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Effects of Homolactic Bacterial Inoculant Alone or Combined with an Anionic Surfactant on Fermentation, Aerobic Stability and *In situ* Ruminal Degradability of Barley Silage

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ABSTRACT : The effect of a homolactic inoculant containing a blend of *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Enterococcus faecium* or, the anionic surfactant, sodium dodecyl sulphate (SDS), alone or in combination on fermentation characteristics, aerobic stability and *in situ* DM, OM and NDF degradability of barley silage was investigated. Barley (*Hordeum vulgare*, L.) was harvested (45% DM), chopped and treated with water at 24 ml/kg forage (Control), inoculant at 1.09×10^5 cfu/g forage (I), SDS at 0.125% (wt/wt) of forage (S) or with the inoculant (1.09×10^5 cfu/g) plus SDS (0.125% wt/wt; I+S). The treated forages were ensiled in triplicate mini silos and opened for chemical and microbiological analyses on d 1, 2, 3, 7, 14, 42 and 77. Silage samples from d 77 were opened and aerobically exposed for 7 d. The *in situ* rumen degradability characteristics of silage DM, OM and NDF were also determined. The terminal concentration of NDF in S and I+S was lower ($p<0.001$) than in other treatments. Lactate concentration was higher ($p<0.001$) and the rate and extent of pH decline were greater ($p<0.001$) in I and I+S than S and Control silages. A homolactic pathway of fermentation in I and I+S was evidenced by reduced ($p<0.001$) water-soluble carbohydrates concentration, higher lactate ($p<0.01$), lower acetate ($p<0.01$) and lower pH values ($p<0.001$) than in S and Control silages. All silages remained stable over 7 d of exposure to air as indicated by lower temperatures and moulds, and by non-detectable yeast populations. The treated silages had lower DM and OM degradability than in the Control but NDF degradation characteristics of I+S were improved compared to other treatments. It is concluded that the inoculant alone improved the fermentation characteristics whereas the combination of the inoculant with SDS improved both fermentation and NDF degradability of barley silage. (**Key Words :** Aerobic Stability, Barley Silage, SDS, Homolactic Inoculant, Ruminal Degradability)

INTRODUCTION

The use of homolactic bacterial inoculants as silage additives is based on their ability to efficiently convert water-soluble carbohydrates (WSC) into lactic acid which rapidly lowers the pH and preserves the silage. Inoculants that possess this characteristic usually contain individual or mixed strains of *Lactobacillus plantarum*, *Pediococcus acidilactici* or *Enterococcus faecium* (Weinberg and Muck, 1996; Muck, 2004; Sucu and Filya, 2006). However, the effect of homolactic bacterial inoculants on the subsequent aerobic stability of silages upon feed out has been far more variable (Muck, 2004; Zahiroddini et al., 2006) and as a result *Lactobacillus buchneri,* a strain that produces acetic acid has been included in many inoculants (Muck, 2004; Reich and Kung, 2010).

Efficacy of silage inoculants depends to some extent on their uniform distribution over the forage during the ensiling process as the bacteria they contain have limited motility (McAllister and Hristov, 1999). Additives that could improve the distribution and promote adhesion of inoculated bacteria, such as surfactants, may improve the efficacy of inoculants. Surfactant molecules accumulate at liquid-solid and liquid-liquid interfaces and reduce interfacial tensions thereby increasing moisture penetration, distribution and interactions among surfaces. Although many microorganisms produce biosurfactants to facilitate their motility, access to substrates and to promote colonization and establishment of new communities (van Hamme et al., 2006; Rahman and Gakpe, 2008), studies to examine these properties in lactate-producing bacteria

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(LAB) have not been undertaken. The addition of chemical surfactants such as SDS to silage to mimic the roles of biosurfactants may improve the distribution of inoculated bacteria on ensiled forage and thereby enhance the fermentation process.

Numerous studies have examined the effects of Tween 80, a non-ionic surfactant on *in situ* (Lee et al., 2004; Baah et al., 2005; Lee et al., 2007) and *in vivo* (McAllister et al., 2000) digestion in ruminants but only a few studies have assessed the effects of SDS on silage fermentation (Kamande, 1994) or digestibility (Lee et al., 2007). Kamande (1994) reported that treatment of orchard grass silage with SDS did not improve fermentation characteristics, but Pauly and Lingvall (1999) observed improvements in fermentation characteristics of grass silage treated with non-ionic and zwitterionic surfactants in combination with an inoculant containing *L. plantarum* and *P. acidilactici*.

The objective of this study was to investigate the effects of a homolactic inoculant and SDS on the fermentation, aerobic stability and *in situ* rumen degradability characteristics of barley silage.

MATERIALS AND METHODS

Silage preparation and sampling

Whole-crop barley forage (*Hordeum vulgare*, L.) was harvested at the mid-dough stage of maturity (45% DM) and chopped to approximately 10 mm with a John Deere 6,610 forage harvester. The chopped forage was divided into twelve 24-kg lots (3 lots per treatment), spread out on separate clean plastic sheets and treated as follows: i) Inoculated with a mixture of *L. plantarum*, *E. faecium* and *P. acidilactici* (SIL-ALL® Alltech Inc., Canada) to obtain 1.0×10^5 cfu of LAB per g of fresh forage and designated as treatment I. ii) Treated with SDS at 0.125% (wt/wt) and designated as treatment S. The SDS powder was evenly distributed over each lot of forage prior to spraying it with 100 ml of water. iii) The third treatment consisted of first treating the forage with the inoculant as in I above followed by treatment with 0.125% (wt/wt) SDS. This treatment was designated as I+S. The inoculant (0.24 g) was suspended in 100 ml of water and sprayed onto each lot of forage in all treatments. The Control forage was ensiled without any additives, but each 24 kg lot was sprayed with 100 ml of water prior to ensiling. All treated forages were uniformly mixed to ensure even distribution of additives. Prior to ensiling, forage samples were randomly collected unto ice and immediately sent to the laboratory for chemical and microbial analyses.

Triplicate mini silos (10.4 cm in diameter and 35.5 cm in height) prepared for each opening day for each treatment (21 silos per treatment) were immediately filled with approximately 2.5 kg of treated forage, compressed to a density of about 240 kg/m^3 using a hydraulic press and capped. Each 24-kg lot of treated forage was used to fill one of three sets of replicate silos (1 to 3) per treatment and the silos (7 silos) in each set were randomly filled.

All silos with their caps (84) were weighed before and immediately after being filled for estimation of DM loss. Silos were stored at ambient temperature (20 to 22°C) and opened after 1, 2, 3, 7, 14, 42 and 77 d of ensiling. The contents of each opened silo were thoroughly mixed and samples were collected for chemical and microbial analyses.

Aerobic stability

After opening the d 77 silos, the contents of each silo were thoroughly mixed and 1 kg of silage samples were transferred into separate 1-L containers (3 containers per treatment). Each container was embedded with two Thermochron iButtons (Embedded Data Systems, Lawrenceburg, KY) in the lower and mid layers of the silage mass to record the temperature every 15 min. The containers were each covered with a double layer of cheesecloth and stored at ambient temperature (20 to 22°C) for 7 d. Ambient temperature was also simultaneously measured at 15 min intervals during this period. Silage was sampled from each container after 1, 3 and 7 d of aerobic exposure for chemical and microbial analysis, and for measurement of pH.

Chemical and microbial analyses

Forage and silage samples were processed and analysed for lactate, WSC, volatile fatty acids, ammonia nitrogen (NH_3-N) and starch as described by Zahiroddini et al. (2004). 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 N×6·25. The DM of the fresh forages and silages was determined by oven drying at 105°C for 24 h. Organic matter was estimated by ashing 1 g of sample in a muffle furnace at 550°C for 5 h. Neutral detergent fibre was analyzed with the addition of sodium sulphite and α-amylase while ADF was analyzed with α-amylase omitted from the procedure, using the Ankom 200® system (Ankom Technology Corporation, Fairport, NY, USA). Nitrogen in ADF residues (ADIN) was measured as described above.

Microbiological evaluation of silages included enumeration of total culturable bacteria, lactobacilli, yeasts and moulds according to protocols described by Zahiroddini et al. (2004).

In situ study

Three ruminally-fistulated Angus-Charolais crossbred cows were adapted to a diet (DM basis) consisting of 60% barley silage, 35% steamed rolled barley grain and 5% supplement for 14 d. Frozen replicate silage samples of each treatment from the final day of ensiling were lyophilized, ground through a 4-mm screen, thoroughly mixed and weighed (5 g per bag) into triplicate polyester bags for each incubation time point. Bags were incubated in the rumen for 1, 2, 4, 8, 16, 24, 48 and 72 h. The bags were retrieved, washed and kinetic parameters of *in situ* degradability of DM (DMD), OM (OMD) and NDF (NDFD) determined by methods previously described by Zahiroddini et al. (2004). The cows were cared for according to the guidelines of the Canadian Council on Animal Care (1993).

Statistical analysis

Data were analyzed by analysis of variance using the PROC MIXED procedure of SAS (2006). The data on microbial populations were transformed to $\log_{10}$ cfu/g of DM of forage or silage prior to statistical analysis except for aerobic stability studies which were expressed on a fresh wt basis.

Silage fermentation characteristics and aerobic stability data were analyzed for the fixed effects of treatments at each time point of ensiling or aerobic exposure as a completely randomized design. Parameters for DM, OM and NDF degradation were generated using non-linear regression (PROC NLIN) of SAS and were analyzed as a completely randomized block design for the fixed effects of treatment with cow as the random (block) variable. Differences among treatments at each time point of ensiling, aerobic exposure and ruminal incubation were tested and compared using the PDIFF option of LSMEANS. Least square means that showed significant differences were separated by a pair-wise *t*-test at $p \le 0.05$.

RESULTS

Fermentation characteristics

The chemical and microbial compositions of the fresh and ensiled whole-crop barley are given in Table 1. Dry matter, OM ADF, starch and CP were similar among treatments but the NDF concentrations of S and I+S silages were lower ($p<0.001$) than those of Control and I.

Silages containing the inoculant (I and I+S) had higher ($p<0.01$) lactate concentrations, and lower acetate ($p<0.01$), WSC ($p<0.001$) and pH ($p<0.001$) values than Control or S silages. The lactate:acetate ratio in I and I+S (1:8) was also 50% higher than either Control or S silages (1:4). The rate and extent of pH decline were greater ($p<0.001$) in I and I+S than in Control and S silages (Figure 1). There were no differences ($p>0.05$) in fermentation characteristics between Control and S silages, and between I and I+S. Propionate concentration in I was lower ($p<0.05$) than Control and S but similar to I+S. Butyrate and NH_3-N were present in low concentrations (≤0.07 and ≤0.23 mg/g DM respectively) in all silages and did not differ among treatments.

Moulds and yeasts were non-detectable (dilution 10^{-1}) while LAB and total culturable bacteria populations peaked to 9 $\log_{10}$ cfu/g DM in all silages as early as 7 d after ensiling (data not shown). In the terminal silages, LAB ($p = 0.230$) and total culturable bacteria ($p = 0.229$) populations were similar among all treatments.

Aerobic stability

Chemical and microbial compositions of silages after 7 d of aerobic exposure are presented in Table 2. All silages remained stable over the 7 d of aerobic exposure as indicated by low silage temperatures (Figure 2) and low mould and non-detectable yeasts populations. Butyrate and ethanol levels in all silages exposed to air were generally low, however, the I silage had 59% and 68% lower ($p<0.05$) ethanol levels than Control and I+S respectively. The pH in I and I+S was lower ($p<0.01$) than Control and S however, the acetate concentration in the Control was higher ($p<0.05$) than I and I+S but similar to S throughout the 7 d of aerobic exposure. Residual WSC was lower ($p<0.05$) and lactate tended ($p = 0.095$) to be higher in both I and I+S than in S and Control silages after aerobic exposure.

In situ DM, OM and NDF degradation

The patterns of *in situ* ruminal DMD and OMD during 72 h of incubation are illustrated in Figure 3. The DMD of the Control silage was higher ($p<0.05$) than the treated silages except at 1, 2 and 72 h of incubation, where it did not differ from S ($p = 0.09$). Control and S silages had similar ($p = 0.522$) DMD at 48 h of incubation. A similar pattern of degradation was observed for OM except that the Control silage had higher OMD at 1 and 2 h of incubation as well. Figure 4 shows the pattern of *in situ* ruminal NDFD during 72 h of incubation. Degradability of NDF was higher ($p<0.05$) for I+S than for other silages throughout the ruminal incubation period. However, the exponential model for NDFD in all silages and particularly in I+S, did not reach their asymptotes during the 72 h of incubation (Figure 4).

The DM, OM and NDF degradation characteristics of silages are shown in Table 3. The rapidly degradable fraction (*a*) of silage DM was higher ($p<0.05$) in the Control than in I and I+S silages but similar ($p = 0.115$) to that in the S silage. The rapidly degradable OM fraction (*a*) of the Control silage was higher ($p<0.001$) than those of all

Table 1. Composition of whole-crop barley forage at harvest and 77 days after treatment and ensilage

Item	Forage at harvest	Treatment[1]				SEM[2]	p
		Control	I	S	I+S		
pH	6.16±0.03	4.51[a]	3.95[b]	4.50[a]	3.99[b]	0.22	<0.001
pH decline (unit/d)[3]	-	0.075[b]	0.142[a]	0.067[b]	0.128[a]	0.006	<0.001
DM (%)	45.28±0.75	42.87	43.12	43.85	44.30	0.755	0.549
Dry matter loss (%)	-	7.89	7.64	6.31	7.76	0.659	0.364
Chemical analyses (% DM basis)							
Organic matter	93.76±0.15	93.20	93.29	93.27	93.37	0.171	0.921
NDF	40.78±0.70	40.92[a]	42.08[a]	36.62[b]	36.92[b]	0.649	<0.001
ADF	20.03±0.28	22.25	22.14	22.03	21.26	0.419	0.358
Starch	23.29±0.75	25.94	26.49	26.71	25.83	1.111	0.929
Crude protein	13.68±0.12	14.26	14.13	14.24	14.23	0.059	0.422
WSC (mg/g)[4]	54.65±3.39	63.08[a]	38.08[b]	57.50[a]	37.57[b]	2.919	0.001
ADIN (% N)[5]	2.25±0.22	2.80	3.39	3.49	2.70	0.276	0.149
End products of fermentation (g/kg DM silage)							
Lactate	0.00	48.26[b]	81.17[a]	43.37[b]	83.59[a]	3.648	<0.001
Acetate	0.25±0.21	13.85[a]	9.81[b]	11.86[a]	9.87[b]	0.636	0.006
Propionate	1.64±0.16	2.38[a]	1.91[b]	2.27[ac]	1.96[bc]	0.105	0.034
Butyrate	0.07±0.02	0.07	0.07	0.06	0.06	0.016	0.927
Succinate	0.92±0.02	5.27	3.70	4.96	4.39	0.535	0.250
Lactate:acetate	-	3.50[b]	8.27[a]	3.67[b]	8.30[a]	0.407	<0.001
Ethanol (mg/g)	0.86±0.31	4.71	4.83	4.18	4.71	0.432	0.726
NH_3 N (mg/g)	0.09±0.08	0.22	0.22	0.23	0.22	0.004	0.247
Microbiology (Log_{10} cfu/g DM silage)							
Total culturable bacteria	7.36±0.10	8.19	7.06	7.31	7.25	0.379	0.229
Lactate bacteria	6.07±0.19	8.17	7.16	7.29	7.27	0.351	0.230
Moulds	4.79±0.10	0.00	1.08	0.63	0.62	0.700	0.754
Yeasts	5.3±0.14	0.00	0.00	0.00	0.00	-	-

[1] Control (treated with water; 0.24 kg/100 ml); I = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage); S = 0.125% (wt/wt) SDS; I+S = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage) plus 0.125% (wt/wt) SDS.
[2] Standard error of least square means. [3] The rate of pH decline was estimated for the first 14 d of ensiling.
[4] WSC = Water-soluble carbohydrates. [5] ADIN = Acid detergent insoluble N; expressed as percent of total nitrogen.
[a, b, c] Least square means with different superscripts differ significantly (p≤0.05).

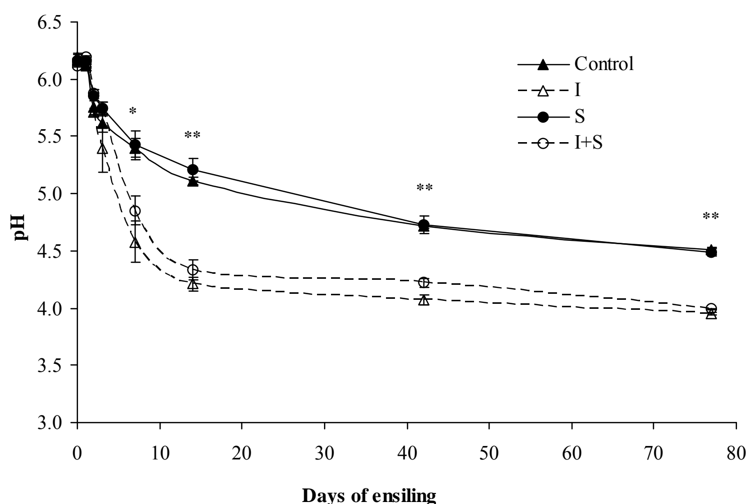

Figure 1. Changes in pH during ensilage of whole-crop barley. Control (treated with water; 0.24 kg/100 ml); I = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage); S = 0.125% (wt/wt) SDS; I+S = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage) plus 0.125% (wt/wt) SDS. Bars, where visible, indicate standard error of least square means. Asterisks indicate days on which I and I+S had lower (* $p<0.05$; ** $p<0.001$) pH than Control and S silages.

Table 2. Composition (fresh basis) of whole-crop barley silage after 7 days of aerobic exposure

Item	Treatments[1]				SEM[2]	p
	Control	I	S	I+S		
pH	4.52[a]	3.99[b]	4.53[a]	4.00[b]	0.021	<0.001
WSC (mg/g)	18.76[a]	10.37[b]	18.22[a]	10.16[b]	1.831	0.0139
Products of Fermentation (g/kg silage)						
Lactate	13.92	23.60	18.80	22.03	2.469	0.095
Acetate	6.00[a]	3.71[b]	5.49[ab]	4.39[b]	0.455	0.028
Propionate	1.15	1.01	1.01	0.98	0.085	0.517
Butyrate	0.04	0.05	0.04	0.03	0.011	0.585
Ethanol (mg/g)	0.54[a]	0.34[b]	0.47[ab]	0.57[a]	0.051	0.048
Microbiology (Log_{10} cfu/g silage)						
Total culturable bacteria	7.43[a]	6.08[b]	7.46[a]	6.23[b]	0.170	0.001
Lactate producing bacteria	7.49[a]	6.04[b]	7.43[a]	6.17[b]	0.196	0.001
Moulds	2.61	2.57	2.95	3.48	1.191	0.942
Yeasts	0.00	0.00	0.00	0.00	-	-

[1] Control (treated with water; 0.24 kg/100 ml); I = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage); S = 0.125% (wt/wt) SDS; I+S = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage) plus 0.125% (wt/wt) SDS.

[2] Standard error of least square means.

[a, b] Least square means with different superscripts differ significantly ($p\leq0.05$).

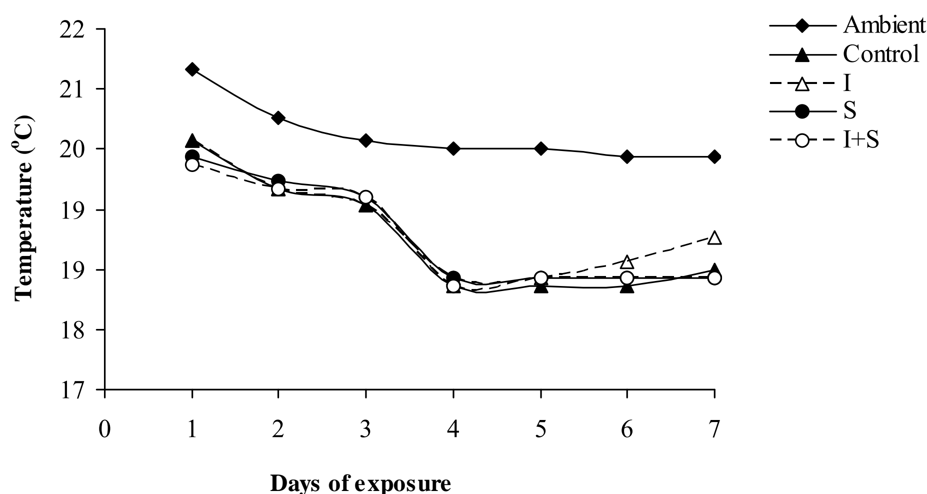

Figure 2. Changes in whole-crop barley silage temperature during 7 days of aerobic exposure. Control (treated with water; 0.24 kg/100 ml); I = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage); S = 0.125% (wt/wt) SDS; I+S = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage) plus 0.125% (wt/wt) SDS.

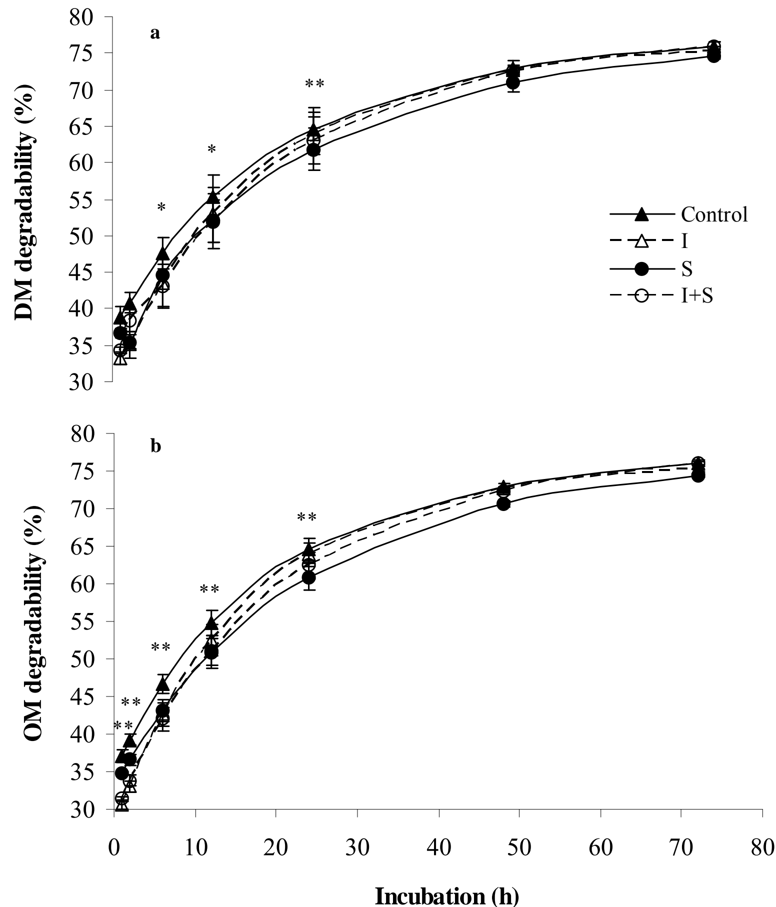

Figure 3. Pattern of *in situ* DM (a) and OM (b) degradabilities of whole-crop barley silages incubated in cows. Control (treated with water; 0.24 kg/100 ml); I = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage); S = 0.125% (wt/wt) SDS; I+S = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage) plus 0.125% (wt/wt) SDS. Bars, where visible indicate standard error of least square means. Asterisks indicate days on which the Control silage had higher (* $p<0.05$; ** $p<0.001$) DMD (a) or OMD (b) than in S, I and I+S silages.

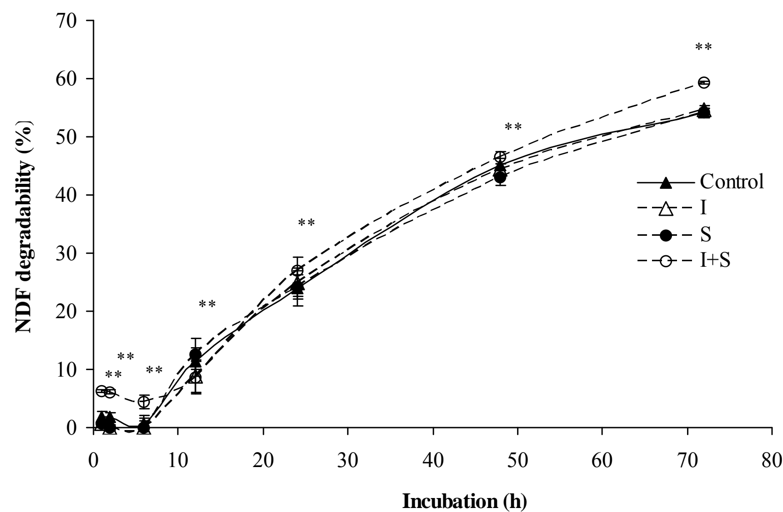

Figure 4. Pattern of NDF degradability of whole-crop barley silages incubated in cows. Control (treated with water; 0.24 kg/100 ml); I = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage); S = 0.125% (wt/wt) SDS; I+S = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage) plus 0.125% (wt/wt) SDS. Bars, where visible indicate standard error of least square means. Asterisks indicate days on which I+S had higher (** $p<0.001$) NDFD than in the Control, S and I silages.

Table 3. *In situ* DM and OM degradability characteristics of treated and untreated whole-crop barley silages

Item[3]	Treatment[1]				SEM[2]	p
	Control	I	S	I+S		
DM						
a	37.79[a]	33.06[b]	34.12[ab]	35.54[b]	1.100	0.035
b	40.47	43.56	41.84	43.92	2.257	0.056
c (h)	0.053[bc]	0.062[a]	0.046[c]	0.055[ab]	0.016	0.017
Lag (h)	0.33	1.38	0.31	1.78	0.450	0.135
Effective degradability[4]	55.18[a]	52.48[b]	52.44[b]	52.06[b]	2.314	0.006
OM						
a	35.96[a]	29.07[c]	33.72[b]	30.17[c]	1.275	<0.001
b	42.21[c]	47.83[a]	43.73[b]	48.51[a]	2.777	<0.001
c (h)	0.056[b]	0.064[a]	0.046[c]	0.054[b]	0.017	0.017
Lag (h)	0.33[a]	0.33[a]	0.31[b]	0.32[b]	0.323	0.006
Effective degradability[4]	54.48[a]	51.78[b]	51.06[c]	51.24[c]	2.422	<0.001

[1] Control (treated with water; 0.24 kg/100 ml); I = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage); S = 0.125% (wt/wt) SDS; I+S = homolactic inoculant (1.09×10^5 cfu of LAB per g of fresh forage) plus 0.125% (wt/wt) SDS.
[2] Standard error of least square means.
[3] Parameters from the fitted equation $p = a+b(1-e^{-c(t-lag)})$ for t>lag; where p is the proportion (%) of DM or OM disappearing from nylon bags after t hours of incubation; a is the rapidly degradable fraction (%); b is the slowly degradable fraction (%); c is the fractional rate of degradability (/h) of fraction b.
[4] Effective degradability calculated at ruminal particulate outflow rate of 5%/h (Zahiroddini et al., 2004).
[a,b,c, d] Least square means with different superscripts differ significantly (p≤0.05).

treated silages. The proportion of OM that degraded slowly in the rumen (fraction *b*) was however, higher (p<0.001) in I and I+S than in the Control. The slowly degradable DM fraction (*b*) of I and I+S silages tended (p = 0.056) to be higher in the treated compared to the Control silage. The lag time for degradation of OM in S and I+S was also shorter (p<0.01) than in Control and S silages. The fractional rates (*c*) of OM and DM degradation were faster (p<0.05) in I than in the other silages.

The effective NDFD of I+S (18.9%) was higher (p<0.001) than those of Control (15.4%), S (14.3%) and I (13.2%) silages. The proportion of slowly degradable NDF was 73.1, 72.9 and 75.4% in I, S and I+S respectively. These were higher (p<0.05) than that of the Control (66.5%). The fractional rate of NDFD in the Control (0.04%/h) was not different (p = 0.056) from I (0.03%/h) but both were higher (p<0.05) than in S (0.02%/h) and I+S (0.02%/h). The lag time for NDF degradation was shorter (p<0.001) in S (0.5 h) than in Control (2.5 h), I (2.7 h) and I+S (2.4 h).

DISCUSSION

Silages containing SDS had lower NDF concentration compared to Control and I. This may be explained by the solubilization of fibre-bound proteins by SDS during ensiling and prior to NDF analysis, that would otherwise have been retained as NDF given that extraction of such proteins during refluxing with NDF solution, which also contain mainly SDS, can be incomplete (van Soest, 1982; Jung, 1997). Hydrolysis of recalcitrant proteins in SDS treated silages thus appeared to have rendered such components more susceptible to further extraction by the NDF solution during NDF analysis.

Homolactic bacterial inoculants are added to silage mainly to induce lactate fermentation thereby accelerating a rapid pH decline which preserves the silage. Treatment of silage with the homolactic inoculant alone or in combination with the surfactant ensured a rapid and lower extent of pH decline, higher lactate concentration, lower concentrations of acetate and propionate, and higher lactate:acetate ratio. All of these are indicative of efficient and favourable homolactic fermentation. While it took 14 d to attain pH of approximately 4.3 in I and I+S, 42 d were required to reach a pH of 4.7 in both Control and S treatments. The efficiency of conversion of WSC to lactate was thus higher in I and I+S than Control and S as the terminal WSC:lactate ratio was 1:2 in I and I+S as compared to 1:1 in Control and S. The combination of two or more lactate-producing bacteria in a single inoculant is intended to promote a synergy among the microbes that accelerates pH decline as conditions for the growth of at least one of the LAB species are favorable throughout ensiling. For example, *P. acidilactici* and some species of *Enterococcus* utilize a wide range of pentoses and hexoses as their substrates, are fast growing and initiate fermentation at the early stages of ensiling when oxygen may still be associated with the forage and pH remains high (i.e., >5). In contrast, *L. plantarum* is acido-psychrophilic, utilizes primarily hexoses as its substrate, but is slow growing, dominating the fermentation process at the latter stage of fermentation (Fitzsimons et al., 1992; Cai, 1999). This synergistic phenomenon may explain the rapid decline in pH of silages containing the inoculant (I and I+S) in the present study. Many studies have shown the capacity of

homolactic inoculants to improve barley silage fermentation (Moshtaghi Nia and Wittenberg, 1999; Hristov and McAllister, 2002; Zahiroddini et al., 2004) although in other studies some inoculants have failed to enhance the fermentation of barley silage to any appreciable level (Zahiroddini et al., 2006). Discrepancies in response of forages to the same inoculant may be explained by differences in forage composition and/or silo types (Keady et al., 1994; Weinberg and Muck, 1996; McAllister et al., 1998; Zahiroddini et al., 2006).

In liquid-solid media, surfactants reduce interfacial tensions by accumulating at the interface and causing wetting and distribution of the liquid, and promoting pseudo-solubility of solids (van Hamme et al., 2006). This can potentially promote the attachment of bacteria to their substrates and thereby improve the fermentation process. However, the favourable ensiling characteristics observed in I+S were not observed in S, suggesting that SDS did not enhance the ability of epiphytic bacteria to contribute to favourable ensiling properties. Other studies have also shown that treatment of silages with surfactants alone, irrespective of the type, did not improve fermentation characteristics. Treatment of grass silage (10 ml/kg) with a combination of Metarin F (0.2 g/L), a zwitterionic surfactant and glyceryl-polyeththylene-glycol ricineoleate (0.01 g/L), a non-ionic surfactant failed to improve fermentation characteristics but combining the surfactants with a homolactic inoculant containing *L. plantarum* and *P. acidilactici* resulted in improved fermentation characteristics (Pauly and Lingvall, 1999). Consistent with the present study, a 0.3% treatment of orchard grass silage with SDS did not improve fermentation characteristics compared to the untreated (Kamande, 1994). These data suggest that the treatment of silage with only SDS is not likely to improve the fermentation characteristics of barley silage without exogenous LAB. The improved fermentation characteristics observed in I+S compared to S can be attributed to higher efficiency of lactate production by the exogenous LAB in I+S compared to the epiphytic LAB in S. Unlike S, it is possible the distribution of inoculated bacteria within the I+S forage was enhanced by the addition of SDS enabling LAB to utilize more WSC and produce higher levels of lactate, higher lactate:acetate ratio and lower WSC and propionate concentrations. Although there were no differences in the fermentation characteristics between I and I+S, the lactate concentration in I+S was 2.4 g/kg higher than in I.

The results of the current study clearly indicate that the homolactic inoculant did not compromise the aerobic stability of barley silage contrary to earlier reports in barley silage (Inglis et al., 1999; Kung et al., 2004) and other cereal silages (Kung et al., 2004; Muck, 2004; Sucu and Filya, 2005). It is worth noting, however, that apart from the lower pH and acetate concentration observed in I silage, the measurements (temperature, ethanol, yeasts and mould) which define aerobic stability of silage did not differ between the Control and other treated silages during the 7 d aerobic stability assessment.

In homolactic fermentation, there is usually an efficient conversion of WSC to lactate. This means less WSC will be utilized for fermentation, thereby preserving residual WSC while at the same time increasing lactate concentration. Residual WSC and lactate serve as substrates for yeasts and moulds when the silage is exposed to air. These organisms metabolize the lactate and/or WSC into carbon dioxide, water and heat, thereby increasing the temperature and pH of the silage. The treatment of silages with heterolactic inoculants such as *L. buchneri* is encouraged, because of the resistance of such silages to aerobic deterioration (Muck, 2004). However, the current study suggests that the aerobic stability of barley silage may be improved or unaffected by homolactic inoculants such as the one used in this study even if the silage had higher residual WSC and lactate concentrations. In our study, the concentration of propionate observed in all the silages was higher than those previously reported by other researchers (McAllister et al., 1995; Zahiroddini et al., 2006), but similar to those of Zahiroddini et al. (2004). This might have also played a role in the stability of the silages.

The combined effects of partial acidic fibre hydrolysis due to the inoculant and increased substrate-enzyme affinity due to the surfactant most likely accounted for the increased rumen and effective NDF degradabilities observed in I+S. This observation is probably due to slow partial acidic hydrolysis of NDF caused by an interaction between hydrogen ions and hemicelluloses in the silo prior to degradation in the rumen (Keady and Steen, 1994; Muck, 1996) given that most LAB contained in traditional commercial inoculants are not known to have fibrolytic activity. This has been confirmed by studies in which *in situ* ruminal NDF degradability of corn silage was increased compared to the unensiled crop (Monteils and Jurjanz, 2005). Surfactant treatment of forges may influence ruminal digestion in one or more of the following ways: increased substrate-enzyme affinity, microbial cell membrane permeability and enzyme release (Lee et al., 2004; Singh et al., 2006), reduced inactivation of enzymes bound to substrate (Kim et al., 2006) and modification of microbial community structure via selective growth inhibition or lyses (van Hamme et al., 2006). The shorter lag times for degradation of OM and NDF in silages containing the surfactant compared to those without in this study, further supports the hypothesis that SDS promoted wetting and pseudo-solubility of feed particles *in situ*.

The effects of surfactants alone or in combination with exogenous enzymes on fibre digestion have been variable

and inconsistent. It appears the type and application rate of surfactants may affect their efficacy of improving feed digestion (McAllister et al., 2000; Lee et al., 2007). In the current study, the synergistic effect of SDS, applied at a rate of 0.125%, plus the inoculant (I+S) resulted in silage with the highest ruminal NDFD throughout the period of incubation. McAllister et al. (2000) observed a decreased total tract NDF digestibility in sheep fed forage treated with enzyme plus Tween 80, a non-ionic surfactant but NDF digestibility was increased when the forage was treated with the surfactant alone. However, in contrast to the present study, Baah et al. (2005) observed reduced ruminal NDFD of orchard grass hay treated with Tween 80 alone or in combination with fibrolytic enzymes compared to the untreated.

In situ ruminal DMD and OMD of all treated silages were decreased by treatment with the inoculant or SDS alone or their combination. The proportion of rapidly soluble DM and OM *in situ* in the Control silage was higher than in the other silages. The Control silage also had a lower fraction of slowly degradable OM while the proportion of the same fraction of DM in the Control silage tended to be lower than in the other silages. This is consistent with the higher concentration of WSC and other soluble fractions in the Control than the treated silages. These factors likely accounted for the increased DMD and OMD observed in the Control compared to the other silages. Consistent with the present study, *in situ* ruminal DMD decreased when rice straw was treated with 1% SDS (Lee et al., 2007) and when orchard grass hay was treated with Tween 80 alone or in combination with exogenous fibrolytic enzymes (Baah et al., 2005). On the contrary, direct ruminal infusion of sorbitant trioleate, a non-ionic surfactant increased rumen bacterial counts, fibrolytic activity and DMD characteristics of rice straw (Lee et al., 2004).

The difference between the results of the present study and those reported with non-ionic surfactants may therefore be due to the level of application and the type of surfactant used. Increasing the surfactant concentration increases the formation of micelle layers at the surface of feed substrates thereby masking the substrate from enzymatic access and degradation (McAllister et al., 2000). While the negative head charge on SDS might have increased enzyme-substrate affinity in I+S treated silage, Tween 80 has been reported to decrease fibrolytic bacterial attachment (Lee et al., 2007) and enzymes absorption (Kim et al., 2006) to substrates.

In conclusion, the results of this study show that the homolactic inoculant containing a blend of *L. plantarum*, *P. acidilactici* and *E. faecium* alone or in combination with SDS may improve barley silage fermentation without impairing aerobic stability. The study also suggests that the combination of the inoculant (containing *L. plantarum*, and *P. acidilactici* and *E. faecium*) and SDS has the potential to increase *in situ* ruminal NDF degradability of barley silage. Further studies are recommended to determine how these improvements will translate into animal growth performance.

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