



Effects of Microbial Inoculants on the Fermentation, Nutrient Retention, and Aerobic Stability of Barley Silage

H. Zahiroddini¹, J. Baah and T. A. McAllister*

Agriculture and Agri-Food Canada, Lethbridge Research Centre, 5403-1st Avenue South
Lethbridge, AB, T1J 4B1, Canada

ABSTRACT : Fermentation characteristics, nutrient retention and aerobic stability of barley silages prepared using 6 commercial inoculants were evaluated using 126 mini-silos (3-L) in a completely randomized design. Whole barley forage was chopped, wilted to 39% DM and treated with water (control, S) or one of six inoculants: A (containing *Lactobacillus plantarum*); B (*L. plantarum* and *Enterococcus faecium*); C (*L. plantarum* and *Pediococcus cerevisiae*); D (*L. plantarum*, *Pediococcus pentosaceus* and *Propionibacterium freudenreichii*, plus hydrolytic enzymes); E (*Lactobacillus buchneri* plus hydrolytic enzymes); F (*L. buchneri* and *P. pentosaceus* plus hydrolytic enzymes). Samples of treated forage were collected for analysis at the time of ensiling, and then 18 silos of each treatment were filled, capped and weighed. Triplicate silos were weighed and opened after 1, 3, 5, 7, 33, and 61 d. On d 61, 400±5 g of material from each silo was placed in 1-L styrofoam containers, covered with cheesecloth and held at room temperature. Silage temperature was recorded hourly for 14 d via implanted thermocouple probes. Chemical composition of the forage at ensiling was consistent with previously reported values. At d 61, pH was lowest ($p < 0.01$) in silage S. Ammonia-N was lower ($p < 0.05$) in silage A than in silages S, B, E, or F. Compared to pre-ensiling values, water soluble carbohydrate concentrations were elevated in silages S, A, B, C and D, and decreased in E and F. Lactic acid concentrations were similar ($p > 0.10$) across treatments. Acetic acid levels were highest ($p < 0.01$) in silage E and lowest ($p < 0.01$) in silage D. Recovery of DM was lower ($p < 0.01$) in silage F than in silages S, A, B, C, or D. On d 61, yeasts were most numerous ($p < 0.01$) in silage D, which was the only silage in which temperature rose more than 2°C above ambient during aerobic exposure. Silage D also had the highest ($p < 0.01$) pH and ADIN content after aerobic exposure. Lactic acid and WSC content of silage D decreased dramatically during the 14-d aerobic exposure period. Yeast counts (at d 14 of exposure) were lowest ($p < 0.01$) in silages E and F. In general, the commercial inoculants did not appear to enhance the fermentation of barley silage to any appreciable extent in laboratory silos. (**Key Words** : Aerobic Stability, Barley Silage, Fermentation, Inoculant)

INTRODUCTION

Bacterial inoculants are the most common type of silage additives used in North America. They are used to enhance the ensiling process and have been reported to occasionally result in improvements in animal performance (Muck, 1993; McAllister et al., 1995; 1998). Most products include one or more homofermentative lactic acid bacterial (LAB) species. *Lactobacillus plantarum*, other *Lactobacillus* species, *Enterococcus faecium*, and various *Pediococcus* species are the most common bacteria that are included in

silage inoculants (Muck and Kung, 1997). The reason for using multiple species in some products is the opportunity of synergistic growth among bacterial species. For example, *Pediococcus* and *Enterococcus* are known to grow more readily during the early stages of fermentation, when the pH is above 5. Combining these species with *L. plantarum*, which grows more readily at a pH below 5, accelerates pH decline and transition to conditions that result in stabilization of the forage (Weinberg and Muck, 1996). Inoculated LAB can complement the epiphytic lactic acid bacteria present on the crop and facilitate the fermentation process (Muck and Kung, 1997; Yahaya et al., 2004; Shao et al., 2005). However, homofermentative LAB have also occasionally been shown to reduce the aerobic stability of silage (Weinberg et al., 1993; Filya et al., 2000), possibly due to a reduction in the production of antifungal compounds such as acetic acid and the conservation of

* Corresponding Author: T. A. McAllister. Tel: +1-403-317-2240, Fax: +1-403-382-3156, E-mail: mcallistert@agr.gc.ca

¹ Alberta Agriculture, Food and Rural Development, Agriculture Centre, 5401-1st Avenue S., Lethbridge, Alberta, T1J 4V6, Canada.

Received October 27, 2005; Accepted March 7, 2006

water soluble carbohydrates. Inoculants containing the heterofermentative species, *Lactobacillus buchneri*, have been marketed mainly on their ability to improve the aerobic stability of silage (Weinberg and Muck, 1996; Ranjit and Kung, 2000; Kung and Ranjit, 2001). Although the fermentation efficiency of heterolactic bacteria is lower than homolactic bacteria (McDonald et al., 1991), any increase in dry matter losses during fermentation may be offset by improvements in the aerobic stability of the silage (Holzer et al., 2003). The acetic acid-associated reduction in silage intake by cattle found in previous studies (Jones et al., 1980; Buchanan-Smith, 1990) has not been reported in more recent studies (Driehuis et al., 1999; Taylor et al., 2002). Consequently, improved stability through elevated acetic acid levels may be possible without a reduction in the intake of silage. Inclusion of propionic acid bacteria in inoculants may also improve aerobic stability as propionate has also been shown to exhibit antifungal activity (Weinberg et al., 1995a; Hegginkbotham et al., 1998). In addition to their ability to improve preservation efficiency, some strains of lactic acid bacteria may have inhibitory activity against hazardous microbes such as *Salmonella typhimurium*, *Listeria monocytogenes* and *Bacillus cereus* and exhibit probiotic properties (Ham et al., 2003).

Application of fibrolytic enzymes, alone or in combination with bacterial inoculants, has been proposed as a means of directly improving fibre digestibility as well as increasing the availability of water soluble carbohydrates (WSC) to serve as a substrate for LAB (McDonald et al., 1991; Weinberg et al., 1995b). The enzymes usually consist of a crude mixture of a number of carbohydrases and their specific activity is often poorly defined.

In the present study, we investigated the effects of six bacterial inoculants on fermentation, nutrient retention and aerobic stability of barley silage. The inoculants contained a variety of bacterial species and 3 of the 6 included exogenous enzymes. The objective of this study was to evaluate the ability of these additives to enhance the fermentation and the aerobic stability of barley silage.

MATERIALS AND METHODS

Silage preparation and sampling

Whole plant barley was harvested from a single field at the soft dough stage, wilted to 39% dry matter (DM) and chopped (10-mm theoretical length of cut) with a forage harvester (1260 Auto-Max, Gehl, Westbend, WI). The chopped barley was mixed thoroughly and divided into seven 60-kg portions. Inoculants were dissolved in distilled water in concentrations sufficient to meet the manufacturers' recommended rates of application when forage was sprayed with inoculant solution at 2.0 ml/kg of forage. Treatments (spraying with inoculant solution) were

applied immediately prior to ensiling. The control forage (S) was sprayed with an equal volume (120 ml) of water. The inoculants contained *Lactobacillus plantarum* (A; Biomax 5, Chr Hansen BioSystems, Milwaukee, WI, USA), *L. plantarum* plus *Enterococcus faecium* (B; Sil-All, Alltech, Inc., Nicholasville, KY, USA), *L. plantarum* plus *Pediococcus cerevisiae* (C; Sil-Edge 50X WS, Chr Hansen BioSystems, Mississauga, ON, Canada), *L. plantarum*, *Pediococcus pentosaceus*, and *Propionibacterium freudenreichii* plus hydrolytic enzymes (D; Biotal Plus, Biotal Inc., Eden Prairie, MN, USA), *Lactobacillus buchneri* plus hydrolytic enzymes (E; Biotal Buchneri, Biotal Inc., Eden Prairie, MN, USA), and *L. buchneri* and *Pediococcus pentosaceus* plus hydrolytic enzymes (F; proprietary blend, Biotal Inc., Eden Prairie, MN, USA). The enzymes in D, E, and F consisted of a mixture of amylases, xylanases, β -glucanases and galactomannases. In order to determine the actual rate of application of LAB (cfu/g) on treated forages, a sample of each inoculant was serially diluted in sterile 70 mM potassium phosphate buffer and plated onto triplicate plates containing semi-selective medium (MRS; Hill and Hill, 1986). Forage samples were randomly collected from each treatment prior to ensiling, after the treated forage had been mixed for 5 minutes.

Barley forage from each treatment was packed into 18 mini-silos measuring 10.5 cm in diameter \times 35.5 cm high, each with a capacity of approximately 2.5 kg. A hydraulic press was used to achieve a packing density of approximately 240 kg/m³. Silos (126 in total) were weighed before and after filling with barley forage and then capped and stored at ambient temperature. Triplicate silos for each treatment on each day were opened after 1, 3, 5, 7, 33, and 61 days of ensiling. Silage samples were taken from each silo after mixing of contents.

Aerobic stability

On day 61, silos were weighed immediately prior to opening to estimate DM losses. After opening, 400 g of silage from each silo were transferred into separate 1-L containers. The containers were implanted with thermocouples to monitor temperature and covered with cheesecloth. After 14 days of aerobic exposure (3 replicate open containers for each silage), chemical and microbial analyses were performed and pH was measured. The thermocouples monitored silage temperature on an hourly basis over the 14-d period.

Chemical and microbial analyses

Forage and silage samples were dried and/or processed for pH determination, chemical analyses, isolation and enumeration of microorganisms (LAB, yeast and molds) according to the methods described by Zahiroddini et al. (2004).

Table 1. Composition of barley forage at harvest and 61 days after application of inoculants and ensiling

Characteristic	Forage at ensiling ²	Treatment ¹							SEM
		S	A	B	C	D	E	F	
pH	6.30±0.21	3.60 ^d	3.78 ^b	3.82 ^b	3.72	3.93 ^a	3.92 ^a	3.92 ^a	0.02
DM (%)	38.77±0.21	35.39	36.65	35.80	35.76 ^c	35.90	35.76	35.71	0.27
Composition (% DM basis)									
Crude protein	12.55±0.08	13.50 ^a	13.38 ^a	13.52 ^a	13.53 ^a	13.48 ^a	13.49 ^a	13.01 ^b	0.06
NDF	49.32±0.52	45.55 ^{bc}	47.54 ^a	44.82 ^c	46.13 ^{bc}	45.34 ^{bc}	46.46 ^{ab}	46.31 ^{ab}	0.44
ADIN (% TN)	4.45±0.09	3.94 ^c	4.24 ^{ab}	3.97 ^c	4.11 ^{abc}	4.02 ^c	4.26 ^a	4.05 ^{bc}	0.07
Starch	19.85±1.07	16.16	16.36	18.11	16.50	18.14	16.93	14.99	1.03
WSC ³	4.80±0.15	7.24 ^c	7.21 ^c	6.79 ^c	8.49 ^b	11.51 ^a	2.14 ^d	1.84 ^d	0.35
NH ₃ -N (% TN) ⁴	0.31±0.01	4.28 ^{ab}	3.63 ^c	4.43 ^a	4.03 ^{abc}	3.87 ^{bc}	4.34 ^{ab}	4.29 ^{ab}	0.18
FAA-N (% TN) ⁵	2.87±0.1	21.09 ^b	21.29 ^b	21.28 ^b	23.03 ^a	20.15 ^b	20.51 ^b	20.84 ^b	0.40
Acetic acid	-	1.95 ^{cd}	1.61 ^d	2.20 ^{bc}	1.63 ^d	0.99 ^e	4.60 ^a	2.57 ^b	0.24
Lactic acid	-	7.13	7.32	6.94	7.07	6.06	6.41	6.40	0.91
Propionic acid ⁴	-	0.025 ^{ab}	0.029 ^{ab}	0.029 ^{ab}	0.028 ^{ab}	0.021 ^b	0.041 ^a	0.038 ^a	0.007
DM Recovery (%)	-	92.48 ^a	92.81 ^a	92.04 ^a	92.34 ^a	91.76 ^a	90.63 ^{ab}	88.59 ^b	0.73
Microbial analysis (log ₁₀ cfu/g silage) ⁶									
LAB	5.74±0.07	7.96 ^b	6.67 ^d	7.73 ^b	7.42 ^c	6.87 ^d	9.16 ^a	8.95 ^a	0.08
Yeasts	3.33±0.10	<2.00 ^c	4.25 ^b	3.63 ^b	2.54 ^c	5.16 ^a	<2.00 ^c	<2.00 ^c	0.29
Molds	4.67±0.07	nd ⁷	nd	nd	nd	nd	nd	nd	-

¹ Treatments denoted as follows: S = control silage (no inoculant), A = *Lactobacillus plantarum*, B = *L. plantarum* plus *Enterococcus faecium*, C = *L. plantarum* plus *Pediococcus cerevisiae*, D = *L. plantarum*, *Pediococcus pentosaceus*, and *Propionibacterium freudenreichii* plus hydrolytic enzymes, E = *Lactobacillus buchneri* plus hydrolytic enzymes, F = *L. buchneri* and *P. pentosaceus* plus hydrolytic enzymes.

² Values shown are means±S.E.

³ WSC: Water soluble carbohydrates.

⁴ For these variables, (p = 0.06).

⁵ FAA: Free amino acids.

⁶ LAB: Lactic acid-producing bacteria. For yeast enumerations, samples positive but below the limits of enumeration (i.e., <2.0) were included in statistical analysis as minimum detectable values.

⁷ nd: Not detected (limit of detection = 100 cfu/g silage). Thus, SEM was not calculated.

^{a-e} Within a row, values bearing different superscripts differ (p<0.05 unless otherwise stated).

Statistical analyses

Data were analyzed as a completely randomized design with seven treatments and three replications per treatment and subjected to ANOVA by the GLM procedure of the SAS Institute (1991). A log₁₀ transformation of the microbiological data was performed before statistical analysis. Differences among reported means were determined using the least square means linear hypothesis test of SAS (1991).

RESULTS

The determined rate of applications of LAB (cfu/g fresh forage) on treated barley forage for products A, B, C, D, E, and F were 2.36×10⁶, 8.40×10⁴, 1.69×10⁵, 1.94×10⁵, 1.98×10⁵ and 1.19×10⁵, respectively. The chemical composition and microbial counts of barley forage (after inoculation, prior to ensiling) and barley silage after 61 days of ensiling in mini-silos are presented in Table 1. Dry matter (DM) content of barley silages was similar across treatments. The rate of decline of silages pH is presented in Figure 1. Final pH was the lowest (p<0.01) in the control silage and the highest (p<0.01) in silages D, E, and F. Crude protein (CP) contents of silages were similar across

treatments except for silage F, which had lower CP content (p<0.01) than other silages. However, the biological relevance of this difference is not clear. The concentration of NDF ranged from 44.8 to 47.5% (of DM) and was higher (p<0.05) in silage A than in control, B, C, and D silages. Also, silage A, along with silage E, had higher (p<0.05) acid detergent insoluble nitrogen (ADIN) content (% TN) as compared to control silage. The concentration of water soluble carbohydrates (WSC) exhibited the greatest variation among treatments and ranged from 1.84 (% DM) for silage F to 11.51 for silage D (p<0.01). Silage A was the only silage with lower (p = 0.06) NH₃-N content (% TN) than control silage. The starch and lactic acid contents (% DM) of silages did not differ among treatments. There was a relatively high variation in acetic acid content among silages with silage D and silage E containing the lowest (p<0.01) and highest amounts, respectively. Although silage D was treated with an inoculant containing propionic acid-producing bacteria (*Propionibacterium*), the propionic acid content (% DM) of this silage was numerically the lowest and was significantly (p = 0.06) lower than in silages E and F. Populations (log₁₀ cfu/g silage) of LAB were highest (p<0.01) in silages E and F, lowest in silages A and D, and intermediate in control, B, and C silages. The

Table 2. Composition of barley silages after 14 days of exposure to air

	Treatment ¹							SEM
	S	A	B	C	D	E	F	
pH	4.28 ^c	5.65 ^b	5.20 ^{bc}	4.11 ^c	8.34 ^a	4.14 ^c	4.14 ^c	0.45
DM (%)	46.34 ^a	45.57 ^a	45.44 ^a	45.96 ^a	43.54 ^b	46.09 ^a	46.62 ^a	0.49
Composition (% DM basis)								
Crude protein	13.14	13.44	13.42	13.12	14.05	13.28	13.18	0.27
NDF	44.23 ^{bcd}	45.95 ^{abc}	44.04 ^{cd}	43.19 ^d	46.75 ^a	46.56 ^{ab}	46.23 ^{abc}	0.78
ADIN (% TN)	4.08 ^{bc}	4.45 ^b	4.24 ^b	3.66 ^c	6.00 ^a	4.34 ^b	4.01 ^{bc}	0.19
Starch	18.48	22.65	20.87	19.77	23.06	19.18	15.88	2.28
WSC ²	5.05 ^a	2.33 ^{bc}	4.12 ^{ab}	5.82 ^a	2.39 ^{bc}	1.99 ^{bc}	1.73 ^c	0.78
NH ₃ -N (% TN)	4.42	4.84	4.04	3.74	6.64	4.82	4.90	0.88
Acetic acid	0.97 ^c	0.26 ^{de}	0.81 ^{cd}	0.70 ^{cde}	0.18 ^e	4.15 ^a	3.32 ^b	0.18
Lactic acid	4.65 ^{bc}	2.75 ^{bc}	4.46 ^{ab}	6.23 ^{ab}	0.89 ^c	5.12 ^{ab}	5.87 ^a	0.81
Microbial analysis (log ₁₀ cfu/g silage) ³								
LAB	9.38 ^a	9.68 ^a	8.39 ^b	8.38 ^b	9.51 ^a	9.15 ^a	9.00 ^{ab}	0.24
Yeasts	7.62 ^b	8.93 ^a	8.29 ^{ab}	7.99 ^{ab}	9.02 ^a	3.14 ^c	<2.00 ^c	0.47
Molds	nd ⁴	nd	nd	7.15	6.26	nd	nd	-

¹Treatments denoted as follows: S = control silage (no inoculant), A = *Lactobacillus plantarum*, B = *L. plantarum* plus *Enterococcus faecium*, C = *L. plantarum* plus *Pediococcus cerevisiae*, D = *L. plantarum*, *Pediococcus pentosaceus*, and *Propionibacterium freudenreichii* plus hydrolytic enzymes, E = *Lactobacillus buchneri* plus hydrolytic enzymes, F = *L. buchneri* and *P. pentosaceus* plus hydrolytic enzymes.

²WSC: Water soluble carbohydrates.

³LAB: Lactic acid-producing bacteria. For yeast enumeration, samples positive but below the limit of enumeration (i.e., <2.0) were included in statistical analysis as minimum detectable values.

⁴nd = not detected (limit of detection = 100 cfu/g silage). Mold population data were not analyzed statistically.

^{a-e} Within a row, values bearing different superscripts differ (p<0.05).

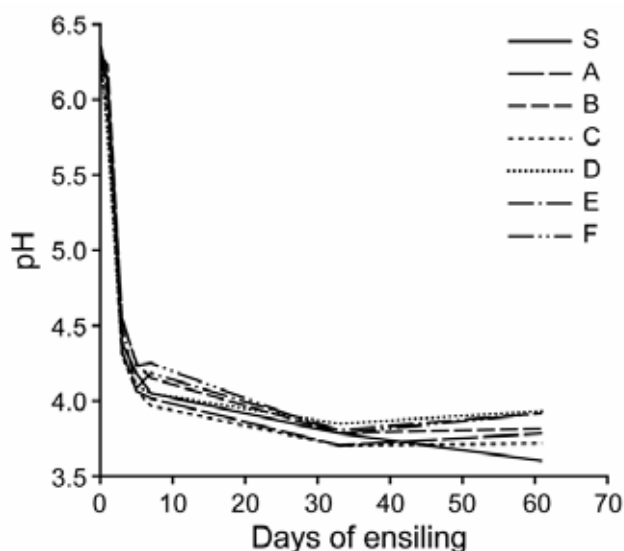


Figure 1. Change in pH during ensiling. S = Control, A = *Lactobacillus plantarum*, B = *L. plantarum* plus *Enterococcus faecium*, C = *L. plantarum* plus *Pediococcus cerevisiae*, D = *L. plantarum*, *Pediococcus pentosaceus*, and *Propionibacterium freudenreichii* plus hydrolytic enzymes, E = *Lactobacillus buchneri* plus hydrolytic enzymes, F = *L. buchneri* and *Pediococcus pentosaceus* plus hydrolytic enzymes.

population of yeast was the highest (p<0.01) in silage D, the lowest in control, C, E, and F silages and intermediate in silages A and B. Silage DM recoveries were similar among treatments except for silage F, in which DM recovery was lower (p = 0.015) than in silages S, A, B, C, and D.

The result of temperature monitoring over the 14-day period of aerobic exposure is presented in Figure 2. This figure shows the differences between daily average of silage temperature and ambient during this period. Silage D was the only one that exhibited a temperature increase that was more than 2°C above ambient temperature (21 ± 2.5°C). The chemical composition and microbial counts of barley silages exposed to air for a period of 14 days are presented in Table 2. The DM content of silage D was lower (p<0.05) than those of other silages. Silage D also had the highest (p<0.01) pH among treatments and the most dramatic drop in WSC content during the 14-day aerobic exposure period. The WSC content of silage A also declined substantially as a result of aerobic exposure. Silage D exhibited the highest (p<0.01) level of ADIN (% TN) and NH₃-N among the treatments examined. Silages E and F, inoculated with *L. buchneri*, had the highest (p<0.01) acetic acid content (% DM) at the end of 14-day period and remained similar to those recorded at the end of the ensiling period. In contrast, the acetic acid content of other silages declined as a result of aerobic exposure. Silage D had the lowest (p<0.01) lactic acid content at the end of aerobic exposure. Microbial enumeration (log₁₀ cfu/g fresh forage) revealed silages E and F as having the lowest (p<0.01) yeast counts at the end of the aerobic phase. Mold population was numerically highest in silage C, followed by silage D.

DISCUSSION

The chemical composition of barley forage used in this

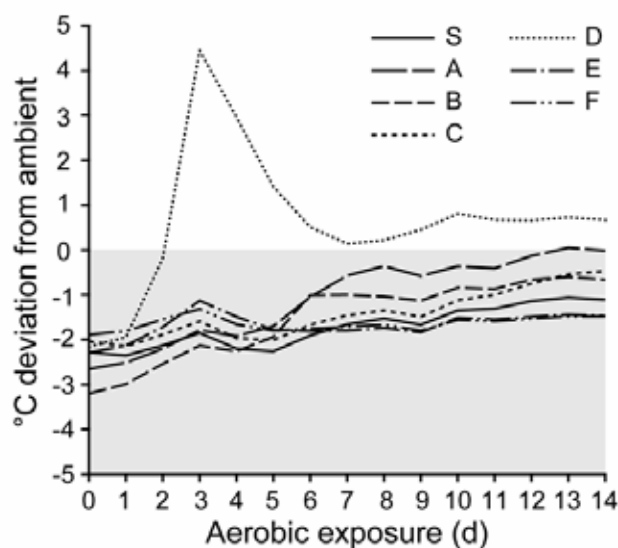


Figure 2. Deviation of silage temperature from ambient ($21\pm 2.5^{\circ}\text{C}$) during 14 d of aerobic exposure. S = Control, A = *Lactobacillus plantarum*, B = *L. plantarum* plus *Enterococcus faecium*, C = *L. plantarum* plus *Pediococcus cerevisiae*, D = *L. plantarum*, *Pediococcus pentosaceus*, and *Propionibacterium freudenreichii* plus hydrolytic enzymes, E = *Lactobacillus buchneri* plus hydrolytic enzymes, F = *L. buchneri* and *Pediococcus pentosaceus* plus hydrolytic enzymes. Shaded area represents temperatures that were below ambient temperature.

study is consistent with values reported in the literature (McCartney and Vaage 1994; McAllister et al., 1995; Zahiroddini et al., 2004). All silages, including the control, appeared to be of good quality, as evidenced by the rapid drop in their pH (Figure 1), and their low (<4.00) terminal pH, high concentration of lactic acid, and low concentration of ADIN. A microbial inoculant should apply at least 90 billion (9×10^{10}) live bacteria/tonne ($9 \times 10^4/\text{g}$) fresh crop to be able to out-compete the epiphytic populations of lactic acid bacteria, and thereby to have a significant impact on the ensiling process (Muck and Kung, 1997). In this study, the recommended level of application was met with all inoculants except B (*L. plantarum* plus *Enterococcus faecium*), which was applied at 8.4×10^4 cfu/g fresh crop. Nevertheless, the inoculants used in this study failed to improve fermentation process in treated silages compared to untreated silage, as evidenced by similar decline in pH among treatments and the fact that terminal pH was lowest in the control treatment. Lactic acid concentrations were also similar across treatments, with the mixture of *L. buchneri* and *P. pentosaceus* plus hydrolytic enzymes (F) even being associated with a reduction in DM recovery. Three major factors influencing the efficacy of silage inoculants include the nature of epiphytic LAB, the water soluble carbohydrate content of the crop, and the characteristics of the bacterial strains included in the inoculant (Muck and Kung, 1997). A survey of inoculant

studies revealed that inoculants most often result in a favourable change in fermentation in alfalfa and grass silages (Muck, 1993). Inoculant-mediated conservation of WSC and suppression of proteolysis and $\text{NH}_3\text{-N}$ production through rapid pH drop have been claimed as benefits of silage inoculation (Muck, 1993). Silage treated with *L. plantarum*, *Pediococcus pentosaceus*, and *Propionibacterium freudenreichii* plus hydrolytic enzymes (D) had the highest WSC content, followed by silage treated with *L. plantarum* plus *Pediococcus cerevisiae* (C) and silages treated with *L. plantarum* plus *Enterococcus faecium* (B), *Lactobacillus plantarum* (A), and control silage (S). The WSC content of these silages was even higher than in the original forage, a phenomenon reported previously by others (Weinberg et al., 1993, 1998; Filya et al., 2000; Dr. Z. G. Weinberg, personal communication) and could be attributed to partial degradation of cell walls. In addition, the rate of fermentation, the LAB load and strain type, and the crop composition and maturity of the plant at harvest may all affect the level of WSC. It has been hypothesized that chopping by itself may not result in complete cell lysis, thus release of soluble sugars during the initial stages of ensiling may also occur. Subsequent cell lysis during the fermentation process may lead to the release of sugars (Muck and Pitt, 1993) and could account for a higher post-ensiling level of WSC, depending on the severity of processing applied to the forage. Silages treated with inoculants containing *L. buchneri* (E and F) contained the lowest WSC content. This effect could be related to heterolactic fermentation occurring in these silages (Henderson, 1993).

Differences in $\text{NH}_3\text{-N}$ content among silages could be attributed to the different rates of decline of pH in various silages during the first few days after ensiling (Muck and Kung, 1997). The inclusion of enzymes with inoculants did not seem to be effective either in decreasing the NDF content or increasing the WSC content of barley silage. Although silage D, which contained an enzyme product, had the highest level of WSC among treatments, we are hesitant to attribute this effect solely to the presence of enzymes, as the NDF and ADF contents of this silage did not differ from those of some of the other silages that contained a lower level of WSC (data not shown). Other researchers (Moshtaghi Nia and Wittenberg, 1999; Ranjit and Kung, 2000; Kung and Ranjit, 2001) have applied enzyme-containing inoculants onto barley silages with no effects on NDF or ADF concentrations. We have previously found higher concentrations of fiber in silages treated with enzyme-containing inoculant and ensiled in mini-silos, but lower concentration of ADF in the same silages ensiled in large bag silos (Zahiroddini et al., 2004). We attributed this effect to the nature of ensiling environment, a factor that could have also influenced the results in the present study.

Lactic acid concentrations were similar among silages and corresponded with terminal pH. These concentrations are consistent with previously reported values (Mahanna, 1993; Zahiroddini et al., 2004). Acetic acid content of silage E, treated with *L. buchneri* as the sole inoculated bacterial species, was the highest among treatments, a result that confirms the ability of this species to increase the production of acetic acid in silage (Weinberg and Muck, 1996; Filya et al., 2003a, b). It has been proposed that acetic acid is produced along with ethanol and 1,2 propanediol (not measured in our study) through anaerobic degradation of lactic acid, a pathway that may provide lactobacilli with a protective mechanism against low pH (Oude Elferink et al., 2001). Acetic acid has strong antifungal properties (Woolford, 1975) and has been shown to improve aerobic stability of silage through suppression of both yeasts and molds (Kung and Ranjit, 2001). The beneficial effects of homofermentative LAB on fermentation and retention of nutrients in silages, along with the ability of heterofermentative LAB to improve the aerobic stability of silage, has led to the development of inoculants consisting of mixtures of these bacteria (Ranjit and Kung, 2000).

Filya (2003) investigated the effects of *L. buchneri* and *L. plantarum*, alone or in combination, on the fermentation and aerobic stability of low dry matter corn and sorghum silages and observed higher levels of acetic acid in silages treated with *L. buchneri* alone and in combination with *L. plantarum* as compared to control silage or silage treated with *L. plantarum* only. In the present study, silage treated with *L. buchneri* plus *P. pentosaceus* (silage F) had the acetic acid level next highest to that of silage treated with *L. buchneri* alone (silage E), although it was much lower and did not differ significantly from silage B, which was treated only with homofermentative LAB (*L. plantarum* and *E. faecium*). This suggests that *L. buchneri* was not able to compete with other silage microorganisms when applied together with a homofermentative LAB. Variation in results using mixtures of *L. buchneri* and homofermentative LAB and others (Filya et al., 2003a, b) may reflect differences in the relative competitiveness of different stains of *L. buchneri* in varying ensiling environments. Although inoculant D contained propionic acid-producing bacteria, the propionic acid content of silage D was either lower or similar to other silages, suggesting that *Propionibacterium* failed to compete with LAB during the ensiling process. This effect has already been observed in other studies and was attributed to the relatively slow growth rate and poor acid tolerance of this species (Higginbotham et al., 1998; Kung and Ranjit, 2001).

Silages treated with *Lactobacillus buchneri* plus hydrolytic enzymes (E), and *L. buchneri* and *Pediococcus pentosaceus* plus hydrolytic enzymes (F) had the highest

number of LAB on d 61 of ensiling. We attribute this effect to the ability of *L. buchneri* to enhance the viability of LAB under acidic conditions (Oude Elferink et al., 2001). Silage treated with *L. plantarum*, *Pediococcus pentosaceus*, and *Propionibacterium freudenreichii* plus hydrolytic enzymes (D) had the highest number of yeasts ($>5 \log_{10}$ cfu/g) among the treatments examined. Silages with yeast populations as high as $5 \log_{10}$ cfu/g are known to be susceptible to aerobic spoilage (Woolford, 1984; Pahlow, 1991; Henderson, 1993). The high concentration of WSC and low concentration of VFA (acetic and propionic acid) in silage D likely promoted the rapid proliferation of yeasts in this silage upon aerobic exposure (Weinberg and Muck, 1996). Silage treated with *L. buchneri* and *P. pentosaceus* plus hydrolytic enzymes (F) had significantly lower DM recovery than control silage and other silages treated with homofermentative LAB. Silage treated with *L. buchneri* plus hydrolytic enzymes (E) also exhibited marginal deterioration. Dry matter losses can increase when heterofermentative LAB are involved in the fermentation (McDonald et al., 1991).

During assessment of aerobic stability, silage treated with *L. plantarum*, *P. pentosaceus*, and *P. freudenreichii* plus hydrolytic enzymes (D) was the only silage that exhibited signs of aerobic deterioration as evidenced by the elevation of its temperature by more than 2°C over ambient temperature ($21 \pm 2.5^{\circ}\text{C}$). This silage also exhibited the highest pH and ADIN content of the silages examined after aerobic exposure. Relatively acid-tolerant, lactate-utilizing yeasts that grow in silage upon exposure to air are known to be primarily involved in aerobic deterioration of silage, which is accompanied by chemical changes, rising temperature, and DM loss (Woolford, 1990; Muck and Pitt, 1993; Bolsen et al., 1996). Although yeast counts at the end of 14-day period are similar among silages treated with homofermentative LAB-containing inoculants, it should be noted that the population of yeasts present upon exposure to air, especially lactate-utilizers, dictates the vulnerability of silage to aerobic deterioration (Woolford, 1990; Inglis et al., 1999). Yeast counts were lowest by far in silages treated with *L. buchneri*. This is probably related to the higher acetic acid content which serves as a fungal inhibitor in these silages (Ranjit and Kung, 2000; Kung and Ranjit, 2001; Taylor et al., 2002). In this study, decomposition of lactate occurred in all the silages to some extent, but it was most dramatic in silage treated with *L. plantarum*, *P. pentosaceus*, and *P. freudenreichii* plus hydrolytic enzymes (D) followed by silage treated with *L. plantarum* (A). These were the two silages in which the highest temperatures were recorded, especially (D). Susceptibility to aerobic exposure was also associated with those silages that contained a higher WSC content. Ranjit and Kung (2000) attributed the

disappearance of readily available nutrients such as lactate and residual WSC to the proliferation of yeasts.

Ammonia-N content in silage D was numerically higher than in the other silages. Proteolysis in silages exposed to air has been attributed to the activity of some bacterial species (Woolford, 1990). Bacteria are known to play a secondary role in aerobic deterioration of whole-crop cereal silages (Woolford, 1990; Inglis et al., 1999). The fact that mold populations were higher in C does indicate that mold population can proliferate without an initial increase in yeast populations. We do not have any explanation for the high population of molds in silage C, given that no concurrent increase in temperature or pH of the silage during aerobic exposure was observed. Typically, molds proliferate after pH has increased and lactic acid concentration has decreased as a result of yeast activity (McAllister et al., 1995; Inglis et al., 1999).

In conclusion, the commercial inoculants used in this study offered only marginal improvements in the fermentation of barley silage as compared to control silage. Although inoculants containing *L. buchneri* improved some indices of aerobic stability during the 14-day aerobic exposure period, the control silage also appeared to be stable for the period that silage would normally be exposed to air under typical production conditions. Considering the adequate WSC content of the barley and its optimal stage of maturity at harvest in this study, together with the uniform packing and high degree of oxygen exclusion that can be obtained in mini-silos, ensiling conditions may have been such that the benefits of inoculants that may occur under commercial ensiling conditions were not apparent.

ACKNOWLEDGEMENTS

The authors wish to thank W. Smart, Z. Xu and Z. Matic for their technical assistance throughout the experiment. This is LRC Contribution no. 38705060.

REFERENCES

- Bolsen, K. K., G. Ashbell and Z. Weinberg. 1996. Silage fermentation and silage additives-Review. Asian-Aust. J. Anim. Sci. 9:483-493.
- Buchanan-Smith, J. G. 1990. An investigation into palatability as a factor responsible for reduced intake of silage by sheep. Anim. Prod. 50:253-260.
- Driehuis, F., S. J. W. H. Oude Elferink and P. G. Van Wikselaar. 1999. *Lactobacillus buchneri* improves the aerobic stability of laboratory and farm scale whole crop maize silage but does not affect feed intake and milk production of dairy cows. In: Proceedings of the 12th International Silage Conference, Uppsala, Sweden. pp. 264-265.
- Filya, I., G. Ashbell, Y. Hen and Z. G. Weinberg. 2000. The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage. Anim. Feed Sci. Technol. 88:39-46.
- Filya, I. 2003a. The effect of *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria, on the fermentation, aerobic stability and ruminal degradability of wheat, sorghum and maize silages. J. Appl. Microbiol. 95:1080-1086.
- Filya, I. 2003b. The effect of *Lactobacillus buchneri* and *Lactobacillus plantarum* on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. J. Dairy Sci. 86:3575-3581.
- Ham, J. S., H. S. Kim, K. H. Hong, J. G. Kim, S. J. Jeong, H. S. Chae, J. N. Ahn, D. K. Kang and H. U. Kim. 2003. Inhibitory activity of lactic acid bacteria against hazardous microbes. Asian-Aust. J. Anim. Sci. 16:1550-1554.
- Henderson, N. 1993. Silage additives. Anim. Feed Sci. Technol. 45:35-56.
- Higginbotham, G. E., S. C. Mueller, K. K. Bolsen and E. J. DePeters. 1998. Effects of inoculants containing propionic acid bacteria on fermentation and aerobic stability of corn silage. J. Dairy Sci. 81:2185-2192.
- Hill, H. A. and J. E. Hill. 1986. The value of plasmid profiling in monitoring *Lactobacillus plantarum* in silage fermentations. Curr. Microbiol. 13:91-94.
- Holzer, M., E. Mayrhuber, H. Danner and R. Braun. 2003. The role of *Lactobacillus buchneri* in forage preservation. Trends Biotechnol. 21:282-287.
- Inglis, G. D., L. J. Yanke, L. M. Kawchuk and T. A. McAllister. 1999. The influence of bacterial inoculants on the microbial ecology of aerobic spoilage of barley silage. Can. J. Microbiol. 45:77-87.
- Jones, G. M., R. E. Larsen and N. M. Lanning. 1980. Prediction of silage digestibility and intake by chemical analyses or *in vitro* fermentation techniques. J. Dairy Sci. 63:579-586.
- Kung, L. Jr. and N. K. Ranjit. 2001. The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. J. Dairy Sci. 84:1149-1155.
- Mahanna, W. C. 1993. Silage fermentation and additive use in North America. In: Silage Production from Seed to Animal. Proceedings of the National Silage Production Conference, Syracuse, New York. pp. 85-95.
- McAllister, T. A., L. B. Selinger, L. R. McMahan, H. D. Bae, T. J. Lysyk, S. J. Oosting and K.-J. Cheng. 1995. Intake, digestibility and aerobic stability of barley silage inoculated with mixtures of *Lactobacillus plantarum* and *Enterococcus faecium*. Can. J. Anim. Sci. 75:425-432.
- McAllister, T. A., R. Feniuk, Z. Mir, P. Mir, L. B. Selinger and K.-J. Cheng. 1998. Inoculants for alfalfa silage: Effects on aerobic stability, digestibility, and the growth performance of feedlot steers. Livest. Prod. Sci. 53:171-181.
- McCartney, D. H. and A. S. Vaage. 1994. Comparative yield and feeding value of barley, oat and triticale silages. Can. J. Anim. Sci. 74: 91-96.
- McDonald, P., A. R. Henderson and S. J. E. Heron. 1991. The Biochemistry of Silage. 2nd edn. Chalcombe Publications, Marlow, Bucks, England. pp. 340.
- Moshtaghi Nia, S. A. and K. M. Wittenberg. 1999. Use of forage inoculants with or without enzymes to improve preservation and quality of whole crop barley forage ensiled as large bales. Can. J. Anim. Sci. 79:525-532.

- Muck, R. E. 1993. The role of silage additives in making high quality silage. In: Silage Production from Seed to Animal. Proceedings of the National Silage Production Conference, Syracuse, New York. pp. 106-116.
- Muck, R. E. and R. E. Pitt. 1993. Ensiling and its effect on crop quality. In: Silage Production from Seed to Animal. Proceedings of the National Silage Production Conference, Syracuse, New York. pp. 57-66.
- Muck, R. E. and L. Kung, Jr. 1997. Effects of silage additives on ensiling. In: Silage: Field to Feedbunk, Proceedings of the North American Conference, Northeast Registered Agricultural Engineering Services Cooperative Extension, Hershey, Pennsylvania. pp. 187-199.
- Oude Elferink, S. J. W. H., J. Krooneman, J. C. Gottschal, S. F. Spoelstra, F. Faber and F. Driehuis. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. Appl. Environ. Microbiol. 67:125-132.
- Pahlow, G. 1991. The role of microflora in forage conservation. In: Forage Conservation Towards 2000-Proceedings of the Conference (Ed. G. Pahlow and H. Honig). Braunschweig: Landbauforschung Volkenrode, FAL, Sonderheft 123. pp. 26-36.
- Ranjit, N. K. and L. Kung, Jr. 2000. The effect of *Lactobacillus buchneri*, *Lactobacillus plantarum*, or a chemical preservative on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 83:526-535.
- SAS. 1991. SAS/STAT User's Guide (version 6). SAS Inst., Inc., Cary, NC, USA.
- Shao, T., M. Shimojo, T. Wang and Y. Masuda. 2005. Effect of additives on the fermentation quality and residual mono- and di-saccharides compositions of forage oats (*Avena sativa* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) silages. Asian-Aust. J. Anim. Sci. 18:1582-1588.
- Taylor, C. C., N. J. Ranjit, J. A. Mills, J. M. Neylon and L. Kung, Jr. 2002. The effect of treating whole-plant barley with *Lactobacillus buchneri* 40788 on silage fermentation, aerobic stability, and nutritive value for dairy cows. J. Dairy Sci. 85:1793-1800.
- Weinberg, Z. G., G. Ashbell, A. Azrieli and I. Brukental. 1993. Ensiling peas, ryegrass and wheat additives of lactic acid bacteria (LAB) and cell wall degrading enzymes. Grass Forage Sci. 48:70-78.
- Weinberg, Z. G., G. Ashbell, Y. Hen and A. Azrieli. 1993. The effect of applying lactic acid bacteria on the aerobic stability of silages. J. Appl. Bacteriol. 75:512-518.
- Weinberg, Z. G., G. Ashbell, K. K. Bolson, G. Pahlow, Y. Hen and A. Azrieli. 1995a. The effect of a propionic acid bacterial inoculant applied at ensiling, with or without lactic acid bacteria, on the aerobic stability of pearl millet and maize silages. J. Appl. Bacteriol. 78:430-436.
- Weinberg, Z. G., G. Ashbell, H. Yaira and A. Azrieli. 1995b. The effect of cellulase and hemicellulase plus pectinase on the aerobic stability and fibre analysis of peas and wheat silages. Anim. Feed Sci. Technol. 55:287-293.
- Weinberg, Z. G. and R. E. Muck. 1996. New trends and opportunities in the development and use of inoculants for silage. FEMS Microbiol. Rev. 19:53-68.
- Weinberg, Z. G., G. Szakacs, G. Ashbell and Y. Hen. 1998. The effect of temperature and *Lactobacillus amylovorus* and *L. plantarum*, applied at ensiling, on wheat silage. J. Appl. Microbiol. 84:404-408.
- Woolford, M. K. 1975. Microbiological screening of the straight chain fatty acids (C₁-C₁₂) as potential silage additives. J. Sci. Food Agric. 26:219-228.
- Woolford, M. K. 1984. The Silage Fermentation. Marcel Dekker, Inc., New York, NY.
- Woolford, M. K. 1990. The detrimental effects of air on silage. J. Appl. Bacteriol. 68:101-116.
- Yahaya, M. S., M. Goto, W. Yimiti, B. Smerjai and Y. Kawamoto. 2004. Evaluation of fermentation quality of a tropical and temperate forage crops ensiled with additives of fermented juice of epiphytic lactic acid bacteria (FJLB). Asian-Aust. J. Anim. Sci. 17:942-946.
- Zahiroddini, H., J. Baah and T. A. McAllister. 2004. Effect of an inoculant and hydrolytic enzymes on fermentation and nutritive value of whole crop barley silage. Anim. Feed Sci. Technol. 117:317-330.