ash.

[y]Glucose equivalent.

For determination of crude protein (CP), lyophilized ball-ground samples (5 mg) were subjected to combustion analysis for total N (Dumas Nitrogen) using an NA1500 Nitrogen/Carbon analyzer (Carlo Erba Instruments, Milan, Italy). Crude protein was calculated as N × 6.25.

Dry matter of the fresh forage, silage samples and TMR was determined by drying at 60 ^{o}C for 48 h in a forced air oven. Organic matter was determined by ashing samples (1g) in a muffle furnace at 550 ^{o}C for 5 h. Subsamples of the fresh forage, silage and TMR were stored at –20 ^{o}C until being lyophilized, and ground through a 1-mm screen prior to analysis of NDF and ADF using an Ankom^{200} system (Ankom Technology Corporation, Fairport, NY, USA). Neutral detergent fiber was analyzed with the addition of sodium sulfite and α-amylase and expressed inclusive of residual ash whereas ADF was analyzed without α-amylase and expressed inclusive of residual ash. Nitrogen in ADF residues (ADIN) was measured as described above for N analysis.

5.2.5. Microbial Analyses

For microbiological analyses, fresh whole-crop barley forage or silage samples (10 g) were added to 90 mL of sterile 70 mM potassium phosphate buffer (pH = 7.0); this mixture was agitated for 60 s at 260 rpm in a Stomacher 400 Laboratory Blender (Seward Medical Limited, London, UK). The suspension was serially diluted (10^{-2} to 10^{-7}) and 100-µL aliquots of each dilution were plated in triplicate onto semi-selective lactobacilli media (de Man-Rogosa-Sharpe, MRS; Oxoid, Basingstoke, Hampshire, UK) for enumeration of LAB (Hill and Hill, 1986); onto nutrient agar (NA; Difco, Detroit, MI, USA) for the enumeration of total culturable bacteria, and onto Sabouraud's dextrose agar (SDA; Difco, Detroit, MI, USA) for the enumeration of yeasts and moulds. Lactobacilli MRS agar and NA were amended with 200 µg mL^{-1} of cycloheximide (Sigma-Aldrich, St. Louis, MO, USA) and SDA with 100 µg mL^{-1} each of tetracycline and chloramphenicol. Lactobacilli MRS agar plates were incubated at 37°C for 24 to 48 h and SDA plates were incubated at ambient temperature (~ 22^{o} C) for 72 h. Colonies were counted from plates containing a minimum of 30 and a maximum of 300 colonies.

5.2.6. Experimental Design and Statistical Analyses

Silage fermentation and aerobic stability data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) as a completely randomized design. Data on microbial populations were transformed to $\log_{10}$ CFU g^{-1} of DM of forage or silage prior to statistical analysis. Silage fermentation characteristics and aerobic stability data were analysed with each mini silo (n = 3) as the experimental unit for the ensiling data and each 4-L insulated container (n = 3) as the experimental unit for the aerobic stability data. Fermentation characteristics were analyzed for the fixed effects of chop length, inoculation and chop length × inoculation. Least-square means of interactions that showed significant differences were separated by a pair-wise Fisher's LSD test. Least square means of daily temperatures of the silages and the corresponding ambient daily temperature were computed. Difference between ambient temperature and silage temperatures on each day of aerobic exposure were separated using the Dunnett's adjustment option of LSMEANS procedure of SAS (SAS Inst. Inc., Cary, NC).

Data on DMI and growth performance of steers (weight gain, ADG and feed efficiency; n = 4) and carcass traits (hot carcass weight, fat thickness, rib eye area, sellable meat and marbling level; n = 4) were analyzed using the PROC MIXED procedure of SAS for the fixed effects of chop length, inoculation and their interaction as a completely randomized design with pen as the experimental unit and initial pen weight as a covariate.

Data for carcass quality grades, and marbling and liver scores were analyzed using the PROC GLIMMIX procedure of SAS for the fixed effects of chop length, inoculation, and their interactions. There were no major differences in carcass grades among treatments; hence data on Prime and Choice grades within each treatment were pooled for statistical analysis.

Differences among least-square means for all fixed effects were declared statistically significant at $P \leq 0.05$ whereas trends were discussed at $P \leq 0.1$.

5.3. RESULTS

5.3.1. Mini Silo Experiment

5.3.1.1. Silage Fermentation Characteristics

The chemical and microbial characteristics of the processed untreated forages prior to ensiling are typical of whole-crop barley silage harvested at similar range of DM (Hargreaves et al. 2009; Table 5.1). After 64 d of ensiling, crude protein content was higher (P = 0.008) for the LC than SC silage (Table 5.3). However, irrespective of chop length, inoculation decreased (P = 0.029) the CP concentration by 8.3 % (131.6 to 120.7 g kg^{-1} DM). Inoculation had no effect (P = 0.277) on acid detergent insoluble N concentration in the SC silage but it increased (P = 0.013) it in the LC silage.

Though it increased (P = 0.047) starch content of the silage, use of the inoculant decreased (P = 0.022) WSC concentration. There was a trend (P = 0.119) towards higher WSC concentration in LC than SC silage. Lactic acid also was higher (P = 0.011) in both LC silages compared to the inoculated SC silage, but its concentrations in both LC silages did not differ from that of uninoculated SC silage. In general, the concentration of lactic acid was consistently higher in the LC silage than in the SC especially between d 2 and d 42 after ensiling (Figure 5.1). There were significant interactions between chop length and inoculation on silage pH at day 7 (P = 0.04) and 64 (P = 0.004) of ensiling (Figure 5.2). Inoculation decreased (P=0.004) the pH in the SC (3.87 *versus* 3.97) but not in LC (4.02 *versus* 4.02) silage at 64 d of ensiling (Table 5.3). There was a significant interaction (P = 0.034) between chop length and inoculation on acetic acid concentration; inoculation decreased acetic acid of SC silage but increased it for LC silage. Consequently, the lactic: acetic acid ratio was higher (P = 0.020) for uninoculated LC silage compared to the other three silages. A chop length by inoculation interaction was also observed for concentration of total VFA as it was increased by inoculation of SC silage but decreased by inoculation of LC silage (P = 0.032). Ammonia concentration was lower (P = 0.003) for inoculated than uninoculated silage, but it was not affected by chop length. With both chop lengths, inoculation increased (P = 0.002) the population of LAB and decreased

(P = 0.030) the population of yeasts. Moulds were not detected (silage diluted to 10^{-1}) after 64 d of ensiling. Neither chop length (P = 0.176), inoculation (P = 0.573) nor their interaction (P = 0.875) affected DM loss.

The concentration of acetic acid produced during ensiling of both SC and LC forage in Ag-Bag® silos (Table 5.4) were generally consistent with those of LC forage ensiled in mini silos (Table 5.3), but acetic acid concentration was greater in uninoculated than inoculated SC silage stored in mini silos (17.3 *versus* 14.4 g kg^{-1} DM; Table 5.3) whereas in the in the Ag-Bag® silages, inoculation acetic acid concentration in both SC and LC.

Table 5.2. Ingredient and chemical compositions, and particle size distribution (mean ± standard deviation) of total mixed rations formulated with uninoculated or inoculated whole-crop barley silage chopped at two lengths and ensiled in Ag-Bag® silos[z]

Item	Short chop (TLC = 0.95 cm; *n* = 4)		Long chop (TLC = 1.95 cm; *n* = 4)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Ingredients (g kg^{-1} DM basis)				
Silage	100	100	100	100
Dry-rolled barley grain	850	850	850	851
Supplement[y]	50	50	50	50
DM content of ingredients (g kg^{-1})				
Silage	290 ± 39.9	324 ± 30.9	306 ± 58.1	328 ± 57.6
Dry-rolled barley grain	868 ± 10.9	868 ± 10.9	868 ± 10.9	868 ± 10.9
Supplement[y]	921 ± 5.1	921 ± 5.1	921 ± 5.1	921 ± 5.1
Chemical composition (g kg^{-1} DM basis)				
Dry matter	723.3 ± 25.4	739.3 ± 20.5	729.2 ± 292	728.3 ± 40.5
Neutral detergent fiber[x]	259.3 ± 19.7	254.8 ± 10.0	260.7 ± 7.3	254.4 ± 13.7
Acid detergent fiber	91.7 ± 17.5	86.5 ± 53.0	90.2 ± 9.9	89.7 ± 10.9
Crude protein	135.0 ± 7.9	134.7 ± 4.8	132.1 ± 8.9	133.3 ± 7.3
Starch	475.3 ± 16.7	515.0 ± 10.6	509.9 ± 15.3	504.6 ± 4.7
Penn State particle size distribution (DM retained, g kg^{-1})				
Top screen (19 mm)	4.8 ± 0.96	3.2 ± 0.3	11.9 ± 5.6	7.3 ± 1.1
Second screen (8 mm)	81.9 ± 4.9	52.3 ± 0.3	83.4 ± 6.2	85.1 ± 6.2
Third screen (1.18 mm)	884.2 ± 22.0	914.2 ± 15.9	858.7 ± 20.1	863.6 ± 13.7
Bottom pan	30.4 ± 8.9	38.2 ± 7.8	33.8 ± 2.9	35.0 ± 8.2
Physically effective fiber[w]	87.0 ± 6.4	55.2 ± 1.2	113.1 ± 22.3	101.4 ± 15.4

[z]Whole–crop barley silage was treated at ensiling without (uninoculated) or with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chatham, ON, Canada) at a combined rate of 2.8×10^{5} CFU g^{-1} of fresh forage (inoculated).

TLC, theoretical chop length.

[y]The supplement contained (g kg^{-1} DM): 562.5 chopped barley grain, 99.9 canola meal, 25 calcium carbonate, 25 molasses, 30 salt, 20 urea, 10 feedlot premix, 0.66 vitamin E and 2.2 monensin-sodium (Rumensin-80®, Elanco Animal Health, Indianapolis, IN).

[x]Neutral detergent fiber assayed with heat-stable amylase and expressed inclusive of residual ash.

[w]The sum of the proportions of NDF retained on the 19 and 8 mm screens determined by multiplying NDF content of the TMR by the proportions of DM retained on the respective screens (Yang and Beauchemin, 2006).

Table 5.3. Effects of inoculation and chop length of whole-crop barley forage on fermentation characteristics of silages after 64 d of ensiling in mini silos[z]

	Short chop (TLC = 0.95 cm; *n* = 3)		Long chop (TLC = 1.95 cm; *n* = 3)		SEM	*P*-value		
	Uninoculated	Inoculated	Uninoculated	Inoculated		Chop	Inoculation	Chop × Inoculation
pH	3.97	3.87	4.02	4.02	0.013	0.001	0.004	0.004
Dry matter (g kg^{-1})	315.1	337.8	296.4	311.2	10.93	0.072	0.124	0.728
Composition (g kg^{-1} DM basis)								
Neutral detergent fiber[y]	450.2	448.8	455.3	449.0	7.36	0.727	0.620	0.749
Acid detergent fiber	264.5	259.3	274.8	276.4	6.32	0.060	0.779	0.609
Acid detergent insoluble N	5.8	5.3	4.4	5.7	0.29	0.149	0.168	0.013
Crude protein	127.7	110.4	135.6	131.0	4.14	0.008	0.029	0.165
Starch	168.9	207.2	135.7	158.7	13.13	0.014	0.047	0.575
Water-soluble carbohydrates[x]	16.7	13.8	22.5	14.6	1.20	0.119	0.022	0.222
End-products of fermentation (g kg^{-1} DM basis)								
Lactic acid	70.6	63.9	84.0	78.9	4.42	0.011	0.255	0.774
Acetic acid	17.3	14.4	14.6	17.7	1.17	0.828	0.952	0.034
Propionic acid	3.6	3.4	4.2	4.1	0.48	0.226	0.703	0.872
Butyric acid	ND	ND	ND	ND	–	–	–	–
Total volatile fatty acids	21.1	17.9	18.9	21.9	1.18	0.464	0.939	0.032
Succinic acid	7.7	6.1	6.7	6.7	0.61	0.758	0.265	0.257
NH_3–N (g kg^{-1} of total N)	48.3	34.1	51.0	40.0	2.96	0.198	0.003	0.642
Lactic: acetic	4.1	4.4	5.8	4.6	0.27	0.011	0.152	0.020
Dry matter loss	78.2	72.7	60.1	50.6	12.2	0.177	0.573	0.875
Microbial counts (Log_{10} CFU g^{-1} DM basis)								
Lactic acid bacteria	8.3	9.1	8.3	9.3	0.04	0.084	0.001	0.020
Yeasts	4.0	3.8	5.6	3.4	0.39	0.187	0.018	0.030

[z]Whole–crop barley silage was treated at ensiling without (uninoculated) or with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chatham, ON, Canada) at a combined rate of 2.8×10^{5} CFU g^{-1} of fresh forage (inoculated).
TLC, theoretical chop length.
[y]Neutral detergent fiber assayed with heat-stable amylase and expressed inclusive of residual ash.
[x]Glucose equivalent.

Table 5.4. Fermentation characteristics (± standard deviation) of uninoculated and inoculated whole-crop barley silages fed to finishing feedlot steers after 294 d of ensiling in Ag–Bag® silos[z]

Item	Short chop (TLC = 0.95 cm; *n* = 4)		Long chop (TLC = 1.95 cm; *n* = 4)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
pH	4.33 ± 0.3	4.34 ± 0.2	4.36 ± 0.2	4.33 ±0.2
Dry matter (g kg^{-1})	283.0 ± 41.8	307.2 ± 40.8	323.1 ± 88.3	312.4 ± 58.0
Composition (g kg^{-1}DM basis)				
Neutral detergent fiber[y]	527.3 ± 24.9	473.1 ± 16.6	501.1 ± 39.5	469.1 ± 1.97
Acid detergent fiber	326.6 ± 22.9	284.4 ± 15.1	312.1 ± 31.6	282.9 ±15.4
Acid detergent insoluble N	53.8 ± 11.7	43.3 ± 25.0	44.8 ± 8.8	42.4 ± 27.6
Crude protein	152.9 ±3.5	146.2 ± 4.3	151.9 ± 12.1	140.6 ± 4.7
Starch	128.7 ± 14.1	176.3 ± 12.4	117.7 ± 19.6	216.7 ± 6.9
Water-soluble carbohydrates[x]	16.8 ± 1.8	9.1 ± 0.7	19.4 ± 3.5	6.74 ± 0.1
End products of fermentation (g kg^{-1}DM)				
Lactic acid	79.8 ± 6.0	54.4 ± 1.48	82.9 ± 28.6	55.9 ± 9.8
Acetic acid	24.3 ± 6.6	45.7 ± 4.6	15.9 ± 3.9	54.3 ± 10.8
Propionic acid[w]	ND	ND	ND	ND
Butyric acid[w]	ND	ND	ND	ND
Succinic acid	7.1 ± 2.7	5.7 ± 2.0	4.5 ± 2.4	3.9 ± 1.4
Lactic: acetic	3.5 ± 1.2	1.2 ± 0.5	5.6 ± 2.6	1.1 ± 0.4
Microbial counts (Log_{10} CFU g^{-1} DM)				
Lactic acid bacteria	9.0 ± 0.1	7.8 ± 0.3	8.5 ± 0.9	8.4 ± 0.3
Yeasts	7.6 ± 0.7	5.2 ± 0.7	6.6 ± 1.6	6.1 ± 1.6
Moulds[a]	ND	ND	ND	ND

[z]Whole–crop barley silage was treated at ensiling without (uninoculated) or with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chatham, ON, Canada) at a combined rate of 2.8×10^5 CFU g^{-1} of fresh forage (inoculated).

TLC, theoretical chop length.

ND, not detected (silage diluted to 10^{-1}).

[y]Neutral detergent fiber assayed with heat-stable amylase and expressed inclusive of residual ash.

[x]Glucose equivalent.

[w]ND, not detected (silage diluted to 10^{-1}).

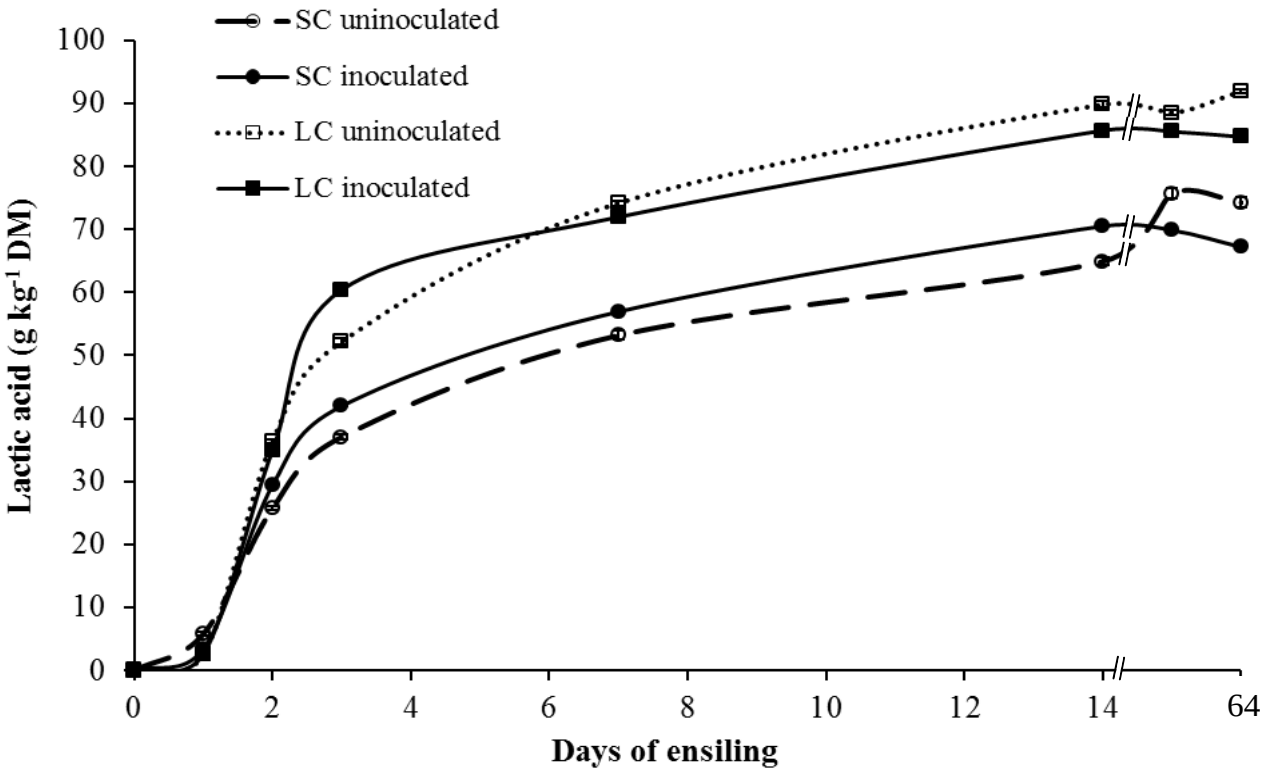

Figure 5.1. Effects of inoculation and chop length (short = 0.95 cm theoretical chop length; long = 1.95 cm theoretical chop length) of whole-crop barley silage on lactic acid concentration after 64 d of ensiling in mini silos. Treatments: SC = Short chop silage; LC = Long chop silage. Uninoculated whole–crop barley silage was treated at ensiling with deionized water whereas inoculated whole–crop barley silage was treated at ensiling with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU/g of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chatham, ON, Canada) at a combined rate of 2.8×10^{5} CFU g^{-1} of fresh forage.

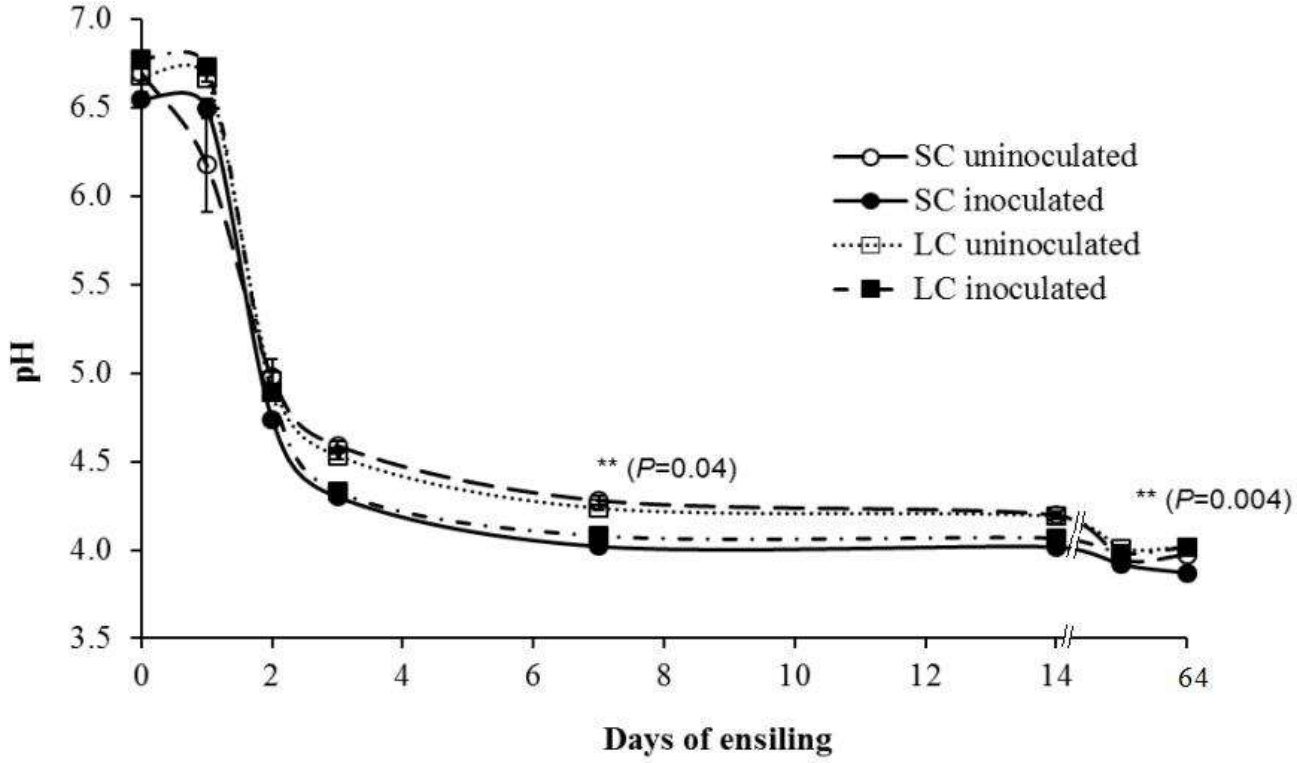

Figure 5. 2. Effects of inoculation and chop length (short = 0.95 cm theoretical chop length; long = 1.95 cm theoretical chop length) of whole-crop barley silage at ensiling on pH after 64 d of ensiling in mini silos. Treatments: SC = Short chop silage; LC = Long chop silage. Uninoculated whole–crop barley silage was treated at ensiling with deionized water whereas inoculated whole–crop barley silage was treated at ensiling with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chatham, ON, Canada) at a combined rate of 2.8×10^{5} CFU g^{-1} of fresh forage. Asterisks with corresponding *P* values indicate days of ensiling on which the interaction of chop length and inoculation was significant

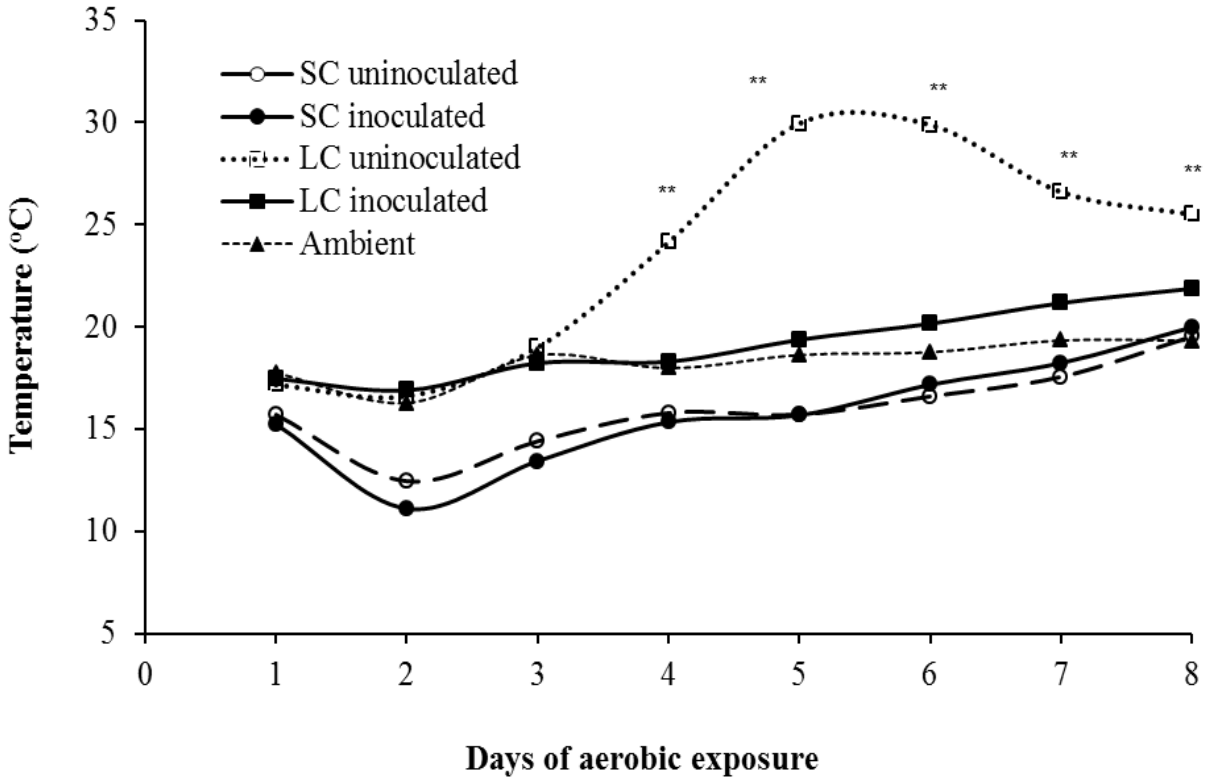

Figure 5.3. Effects of inoculation and chop length (short = 0.95 cm theoretical chop length; long = 1.95 cm theoretical chop length) on temperature of whole-crop barley silage exposed to air for 8 d after 64 d of ensiling in mini silos. Treatments: SC = Short chop silage; LC = Long chop silage. Uninoculated whole–crop barley silage was treated at ensiling with deionized water whereas inoculated whole–crop barley silage was treated at ensiling with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU/g of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chatham, ON, Canada) at a combined rate of 2.8×10^{5} CFU g^{-1} of fresh forage. Asterisks indicate days of ensiling on which the interaction of chop length and inoculation was significant ($P < 0.001$).

5.3.1.2. Aerobic Stability

The effect of chop length, inoculation or their interaction on the aerobic stability of barley silage is illustrated in Figure 5.3. Both the inoculated and uninoculated SC silages remained stable during the period of aerobic exposure, but the uninoculated LC silage became unstable (more than 2^{o}C above ambient temperature; $P < 0.001$) after 4 of aerobic exposure.

5.3.2. Feed Intake, Growth Performance and Carcass Characteristics

Neither chop length, inoculation nor their interaction had a significant ($P > 0.05$) effect on DMI or animal performance (Table 5.5). However, rib eye area of carcasses from steers fed the SC averaged 4.3 cm^2 greater ($P = 0.035$) than that of steers fed the LC silage. This resulted in greater ($P = 0.017$) saleable meat yield for the steers fed SC silage. Neither the main effects ($P = 0.531$) nor their interaction ($P = 0.403$) altered marbling score (Table 5.5).

Table 5.5. Effect of inoculation and chop length of whole-crop barley silage ensiled in Ag–Bag® silos on feed intake, growth performance and carcass characteristics of finishing feedlot steers fed a total mixed finishing diet for 108 d[z].

Item	Short chop (TLC = 0.95 cm; *n* = 4)		Long chop (TLC = 1.95 cm; *n* = 4)		SEM	*P*-value		
	Uninoculated	Inoculated	Uninoculated	Inoculated		Chop	Inoculation	Chop × Inoculation
DMI and growth performance								
Initial weight (kg)	495.9	491.8	486.7	492.1	0.59	0.004	0.157	0.001
Final weight (kg)	711.6	703.0	702.7	706.1	0.42	0.616	0.378	0.748
Weight gain (kg)	215.6	211.2	216.0	214.0	0.79	0.616	0.378	0.748
Dry matter intake (kg)	11.2	11.1	11.5	11.2	0.11	0.257	0.395	0.782
Average daily gain (kg)	2.00	1.96	2.00	1.98	0.007	0.599	0.382	0.763
Gain: feed ratio	0.182	0.179	0.176	0.198	0.002	0.600	0.937	0.636
Carcass yield and characteristics								
Warm carcass weight (kg)	422.8	416.8	415.4	416.7	5.23	0.352	0.519	0.332
Dressing percentage[y]	61.0	61.2	60.8	60.9	0.33	0.427	0.700	0.934
Rib eye area (cm^2)	94.0	92.8	88.7	89.5	1.75	0.035	0.974	0.599
Sellable meat (%)[x]	54.5	54.8	53.1	52.7	0.70	0.017	0.937	0.587
Grade fat (mm)	14.3	13.5	14.7	15.6	0.75	0.138	0.935	0.265
Liver score (%)[w]								
0	16.2	14.1	17.6	16.2	0.352	0.587	0.728	0.976
1	1.4	2.8	0.7	1.4	0.352	0.587	0.728	0.976
2	0.0	2.1	1.4	0.7	0.352	0.587	0.728	0.976
3	7.0	5.6	6.3	6.3	0.352	0.587	0.728	0.976
Quality grade (%)[v]								
Prime and choice	19.0	16.2	19.7	19.0	3.73	0.531	0.531	0.403
Select	5.6	8.5	6.3	5.6	3.73	0.531	0.531	0.403
Marbling level[u]	468.6	444.3	480.8	470.0	16.91	0.260	0.298	0.689

[z]Whole–crop barley silage was treated at ensiling without (uninoculated) or with a ferulic acid esterase-producing inoculant containing 1.0×10^{11} CFU g^{-1} of *Lactobacillus buchneri* LN4017, 2.0×10^{10} CFU g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0×10^{10} CFU g^{-1} of *Lactobacillus casei* LC3200 (Pioneer Hi-Bred Ltd., Chatham, ON, Canada) at a combined rate of 2.8×10^5 CFU g^{-1} of fresh forage (inoculated). TLC = theoretical chop length.

[y]The hot carcass weights were recorded with kidney, heart and pelvic fats present. Dressing percentage was estimated as warm carcass weight divided final live weight before shipping adjusted for a 3% shrinkage multiplied by 100.

[x]Expressed as a percent of warm carcass weight.

[w]Liver abscesses scores: 0 = no abscesses evident; 1 = one or two small abscesses or scars; 2 = two to four well-formed abscesses less than 2.5 cm in diameter; or 3 = one or more large active abscesses greater than one 2.5 cm in diameter (Brink, et al. 1990).

[v]Prime = slightly abundant; Choice = small; Select = slight marbling (USDA, 1997).

[u]Marbling level within each score = 0 – 1000 (USDA, 1997).

5.4. DISCUSSION

5.4.1. Silage Fermentation Characteristics and Aerobic Stability

The addition of the inoculant to either SC or LC forages at ensiling ensured that the exogenous LAB dominated the fermentation process as reflected by the greater population of LAB and lower population of yeast in inoculated silages throughout the ensiling period (data not shown) and on the final day of ensiling (Table 5.3). Even though the concentrations of NDF in silages ensiled in the mini silos were not significantly lower in the inoculated compared to the uninoculated silages, the reduction in NDF of inoculated barley silage ensiled in farm-scale Ag-Bag® silos was substantial. The NDF concentrations of Ag-Bag® silages with and without inoculation for the SC were 473 versus 527 and for LC were 469 versus 501 g kg^{-1} DM, respectively (Table 5.4). Treatment of corn silage solely with a fibrolytic enzyme containing cellulase and hemicellulase activity decreased the NDF content of silages ensiled in mini silos but not for silage in bag silos (Sheperd and Kung, 1996). Stokes and Chen (1994) observed lower NDF concentration in corn silages treated with a fibrolytic enzyme-homolactic inoculant mixture and ensiled in mini silos. Other studies with FAE-producing inoculants similar to the one used in the current study reported that NDF content for inoculated silage was either similar to (Kang et al. 2009) or higher (Nsereko et al. 2008; Addah et al. 2012a) than uninoculated silage. Factors such as crop maturity (structure of feruloylated fiber), silo type, duration of ensiling, exposure to oxygen, and loss of non-NDF DM during fermentation or drying may account for the discrepancies among these studies.

Inoculation reduced the level of residual WSC in silage, an outcome that presumably reflects greater utilization of WSC by the inoculated LAB, but starch was greater for inoculated silages. The higher concentration of total VFAs, absence of butyric acid and lower pH in all silages after 64 d of ensiling indicate that barley forage was well fermented in all treatments (Table 5.3). The lower CP and WSC concentrations for SC silages may reflect seepage loss during ensiling as effluent loss is enhanced in short-chop silages (Muck et al. 2003). This hypothesis is further supported by the fact that average DM losses was 75.5 g kg^{-}

[1]for SC versus 55.4 g kg^{-1} in LC. Lower concentrations of soluble protein and sugar in SC as compared to LC silage could also be explained by greater effluent loss. However, starch concentration was greater in SC than LC silage and greater for inoculated than uninoculated SC and LC silages.

Although inoculation did not affect the concentration of lactic acid, chopping the silage to 1.95 cm (LC) resulted in increased lactic acid as compared to that chopped to 0.95 cm (81.9 *versus* 67.3 g kg^{-1} DM; Table 5.3). These data are consistent with those from the Ag-Bag® silos (LC = 69.4 g kg^{-1} DM *versus* SC = 67.1 g kg^{-1} DM). Inoculation did not affect the lactic:acetic ratio in the SC silage, but the ratio was higher in uninoculated than inoculated LC. The inoculated LC silage also had a higher lactic:acetic acid ratio than both SC silages. These results were unexpected because chopping corn silage to a TLC of 0.95 cm presumably should enhance packing density and increase carbohydrate availability to LAB and lactic acid concentration as compared to chopping it at 1.90 cm or 1.45 cm (Bal et al. 2000). Reducing the TLC from 1.9 cm to 0.6 cm also increased the concentration of lactic acid in alfalfa and oat silages stored in plastic-covered piles (Bhandari, et al. 2008). In the present study, all silages were packed to a similar density (240 g m^{-3}) in mini silos. Compaction of forage in mini silos might obliterate the effects of chop length on fermentation characteristics except for forage of high DM (> 400 g kg^{-1} DM) for which fine chopping will improve consolidation of the forage (Marsh, 1978). In a review of postharvest factors affecting ensiling, Muck et al. (2003) found that the effects of chop length on the final silage characteristics depended on the silo type and the level of compaction. Under some conditions, longer TLC resulted in better quality silage, while in others it did not. They concluded that packing with greater pressure may exclude oxygen and provide bacteria with the sugars and other substrates needed for efficient fermentation across a wide range of TLC (0.6-10 cm).

The fermentation characteristics of inoculated LC silages ensiled in both mini and Ag-Bag® silos were consistent with the fermentation pattern of silages expected for *L. buchneri* inoculants. *Lactobacillus buchneri* converts some lactic acid into equimolar amounts of acetic acid and 1,2-propanediol (Oude Elferink et

al. 2001). However in SC silages, inoculation increased acetic acid concentration only for silages ensiled in Ag-Bag® silos but, not in mini-silos. The reason for the lower acetic acid in the inoculated SC silage ensiled in mini silos is unknown. In some studies, acetic acid concentration has not been altered by treatment with *L. buchneri* even though yeasts counts were lower and aerobic stability was improved by inoculation (Mari et al. 2009; Arriola et al. 2011; Tabacco et al. 2011a). In those studies the equally high concentration of acetic acid in the control silages was attributed to greater populations of epiphytic heterolactic LAB. Kang et al. (2009) treated two corn hybrids with an esterase-producing inoculant similar to the one used in the present study and found no effect of inoculation on acetic acid concentration in either hybrid. They attributed this to the presence of homolactic *L. casei* in the inoculant. Silage inoculants are expected to offer the greatest improvement in silage quality when ensiling conditions are suboptimal. Examples of this would include; forage that is extremely wet or dry, low WSC concentrations, long chop length, insufficient packing density or silos that fail to exclude oxygen. However, when conditions are optimum and oxygen exposure is prevented, as is the case with most mini silo experiments, the impact of silage inoculants would be less obvious than studies using commercial-scale silos (McAllister et al. 1998; Cherney et al. 2004).

With homolactic fermentation, WSC are converted only to lactic acid, dry matter loss is miminized and conservation of energy from fermented glucose in products is near 970 g kg^{-1} even though some of the lactate can be lost during drying. In contrast, with heterolactic fermentation WSC may be converted to acetate, ethanol, mannitol, and carbon dioxide as well as lactic acid. With formation and loss of carbon dioxide, up to 240 g kg^{-1} of dry weight of sugars fermented to acetate can be lost. Despite this dry weight loss, energy loss remains minimal (20 to 60 g kg^{-1}). *Lactobacillus buchneri* converts lactic acid to near equimolar amounts of acetic acid and 1,2 propanediol (propylene glycol) resulting in over a 240 g kg^{-1} loss in weight of the lactate catabolised. Yet, under these conditions energy loss is less than 20 g kg^{-1}, so compared to homolactic fermentation, heterolactic fermentation results in greater loss of DM during

fermentation even though energy loss by biochemical pathways used by silage bacteria (Woolford, 1984) never exceeds 46 g kg^{-1}. Yet, final silage pH remains greater with heterolactic fermentation because the pKa is higher for acetic than lactic acid. In the present study, neither chop length, inoculation nor their interaction altered extent of DM loss during the fermentation process. Dry matter losses in stack silos generally are lower in silage chopped to medium (1.3 and 2.5 cm) than lower (0.63 cm) or higher (3.8 cm) TLC (Savoie et al. 1992). Co-culturing *L. buchneri* with homolactic strains of LAB in a single inoculant reduced DM loss compared with a single strain or mixed strains of *L. buchneri* alone (Driehuis et al. 2001). This benefit has been attributed to the homolactic strain (*L. plantarum*) co-cultured with *L. buchneri* in the inoculant. Most of losses of DM in silages inoculated solely with *L. buchneri* likely occur at the initial stages of ensiling when *L. buchneri* is less active and conditions such as higher pH and remnants of trapped oxygen, are suitable for enterobacteria resulting in silage DM loss.

Inoculation with *L. buchneri* alone has increased the ammonia-N concentration of corn (Driehuis et al. 1999) and grass silages (Driehuis et al. 2001). In studies where *L. buchneri* has been used alone, the higher ammonia-N concentrations have been attributed to higher silage pH allowing the activity of proteolytic bacteria to continue. However, in studies where *L. buchneri* was co-inoculated with *L. plantarum,* a homolactic LAB (Reich and Kung, 2010; Addah et al. 2012a), neither ammonia-N concentrations nor pH were increased significantly. The lower pH induced by the activities of homolactic LAB accompanying *L. buchneri* likely inhibits the activity of proteolytic microbes during ensiling. With ryegrass silage, Nsereko et al. (2008) observed no increase in ammonia-N concentration even when *L. buchneri* served as the sole LAB in the inoculant.

The primary advantage from inoculating ensiled forages with *L. buchneri* is the prolonged aerobic stability associated with the inhibitory effects of acetic acid or 1,2 propanediol on yeasts. Consistent with this hypothesis, the inoculated LC silage was stable for 7 d whereas the uninoculated LC was stable for only 4 d

(Figure 5.3). Although 4 d of exposure to air may seem long, exposure time in field scale silos will include not only the time from removal from storage to consumption by livestock, but also the time that silage on the silo face is exposed to air, conditions not simulated with mini-silo experiments. Both the inoculated and uninoculated SC remained stable for the entire 8 d of aerobic exposure. Although all silages were compacted to similar densities in the silos, silage was thoroughly mixed before it was loosely placed in each container for the aerobic stability test. This procedure may have increased the porosity and ingress of air to a greater degree in LC than SC silage. Increased exposure of silage microorganisms to oxygen will increase the rate of deterioration and heating of the silage (Wilkinson and Davies, 2012). Consequently, greater oxygen ingress would make benefits from *L. buchneri* more evident with the LC silage and might explain why inoculation improved aerobic stability with the LC but not SC silage. Data from Ruppel et al. (1995) indicated that a greater silage packing density also improves aerobic stability across the feed-out face of bunker silos. Heating during aerobic exposure can deplete volatile fatty acids and reduce silage digestibility and nutritional value. Depletion of potentially digestible nutrients caused by heating during feed-out has been reported to decrease the nutritive value of the silage by as much as 16% as compared to its nutritive value at the time the silo is opened (Tabacco et al. 2011b). With wheat silage, exposure to air for 7 d increased silage temperature and carbon dioxide release, and reduced DM and NDF digestibility of the silage by 14% and 7%, respectively (Chen and Weinberg, 2009). Inoculation of whole-crop barley forage with a FAE-producing third-generation inoculant also increased the aerobic stability of silages stored in mini silos (Addah et al. 2012a) as well as the aerobic stability and in situ NDF digestibility of those stored in bag silos by 7% and 9% after 24 and 48 h of incubation, respectively (Addah et al. 2012b).

Fine chopping of forage prior to ensiling increases both the cost (tractor power and labour) and the time required for harvest. Although increasing the chop length can reduce operational cost, fermentation characteristics and oxidative losses during feeding may be compromised when chop length is too long. Results of our

studies indicate that the chop length of barley silage could be increased from the conventional 0.95 cm TLC to approximately 2.0 cm without adversely affecting fermentation characteristics or aerobic stability with the appropriate inoculant.

5.4.2. Feed Intake, Growth Performance and Carcass Characteristics

Neither chop length, inoculation nor their interaction had a significant effect on DMI or growth performance of steers. The major mechanism by which inoculation of silages with third-generation ferulic acid esterase-producing inoculants may improve animal performance is through increasing fiber digestibility (Nsereko et al. 2008; Kang et al. 2009; Addah et al. 2012a; 2012b). Fiber digestibility was not measured in this study. Silage comprised only 100 g kg^{-1} of diet DM and only 208 g kg^{-1} of the dietary NDF (49 g kg^{-1} NDF from silage out of 257 g kg^{-1} NDF in the diet; Tables 2 and 4). Therefore, one would not expect to detect effects of inoculation as compared to previous backgrounding studies where improvements due to inoculation were observed when the barley silage constituted 770 g kg^{-1} DM of the diet (Addah et al. 2012a) or when barley silage treated with a first-generation homolactic inoculant constituted 870 g/kg DM of the diet (Zahiroddini et al. 2004). Though very few studies examining the interaction of inoculation with chop length of barley silage have been completed, several studies with corn silage have been published. Inoculation of corn silage with a homolactic silage inoculant alone decreased DMI and ADG of finishing steers when the diet contained 250 g kg^{-1} silage (Schaefer et al. 1989). Bal et al (2000) fed a diet containing corn silage chopped to 0.90 cm, 0.95 cm or 1.45 cm to Holstein cows at 330 g kg^{-1} DM inclusion level. They found no effect of chop length on DMI. In the present study, any response in fiber digestibility may have been obviated by low pH within the rumen. Dry rolled barley grain is rich in starch, and fermentation of starch in the rumen depresses ruminal pH. Low pH in turn inhibits the growth and activity of fibrolytic bacteria needed for ruminal fiber digestion (Russell and Wilson, 1996). As dietary concentrate increased, the extent of ruminal fiber digestibility is depressed based on both in situ (Miller and Muntifering, 1985) and in vitro (Mertens and Loften, 1980) results.

Forage particle length can affect feed intake, rate of digestion and the pattern of rumen fermentation. Increasing the TLC of forage increases bulkiness and rumen fill when diets high in forage are fed. But increasing the TLC of forage will also increase the physical effectiveness of fiber that in turn increases the time spent chewing and saliva production thereby modulating ruminal pH and increasing DMI (Soita et al. 2002). However, the magnitude of difference in chop length of forages evaluated in this study was likely too small to elicit a response in feed intake. The 1.0 cm difference between short (of 0.95 cm) and long (1.95 cm) TLC coupled with the low inclusion level of silage (100 g kg^{-1} DM) may have been insufficient to alter either DMI or growth performance. Effects of chop length of whole-crop barley silage on the DMI and growth performance of finishing feedlot steers has not been previously investigated, but Einarson et al. (2004) reported that with dairy cows, reducing the TLC of barley silage from 1.9 cm to 1.0 cm, increased DMI.

However, this increase in DMI was slightly less when the diet contained 580 g kg^{-1}*versus* 410 g kg^{-1}concentrate (4% *versus* 5%). Reducing the TLC of barley silage from 1.88 to 0.47 cm significantly reduced ruminal and total tract retention times, but despite this reduction, the lower TLC increased apparent digestibility of DM and NDF and increased DMI of steers fed an all-forage diet by 18% (Soita et al. 2002). In contrast, DMI by dairy cows fed 540 g kg^{-1} concentrate containing corn silage chopped at short (0.48 cm), medium (1.59 cm) or long (2.86 cm) TLC was not altered even though silage accounted for 460 g kg^{-1} of diet DM (Yang and Beauchemin, 2006). However, in this study, differences in chop length only had a marginal impact on the physically effective NDF content of the diet. Within a TLC range of 0.6 -3.8 cm for grass silage stored in stack silos, Savoie et al. (1992) found no consistent effect of chop length on silage quality or on DMI by dairy cattle when silage constituted 560 g kg^{-1} of the diet. Similarly, reducing the TLC of alfalfa (1.9 cm to 0.6 cm) failed to affect feed intake of dairy cows, however, reducing TLC by this degree increased DM intake of oat silage from 19.4 to 21.2 kg d^{-1} (Bhandari et al. 2008). Increasing the TLC of forage in a diet reduces the surface area available for rumen bacterial attachment, an outcome that may

decrease digestibility and increase rumen retention time (Soita et al. 2002), However, this response may be offset by the increase in physically effective fiber stimulating chewing and salivation (Beauchemin and Yang, 2005), thereby reducing the impact of chop length on rumen fermentation, passage rate, and DMI.

In this study the proportion of DM retained on a 1.18 mm sieve averaged 899.2 g kg^{-1} for SC silages and 861.2 g kg^{-1} for LC silages (Table 5.2). Increasing the TLC from 0.95 to 1.95 cm only increased the proportion of retained DM on the 1.18 mm screen by less than 4%. Therefore, peNDF values obtained using a 1.18-mm sieve does not adequately reflect differences in particle length of diets with varying TCL (Yang and Beauchemin, 2006). This is because finishing diets contain a high proportion of grain (850 g kg^{-1}) and most DM retained on the 1.18 mm screen originated from the barley grain that passed through 19 and 8 mm sieves. Consequently others (Lammers et al. 1996) have considered only the proportion of dietary NDF retained on the 19 mm and 8 mm sieves to represent physically effective fiber. Even though inoculation appeared to reduce the peNDF of both SC and LC diets, the differences were small. In general, the coarser diets (LC) were more physically effective as they had more peNDF retained on the 19 and 8 mm screens than SC diets. Beauchemin and Yang (2005) recommended a peNDF threshold of 100 g kg^{-1} to avoid a reduction in chewing activity. In this study, only the LC diets were within this recommended range, but this did not result in difference in DMI or the occurrence of clinical acidosis.

Carcasses are composed primarily of muscle, fat and bone. As external and internal trimmable fat increases, the proportion of saleable or edible lean tissue decreases if and when fat is trimmed and removed. In the present study, there was a trend (P = 0.138) towards greater fat content of carcasses for steers fed the LC diet than for steers fed the SC diet. Concurrently, longissmus muscle (rib eye) area of carcasses of steers was greater for steers fed SC silage, even though no corresponding improvements in growth performance were observed. Differences in site of digestion or in end products absorbed may be involved with

these changes. If such changes were consistent across trials they would have economic implications.

Manipulation of the relative proportions of ruminal VFAs available can affect intramuscular fatty acid synthesis and deposition. Acetic acid provides 70-80% of the acetyl units for lipogenesis in subcutaneous adipocytes but only 10-25% in intramuscular adipocytes. Instead, a greater proportion (50-60%) of the acetyl units for lipogenesis in intramuscular adipocytes are derived from glucose that is derived largely from propionic acid produced in the rumen (Smith and Crouse, 1984). The reduction in silage particle size can impact the pattern of ruminal fermentation. Indeed, Soita et al. (2002) observed that steers fed barley silage had a greater ratio of propionate to acetate when fed diets rich in barley silage chopped at a short (0.47 cm) rather than long (1.88 cm) length. An increase in the propionate to acetate ratio associated with the reduced forage chop length could account for the higher saleable meat and greater rib eye area for steers fed the SC silage diet in our trial. Unfortunately, ruminal VFA concentrations were not measured in this trial. However, increasing the TLC of barley silage from 1.59 cm to 2.86 cm (Yang and Beauchemin, 2006) or the TCL of corn silage from 0.9 cm to 1.45 cm (Bal et al. 2000) failed to alter the molar proportions or total concentrations of ruminal VFA. Likewise, reducing the TLC of alfalfa and oats silage from 1.9 cm to 0.6 cm had no effect on rumen fermentation characteristics of dairy cows (Bhandari et al. 2008). However, with increased chewing and saliva flow associated with a greater TLC, more glucogenic compounds from other components of the TMR of the diet might be expected. Further studies examining the effect of forage chop length on carcass characteristics of finishing feedlot steers seems warranted.

Such carcass benefits from a shorter TLC should be weighed against the additional cost of chopping silage to a shorter TLC prior to ensiling. A mathematical model has been developed to calculate the total horsepower required to operate a cylinder-type forage harvester (Tremblay et al. 1990). It indicates that increasing the TLC of forage from 1.0 cm to 5.0 cm would reduce

power consumption by 20% (Tremblay et al. 1990). Extrapolated to the current trial, the coarser particle size should reduce power consumption by about 5%.

5.5. CONCLUSION

Inoculation of the LC, but not SC silage, increased acetic acid concentration and extended its aerobic stability in the mini silo experiment. Fermentation characteristics of both SC and LC silages stored in Ag-Bags® were consistent with higher concentration of acetic acid, the hallmark of silage inoculated with *L. buchneri*. Neither inoculation, chop length nor their interaction had a significant impact on growth performance of finishing feedlot steers. However, any possible economic benefits of greater saleable meat yield and *longissmus* muscle area for SC barley silage should be weighed against the operational cost of chopping silage to a shorter TLC. This study indicates that whole-crop barley silage could be ensiled at a TLC of 1.9 cm and inoculated with this ferulic acid-producing inoculant without impeding fermentation, or compromising the aerobic stability of the silage.

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CHAPTER 6: GENERAL CONCLUSIONS AND IMPLICATIONS

6.1. An Overview of the Present Findings

6.1.1. Overview

First-generation silage inoculants consist mainly of homolactic lactobacilli (LAB) that produce lactic acid as the end product of fermentation of hexose sugar. The lactic acid formed induces a rapid decline in silage pH that preserves the silage, but residual lactic acid in silage can serve as a substrate for silage spoilage microorganisms upon exposure of the silage to air. Co-culturing of these homolactic strains with heterolactic LAB such as *Lactobacillus buchneri* and *Propionibacteria* that produce other volatile fatty acids (VFA) was used as a strategy to overcome this problem. Acetic acid produced by *L. buchneri* or propionic acid produced by *Propionibacteria* extends the aerobic stability of silage by inhibiting growth of yeasts and moulds. These inoculants have been classified as second-generation inoculants.

Inoculation of forages with first- and second- generation inoculants may improve the fermentation characteristics of silages, but their ability to improve the digestibility and nutritional value of silage has not been consistently supported by available literature as they lack fibrolytic enzyme activity during ensiling. Ferulic acid is a phenolic acid that forms ester linkages with potentially soluble fibre and reduces its digestibility. The development of strains of LAB capable of de-esterifying the ester linkages in forages has therefore become the target of third-generation silage inoculants.

Fermentation responses to first-generation inoculants in barley and corn silages have been studied extensively. Although fermentation responses are of interest, responses in digestibility and in animal performance ultimately dictate the commercial value of any silage inoculant. Unfortunately, data documenting responses in digestibility and animal performance to silage inoculants are very limited. However, in this thesis, not only were fermentation characteristics and aerobic stability of inoculated and uninoculated of corn and barley silage examined, but also ruminal digestibility (*in situ*) and animal growth performance

responses were investigated. Responses within each crop were generally consistent with the literature; inoculants failed to alter digestibility, animal growth and/or performance even though favourable alterations in fermentation characteristics and aerobic stability were detected (Chapter 2). In subsequent studies, responses to a third-generation FAE-producing inoculant were evaluated (Chapter 3 and Chapter 4). Beneficial effects of this inoculant on *in situ* digestibility and on feed efficiency by growing steers fed a high silage diet were observed. Finally, fermentation and steer performance responses both to inoculation and to increasing the chop length from the conventional 1.0 cm to 2.0 cm as a means of increasing physically effective fibre were examined.

6.1.2. Effects of First-generation Inoculants on Silage Fermentation and Animal Performance

Livestock producers often use a single commercial silage inoculant for treating different forage crops, assuming that an inoculant that improves fermentation characteristics of one crop will also be effective on others. Although many studies have evaluated the effects of first-generation inoculants on fermentation characteristics of barley and corn silage, consistency of response across crops has never been addressed directly. In Chapter 2, improvements in the fermentation characteristics and nutritional value of barley and corn silages when inoculated with a single homolactic inoculant were compared. Responses in fermentation profiles to a single inoculant differed between these two cereal forages, likely due to differences in their chemical composition and characteristics of the epiphytic microbes present. Such differences presumably account for the greater homolactic fermentation observed in barley and improved aerobic stability as compared to corn. The microbial composition both before and after fermentation probably plays a significant role in determining the stability of silage during feed-out. Results illustrate that while traditional (non-fibrolytic) fermentation characteristics of silage considered desirable, may potentially result in retention of energy in the silage for feeding, it may not translate into improved digestibility or animal performance. Response differences and inherent differences among the

silages in their ease of ensiling and synergy between an inoculant and the epiphytic population suggests that there may be merit in formulating inoculants that are designed to be specific for each forage type. Unfortunately, because chemical and biological characteristics of forage are also impacted by the environment, broad-based inoculants capable of producing desirable fermentation qualities under sub-optimum ensiling conditions are also needed.

Barley silage is the predominant ensiled forage fed to growing and finishing cattle in feedlot in western Canada. Few feeding trials testing response of barley silage to first-generation inoculants have detected any increases in rate or efficiency of gain. This failure indicates that digestibility of the silage generally is not improved by inoculation with such first-generation inoculants. One biochemical process that potentially could enhance ruminal solubility of silage fibre during ensiling is acid hydrolysis of fibre (Keady et al. 1994), but this process has a negligible impact on the digestibility of silage or animal performance. The fact that performance responses could not be detected despite evidence of the occurrence of the phenomenon (Chapter 2; Figure 2.2) might indicate that hydrolysis of the specific bonds by acid hydrolysis are insufficient to significantly improve fibre digestibility in the animal.

6.1.3. Effects of Third-generation Inoculants on Silage Fermentation and Animal Performance

Third-generation silage inoculants with microbes that produce ferulic acid esterase were first released commercially in 2008 (11CFT; Corn Fiber Technology) followed in 2009 by 11GFT (Grass Fiber Technology; Pioneer Hi-Bred, Johnston, IA). Research on the effects of third-generation inoculants on silage fermentation and silage fibre digestibility has been limited (Nsereko et al. 2008; Kang et al. 2009). Corn was the first crop examined for the ability of an inoculant with ferulate esterase activity (11CFT) to improve the feeding of silage. None of these experiments measured animal growth or performance responses to determine whether increases in fiber digestibility translated into improved animal performance. In Chapters 3 and 4 of this thesis, barley silage was inoculated with

a FAE-producing inoculant (11GFT). Fermentation characteristics, aerobic stability, and digestibility and growth performance of feedlot steers fed diets containing these silages was determined. The fermentation trajectory of silages stored in mini silos depended on the degree of acidity attained in the silage during the initial stages of ensiling. *L. buchneri*, a heterolactic LAB, is more metabolically active and competitive with other microbes after silage has attained a low pH. As opposed to homolactic LAB, most heterolactic LAB are not tolerant of very low pH. Undissociated organic acids formed during the initial stages of homolactic fermentation diffuse through cellular membranes and the acidity within the cytoplasm can disrupt the integrity of purine bases, resulting in cell death. Heterolactic LAB present in third-generation inoculants counteract this problem by degrading the primary organic acid, lactic acid, into acetic acid and propanediol, thus resulting in the heterolactic fermentation pattern (Chapters 4 and 5; Figures 4.1-4.2 and 5.1). Though less efficient than homolactic fermentation in terms of DM retention, heterolactic fermentation by *L. buchneri* and the production of acetic acid and propanediol reduces energy loss through inhibition of spoilage microorganisms upon feed-out. Inoculation of whole-crop barley silage with a FAE -producing third-generation inoculant extended the aerobic stability of silage while also increasing ruminal fibre digestibility and improving the feed efficiency of growing feedlot steers. These benefits should improve animal productivity and enhance the profit margins for feedlot operators (Appendices).

The improved feed use in Chapter 4 was likely mediated by an increase in ruminal fibre digestibility (Chapter 3) due to esterase activity during storage of the silage. Although inoculation of whole-crop barley silage improved NDF digestibility (Chapter 3) and feed efficiency of growing steers (Chapter 4), the concentration of NDF in the silage was not reduced by inoculation. Fibre hydrolyzed by FAE may not reduce NDF content if the ferouylated fibre is only partially hydrolyzed. Even though residual ferulic acid residues in silage were not measured in the present thesis, free ferulic acid, an end-product of de-esterification of ferouylated fibre should reflect FAE activity in silage. However,

as ferulic acid is readily metabolized to vanillic acid (Huang et al. 1993; Rosazza et al. 1995; Civolani et al. 2000), ferulic acid may also not be detected in silage. The most reliable method for assessing the efficacy and biological significance of third-generation FAE-producing inoculant should be an increased solubility of silage fibre measured as an increase in ruminal or total tract fibre digestibility and/or animal growth performance.

Real-time information about the quality of silage offered to animals is needed for appropriate decision-making by feedlot managers. The aerobic stability of silage being delivered to the feed bunk cannot be obtained immediately using conventional methods for assessing aerobic stability through measurement of temperature. However, thermal images can be employed to assess aerobic stability of silages in real-time (Chapter 3). This approach enables managers to make quick management decisions unlike time consuming and laborious conventional methods such as microbial or chemical analysis where results are only available after the feed has been fed. Thermal imaging is an advanced and novel method for detecting silage deterioration at the feed-out face in a format that is easily understood at the farm level. Thermal imaging also may prove useful for identifying problems with the ensiling process such as identifying areas of the silo where anaerobicity was compromised during the ensiling process.

Laboratory-sized silos are used extensively by researchers for testing responses to additives and inoculants. Differences between bag silos and mini silos in response to inoculation of barley silage were observed in Chapter 5. Inconsistencies in ensiling responses between mini- and large-scale silos are indicative of the need to confirm results from mini silos in farm-scale silos as results from mini silos cannot always be extrapolated to farm-scale ensiling methods (Woolford and Sawczyc 1984; Cherney et al. 2004). Although laboratory silos can are useful in assessing silage inoculants under tightly controlled conditions, they do not reflect responses under field conditions where many variables are not as easily controlled (e.g., packing, gradual filling and anaerobiosis, oxidative deterioration during feeding).

Neither inoculation with 11GFT nor its interaction with chop length affected animal growth performance in finishing feedlot diets (Chapter 5). The magnitude of the difference between the theoretical chop lengths that were tested (0.95 versus 1.95 cm) and the level of inclusion of silage in finishing feedlot diets (10% DM) presumably was too small to induce significant responses either to the main factors or their interaction. Unlike the animal performance responses to inoculation that were clearly apparent during the growing stage where ~76 % of diet DM was barley silage, response to the inoculant was not detected when included in the diet during the finishing stage when the diet consisted of only 10 % (DM) barley silage.

6.2. Implications and Future Directions

Future development of first-generation (rapid acidification lactic acid bacteria) inoculants should proceed on a forage-specific basis. When developed for a particular forage crop, selected LAB are more likely to prove effective as the selected bacterium are more able to adapt to the nutrient profile and tolerate inhibitors that may be present. Nevertheless, precarious interactions between weather conditions and crop harvest make it impossible to always harvest a crop at the optimum maturity for ensiling. Consequently, silage inoculants capable of eliciting favourable responses across a wide range of crop compositions, epiphytic microbial populations, and ensiling conditions are needed.

Previous studies have confirmed that certain LAB in some second-generation silage inoculants survive within the rumen environment and may modify rumen fermentation patterns (Weinberg et al. 2003; 2004) and potentially could improve DM and NDF digestibility (Weinberg et al. 2007). The extension of FAE activity from the silo to the rumen could form the basis for the development of fourth-generation silage inoculants if selected LAB remained present and active within the ruminal environment. This offers further opportunities for future research to investigate the dual effects of fibrolytic LAB in the silo and rumen. Studies on the survival and persistence of FAE-producing LAB through metagenomic tools also will offer an opportunity to further engineer

the FAE capability of third-generation inoculants. Specific traits that could therefore be useful for selection of these fourth-generation LAB strain include 1) ability to survive and persist in the rumen without losing the desired primary traits of improvements in silage fermentation and fibre hydrolysis during ensiling; this may include the absence of antagonistic interaction with beneficial rumen microorganisms. For example, such LAB should be able to produce bacteriocins that inhibit the growth of pathogenic bacteria, or produce enzymes capable to hydrolysing toxins produced by pathogenic microorganisms 2) they should be non-pathogenic to the host and humans; and 3) given that they will have to persist in the silage and diet offered to animals, they should be facultative anaerobes that are robust and capable of surviving long hours in the feed bunk. These conditions are likely achievable given that molecular tools for engineering the lactobacillus genus, which has a wide range of species most of which have already been demonstrated to have probiotic effects in livestock, already exist . Genetically modified *Lactococcus lactis* (Shinkawa et al. 2011) and *L. plantarum* (Okano et al. 2009) engineered to produce greater amounts of lactic acid and only trace amounts of acetic acid from xylose and arabinose respectively, by blocking the phophoketolase pathway and enhancing the pentose-phosphate pathway already exist. Development of transgenic inoculants that have the ability to enhance silage fermentation in silo, and fibre digestibility and efficiency of product utilization in the rumen may therefore be possible. However there is a challenge of losing known desirable traits such as bacterial growth rate and efficiency of fermentation when introducing a transgene.

In western Canada, whole-crop silages typically constitute from 60 to 80% of the DM in diets for growing cattle. Therefore, silages are a major source of digestible energy for growing steers. Cost of forage is ranked among the most important factors affecting the profitability of feedlot operations during the growing phase. Consequently, the improved aerobic stability, digestibility of fibre and efficiency of gain associated with third-generation inoculants offers considerable economic potential to improve the profitability of using inoculated whole-crop barley silage in feedlot operations during the growing phase. As the

global demand for grain and its by-products such distillers' grains continue to soar, feedlot operators can be expected to increase the proportion of forages fed in total mixed diets. This makes strategies that increase the nutrient availability of forages particularly attractive as an avenue for reducing feed costs. At an estimated cost of $ 2.0 per tonne of treated silage, inoculation of whole-crop barley silage with a third-generation inoculant could reduce the cost per kg live weight gain by 6%, representing a net return of up to $ 10.70 per head (Appendix 5). This increase in return on investment could greatly impact the profit margins of feedlot operations during the growing phase.

Thermal imaging technology has prospects in helping to detect energy losses associated with deterioration of silage along the face of an opened silo. Thermal imaging can identify areas of elevated temperature which correspond to the heat released by spoilage microorganisms during aerobic decomposition of silage. Measurement of *in situ* digestibility of silages obtained from areas specifically identified by thermal imaging could provide insight into the impact of aerobic deterioration on energy losses and the nutritional quality of silages at the silo or feed bunk. Being photographic, results are easily obtained even though energy losses or digestibility differences associated with temperature differences alone are very difficult to quantify.

To increase the concentration of physically effective fiber in feedlot diets, the theoretical chop length (TLC) of whole-crop barley silage was increased from the conventional 0.95 cm to at 1.95 cm when the barley forage was ensiled. Inoculation of longer chopped silage with a third-generation esterase inoculant appeared to avoid the adverse effects of a longer chop length on fermentation characteristics and aerobic stability of the silage (Chapter 5). An increase in TLC also will reduce fuel consumption and accelerate forage processing, increasing the number of harvestable hectares for each silage chopper. Yet, no main effects or interactions of chop length and inoculation on feed intake or growth performance of finishing steers were detected when the silage was chopped within a range of 1.0-2.0 cm prior to ensiling. If the concentration of physically effective fiber is increased, nutritionists would expect that 1) less forage would be needed in

finishing diets and 2) animal performance and health should be improved due to a reduction in the incidence of metabolic disorders such as lactic acidosis. In future studies, the impact of extending the TLC from 1.0-2.0 cm (short) to 3.0-5.0cm (medium) or 6.0-7.0 cm (long) with and without a FAE-producing inoculants on silage fermentation, animal health and performance could be investigated. Such studies could help to explain why the longer chop length in this study increased rib eye area and the proportion of saleable meat of steers without altering growth rate or feed efficiency.

Even though barley is the most common silage crop produced in Western Canada today, cultivation of corn in this region has increased steadily as grain-yielding hybrids requiring fewer growing degree days are developed. Mutant corn hybrids selected for low concentrations of ferulic acid esters have been developed that have more rapid *in vitro* ruminal NDF degradation compared to their isogenic hybrid (Jung and Philips 2010; Jung et al. 2011). Such hybrids are still in the experimental stage of development and are not commercially available as of yet. Brown midrib hybrids of corn and sorghum currently are marketed commercially that have lower lignin content and higher rates of fiber digestion, but to later maturation these hybrids are not readily available in western Canada. A comparison of the fermentation characteristics and nutritional value of barley and corn silages using the same third-generation inoculant as the one used in this study should offer feedlot managers the knowledge required to make a choice between these two crops when considering third-generation inoculants.

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APPENDICES (COST-BENEFIT ANALYSIS OF USING A THIRD-GENERATION INOCULANT MODELLED ON CHAPTER 4)

Appendix 1: Production performance of growing steers fed a diet containing silage inoculated with a third-generation inoculant for 112 d

Performance	Uninoculated	Inoculated	Benefit
Initial body weight (kg)	242.70	242.15	-0.55
Final body weight (kg)	386.90	389.6	2.70
Total body weight gain (kg)	144.80	147.50	2.70
Dry matter intake (kg d^{-1})	7.62	7.14	93.7%
Average daily gain (kg d^{-1})	1.29	1.31	101.6%
Feed Conversion (kg gain kg^{-1} DM intake)	0.169	0.184	108.9%

Appendix 2: Production cost (variable) of growing steers fed a diet containing silage inoculated with a third-generation inoculant for 112 d

Variable Cost	Uninoculated	Inoculated
Inoculant cost ($ ton^{-1} silage)	0.00	2.00
Ration cost ($ ton^{-1} DM)	125.78	125.29
Initial price of cattle ($ kg^{-1} live weight)[1]	2.34	2.34
Final price of cattle ($ kg^{-1} live weight)[2]	2.14	2.14
Yardage ($ hd^{-1} d^{-1})	0.30	0.30
Animal health ($ d^{-1})	11.39	11.39
Interest (% yr^{-1})	5.00	5.00
Shipping cost ($ hd^{-1})	10.00	10.00

Appendix 3: Silage inclusion level and live weight gain of growing steers fed a diet containing silage inoculated with a third-generation inoculant for 112 d

Gain per ton	Uninoculated	Inoculated	Benefit
Silage in the ration (% DM basis)	76.00	77.00	
DM of silage (%)	41.00	44.00	
Amount of silage fed (kg DM hd^{-1})	651.20	613.40	
As-is tonnes silage fed (tonnes hd^{-1})	0.79	0.71	
Gain per ton of silage DM fed (kg)	444.80	481.06	36.26
Gain per ton of as-is silage fed (kg)	182.81	209.26	26.45

Appendix 4: Silage and non-forage feed costs

Ingredient Cost	Uninoculated	Inoculated
Cost of fresh forage ensiled ($ ton^{-1})	45.00	47.00
Silage DM Loss (%)	10.00	11.00
Cost of fresh silage ($ ton^{-1})	50.01	52.69
Silage cost per ton DM ($)	121.67	121.13
Non-forage ingredients cost ($ ton^{-1} DM)	139.00	139.00

Appendix 5: Cost-benefit analysis of inoculating of growing steers fed a diet containing silage inoculated with a third-generation inoculant for 112 d

Profit/loss	Uninoculated	Inoculated	Benefit
Initial cost of cattle ($ hd^{-1})[z]	567.16	565.88	1.29
Total feed (kg DM hd^{-1})[y]	853.44	799.68	-53.76
Total feed cost ($ hd^{-1})	53.67	50.10	3.57
Total yardage cost ($ hd^{-1})	33.60	33.60	0.00
Interest cost ($ hd^{-1})	10.12	10.04	0.07
Miscellaneous cost (bedding; $ hd^{-1})[y]	5.00	5.00	0.00
Cost of gain ($ kg^{-1})	0.672	0.635	0.037
Final cattle value ($ hd^{-1})[y]	827.97	833.74	5.78
Net benefit ($ hd^{-1})[x]	192.01	202.73	10.71

[z]Weekly livestock review (November 13, 2009), Alberta Agriculture and Rural Development

[y]Weekly livestock review (March 5, 2010), Alberta Agriculture and Rural Development

[x]For 112 d on feed