Effect of Lactic Acid Bacteria Inoculants on In Vitro Digestibility of Wheat and Corn Silages

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ABSTRACT

The aim of the study was to determine the effect of 10 sources of lactic acid bacteria (LAB) on dry matter digestibility (DM-D) and neutral detergent fiber digestibility (NDF-D), in various combinations with starch, in vitro. The soluble starch represented a concentrate feed, whereas silage represented feeding only roughage. The DM-D and NDF-D were determined after 24 and 48 h of incubation to represent effective (24 h) and potential (48 h) digestibility. Addition of LAB was both by direct application of the inoculants to rumen fluid (directly fed microbials) and by the use of preinoculated silages. For each feed combination, tubes without added LAB served as controls. The results indicate that, overall, some LAB inoculants applied at ensiling or added directly to the rumen fluid had the potential to increase the DM-D and NDF-D. The major significant inoculant effect on NDF-D was obtained after 24 h of incubation, whereas the effect after 48 h was mainly nonsignificant. The effective inoculants seemed to minimize the inhibitory effect of the starch on NDF-D within 24 h, perhaps by competition with lactate-producing rumen microorganisms.

Key words: wheat silage, corn silage, in vitro digestibility, lactic acid bacteria silage inoculant

INTRODUCTION

Silage, which is forage preserved through lactic acid fermentation, is a major component in the diets of dairy cattle. Inoculants, comprising mainly lactic acid bacteria (LAB), are used as silage additives to improve preservation efficiency. They are used because of their efficient utilization of the water-soluble carbohydrates of the crop, intensive production of lactic acid, and rapid reduction of pH (Weinberg and Muck, 1996). In some cases, silage inoculation has been observed to affect

animal performance. Feed intake, live weight gain, feed efficiency, or milk production were improved in about one-third of the reviewed studies. The improvements of these parameters ranged from 5 to 11% (Muck, 1993; Kung et al., 2003). In many cases, the justification for using a silage inoculant is the expected effect on animal performance.

The cause of the improvement in animal performance that follows feeding with inoculated silage is unclear, but the results of feeding experiments suggest a possible probiotic effect of the LAB used in inoculants. According to Fuller (1989), a probiotic is a “live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.” One hypothesis regarding the mode of action of LAB silage inoculants in ruminants is that certain LAB strains interact with rumen microorganisms to enhance rumen functionality and animal performance. This hypothesis is consistent with Fuller’s definition of probiotics.

In Northern Ireland, cattle-feeding experiments have been performed in which moist grass silage was treated with a single LAB strain, *Lactobacillus plantarum* MTD1. The results of these studies showed that silage inoculated with this strain improved animal performance, regardless of fermentation quality (Keady and Steen, 1994, 1995; Keady et al., 1994). The authors attributed the improved animal performance to the increase they observed in digestibility, particularly in fiber digestibility, and changes in rumen fermentation. When the inoculant was added to silage immediately before feeding, there was no significant effect on digestibility of DM, nitrogen, NDF, or modified ADF (Keady and Steen, 1996). This finding might suggest that the benefits resulted from the silage fermentation rather than from probiotic effects in the rumen itself. In another study (Khuntia and Chaundhary, 2002), DMI, weight gain, and DM digestibility (DM-D) were improved by dietary addition of a mixed LAB culture to calves (i.e., a direct probiotic effect). All of these studies were performed in vivo. In other studies, the use of directly fed LAB also had positive effects on animal

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performance (e.g., Wallace and Newbold, 1993; Nocek and Kautz, 2006).

Our previous in vitro studies (Weinberg et al., 2003, 2004a,b) showed that LAB pass from silage into the rumen fluid (**RF**) and survive there; some LAB silage inoculants resulted in an increase in the RF pH, which may suggest a positive effect on the rumen environment. Gollop et al. (2005) showed that LAB silage inoculants and inoculated silages exhibited bacteriocin-like antibacterial activity, which could inhibit detrimental microorganisms in the silage, the rumen, or both.

The objective of the present study was to further study the mechanism by which LAB silage inoculants affect the rumen environment by focusing on their effects on in vitro DM-D and NDF digestibility (**NDF-D**), both by direct application to RF and by use of preinoculated silages.

MATERIALS AND METHODS

Inoculated Silage Preparation

Wheat harvested at the milk stage of ripening (DM 350 ± 9 g/kg) and corn harvested at the half milk line (DM 377 ± 4 g/kg) were chopped to 2- to 3-cm-long pieces with a laboratory chopper (Wintersteiger, Ried, Austria) and ensiled in 1.5-L anaerobic glass jars (Weck, Wehr-Ofingen, Germany). The chopped forages were treated without and with each of 10 LAB inoculants, which were applied at 10^6 cfu/g (i.e., 11 treatments for each crop). The inoculation rate was based on the numbers of colony-forming units per gram in the inoculant powders, which had been determined in advance. The inoculants were applied by suspending the appropriate weight of inoculant powder in 20 mL of deionized water, spraying it over 2-kg batches of forage, and mixing thoroughly. Control silages were prepared at the same time without inoculant addition; there were 3 jars (replicates) per inoculant treatment of each of the 2 silages. The silages were stored for 2 mo at room temperature (25 to 28°C). At the end of the storage period, the silages were analyzed for pH, fermentation products, and enumeration of LAB. Samples of the untreated and inoculated silages were dried for 48 h at 60°C in an aerated oven, ground into 1-mm particles, pooled, and used for chemical analysis and in vitro digestibility measurements.

Inoculants

The following commercial LAB inoculants were used: 1) *L. plantarum* MTD1 and 2) *Pediococcus pentosaceus*, obtained from Ecosyl (Yorkshire, UK); 3) *L. plantarum*, 4) *Lactobacillus pentosus*, 5) *P. pentosaceus*, 6) *Enterococcus faecium* (C), and 7) *E. faecium* (Q), obtained from

Agri-King (Fulton, IL); 8) *Lactobacillus buchneri* 40788, obtained from Biotol (Lallemand Animal Nutrition, Milwaukee, WI); 9) *L. buchneri* 11A44 and 10) 1188, a mix of *L. plantarum* and *E. faecium*, obtained from Pioneer Hi-Bred International (Des Moines, IA).

The numbers of LAB cells in the dry products were determined before the experiments by suspending inoculants in deionized water and pouring plating serial dilutions into Rogosa or de Man, Rogosa, and Sharpe agar (MRS, Oxoid, Basingstoke, UK). The LAB in the silage samples were enumerated similarly. de Man, Rogosa, and Sharpe agar was used for all products that contained *E. faecium*. Plates were incubated at 30°C for 3 d for enumeration of colony-forming units.

In Vitro Digestibility Measurements

The in vitro experiments included either inoculants that were added directly to the tubes (directly fed microbials, **DFM**) or preinoculated silages. In the DFM experiments, the tubes contained dried control silage samples, with or without an adequate amount of starch and with or without an adequate weight of inoculant powders. In the experiments with preinoculated silages, the tubes contained dried control or preinoculated silages, with or without starch. Each experiment was performed in 2 separate runs, which included control silage plus inoculants 1 to 5 and control silage plus inoculants 6 to 10, at all starch levels. There were 3 tubes (replicates) for each inoculant (DFM or preinoculated silage) plus starch combination, which contained pooled control silage plus DFM inoculant or pooled preinoculated silage, and for each incubation time (24 or 48 h).

Each in vitro experimental run consisted of 114 tubes: 6 inoculant $\times$ 3 starch $\times$ 2 times $\times$ 3 replicates, plus 3 blanks (rumen fluid only) and 3 standardization tubes. The latter contained samples of wheat or corn silage with known in vitro DM-D, which served as reference samples, according to which the results were adjusted as follows: the control DM-D and NDF-D values of the 2 runs of each experiment were averaged and the results were factored accordingly. In addition, adjustments were done by calculating DM-D and NDF-D correction factors for each in vitro run according to the values of the replicate standard tubes.

Rumen fluid was collected from 2 fistulated dry Holstein cows that were fed at maintenance level as follows: 6 kg of DM of wheat hay and 4 kg of DM of TMR containing 30% concentrated grains, 35% wheat and corn silages, 15% soybean and sunflower meal, 20% by-products (cottonseed, wheat bran, and gluten feed), and a vitamin and mineral supplement. The RF was collected before the morning feeding and was strained

Table 1. Composition of the wheat silages¹

Silage treatment	pH	DM, g/kg	NDF	Lactic acid	Ethanol	Acetic acid	LAB
Control	3.8	351 ^b	578 ^d	36 ^{bc}	26 ^d	12 ^e	6.8 ^d
1. <i>Lactobacillus plantarum</i> MTD1 (Ecosyl)	3.9	343 ^{bc}	604 ^b	38 ^b	41 ^c	11 ^e	7.4 ^b
2. <i>Pediococcus pentosaceus</i> (Ecosyl)	3.9	329 ^d	623 ^a	36 ^{bc}	42 ^c	10 ^e	6.6 ^d
3. <i>L. plantarum</i> (Agri-King)	3.8	328 ^d	592 ^{bcd}	38 ^b	37 ^{cd}	12 ^e	7.1 ^c
4. <i>Lactobacillus pentosus</i> (Agri-King)	3.7	332 ^{cd}	618 ^a	37 ^{bc}	46 ^{bc}	7 ^f	6.4 ^d
5. <i>P. pentosaceus</i> (Agri-King)	3.8	337 ^{cd}	584 ^d	30 ^d	64 ^a	11 ^e	7.3 ^{bc}
6. <i>Enterococcus faecium</i> (C) (Agri-King)	3.8	338 ^e	598 ^{bc}	34 ^{cd}	52 ^b	23 ^c	7.3 ^{bc}
7. <i>E. faecium</i> (Q) (Agri-King)	3.8	334 ^{cd}	594 ^{bcd}	32 ^{cd}	40 ^c	20 ^d	7.3 ^{bc}
8. <i>Lactobacillus buchneri</i> (Biotal)	3.7	365 ^a	539 ^f	29 ^d	14 ^e	43 ^b	8.3 ^a
9. <i>L. buchneri</i> 11A44 (Pioneer)	3.8	371 ^a	557 ^e	31 ^d	8 ^e	55 ^a	8.3 ^a
10. <i>L. plantarum</i> and <i>E. faecium</i> 1188 (Pioneer)	3.7	344 ^{bc}	587 ^{cd}	44 ^a	31 ^{cd}	21 ^{cd}	8.3 ^a

^{a-f}Means in the same column followed by different letters are different ($P < 0.05$).

¹Chemical constituents are given in g/kg (DM) and lactic acid bacteria (LAB) are given in log₁₀ cfu/g (DM).

through 4 layers of gauze and flushed with CO₂ before use in the in vitro digestibility tubes, according to the 2-stage fermentation technique of Tilley and Terry (1963). The procedure included incubation of silage-starch combinations in 20 mL of buffer and 5 mL of RF in 50-mL sealed glass tubes, for 24 or 48 h at 39°C, followed by an additional incubation with 20 mL of 0.2% pepsin in 0.1 N HCl for 48 h at 39°C. At the end of this procedure, the undigested solids were precipitated by centrifugation at 1,000 × g for 10 min and dried in an aerated oven at 60°C for 48 h, and digestibility of the residual DM was determined. The precipitate from the triplicate tubes, which had been dried at 60°C, was pooled into Ankom bags (Ankom, Fairport, NY) for determination of residual NDF content, to be used in calculating NDF-D. The differences between the NDF content in the individual original triplicates (before the in vitro digestibility test) and the residual NDF content were used in the statistical analysis.

The following combinations of silage and starch were measured in the in vitro experiment:

1. **S0**: wheat or corn silages (250 to 270 mg per tube, dried and ground through a 1-mm sieve).
2. **S1**: silages plus cornstarch (S-4126, Sigma, St. Louis, MO) at a 2:1 ratio (180 mg of silage + 90 mg of starch per tube).
3. **S2**: silages plus cornstarch at a 1:2 ratio (90 mg of silage + 180 mg of starch per tube).

At the beginning of the incubation period of the DFM experiments, each of the 10 LAB silage inoculants was added directly to the tube at a calculated dose of 10⁶ cfu/mL. This rate is comparable to the LAB numbers found in the preinoculated wheat and corn silages (Tables 1 and 2). In addition, this high rate was applied to ensure any possible inoculant effect. For each feed combination, tubes without added LAB served as con-

trols. These experiments simulated the use of probiotics as DFM.

In an additional set of experiments, control silage and silages that had been preinoculated during ensiling with each of the 10 LAB silage inoculants were used instead of directly adding the inoculants to the digestibility tubes. These experiments simulated feeding inoculated silages treated with these inoculants.

Analyses

Dry matter was determined by oven-drying for 48 h at 60°C. Lactic acid in water extracts of the silages was determined spectrophotometrically according to Barker and Summerson (1941). Volatile fermentation products in the water extracts of silages were determined by gas chromatography according to Weinberg et al. (2004b). The NDF content was determined according to Van Soest et al. (1991) by using α -amylase without sodium sulfite and an Ankom apparatus. The NDF-D was determined according to the NDF content before and after incubation.

Statistical analysis was performed by experiment and sampling time and included 2-way ANOVA (for inoculant and starch) performed with JMP-5 software (SAS Institute, 1996) to calculate the significance of the effect of inoculant and starch on DM-D and NDF-D of the silages. Duncan's multiple range test was used to differentiate between means (SAS Institute, 1996).

RESULTS

Tables 1 and 2 give the results of the chemical analyses and the LAB populations of the wheat and corn silages. Some of the wheat silages had unusually high ethanol contents (40 to 60 g/kg), which might indicate yeast activity in the initial stages of ensiling. High-ethanol silages have been reported in some studies (e.g.,

Table 2. Composition of the corn silages¹

Silage treatment	pH	DM, g/kg	NDF	Lactic acid	Ethanol	Acetic acid	LAB
Control	3.7 ^c	365 ^a	402 ^d	39 ^c	1 ^a	11 ^e	4.2 ^e
1. <i>Lactobacillus plantarum</i> MTD1 (Ecosyl)	3.7 ^c	328 ^{cd}	396 ^d	29 ^e	2 ^c	13 ^d	5.3 ^b
2. <i>Pediococcus pentosaceus</i> (Ecosyl)	3.7 ^c	349 ^b	392 ^d	45 ^a	2 ^c	3 ^f	5.3 ^b
3. <i>L. plantarum</i> (Agri-King)	3.7 ^c	338 ^c	459 ^{bc}	40 ^b	4 ^b	9 ^e	4.7 ^{cd}
4. <i>Lactobacillus pentosus</i> (Agri-King)	3.9 ^b	332 ^{cd}	444 ^c	46 ^a	4 ^b	14 ^d	4.5 ^d
5. <i>P. pentosaceus</i> (Agri-King)	3.7 ^c	321 ^d	508 ^a	35 ^d	5 ^b	18 ^c	5.7 ^a
6. <i>Enterococcus faecium</i> (C) (Agri-King)	3.8 ^{bc}	333 ^{cd}	432 ^c	39 ^c	6 ^{ab}	19 ^c	4.8 ^{cd}
7. <i>E. faecium</i> (Q) (Agri-King)	3.9 ^b	323 ^d	437 ^e	37 ^{cd}	8 ^a	23 ^b	5.0 ^c
8. <i>Lactobacillus buchneri</i> (Biotol)	4.1 ^{ab}	301 ^e	469 ^b	36 ^{cd}	4 ^b	29 ^a	3.5 ^f
9. <i>L. buchneri</i> 11A44 (Pioneer)	4.3 ^a	305 ^e	478 ^b	30 ^e	2 ^c	29 ^a	5.3 ^b
10. <i>L. plantarum</i> and <i>E. faecium</i> 1188 (Pioneer)	4.2 ^a	323 ^d	473 ^b	38 ^{cd}	5 ^b	30 ^a	4.6 ^d

^{a-f}Means in the same column followed by different letters are different ($P < 0.05$).

¹Chemical constituents are given in g/kg (DM) and lactic acid bacteria (LAB) are given in log₁₀ cfu/g (DM).

Driehuis and Wikselaar, 1999). In the present study, silages treated with *L. buchneri* contained higher concentrations of acetic acid, as expected.

Results of 24-h Incubation

Effects of LAB inoculants, applied as DFM or preensiled with the inoculants, on DM-D and NDF-D after 24 h of incubation of wheat and corn silages are given in Tables 3 to 6.

Starch Effect. As expected, addition of soluble starch significantly increased DM-D in both wheat and corn experiments in both the preinoculated and DFM silages. In the experiments with wheat silages with both inoculated and DFM (Tables 3 and 4, respectively), the NDF-D tended to decrease as the starch level increased. This was expected because the presence of soluble starch reduces the NDF-D (Miron et al., 1996). In some inoculant treatments, NDF-D did not decrease as a result of addition of starch (e.g., inoculant 6, Table 3; inoculants 7 and 8, Table 4).

In the experiments with preinoculated corn silage (Table 5), NDF-D also decreased as the starch level increased, except in inoculant treatments 1 and 6. In the experiment with DFM corn silages (Table 6), half of the inoculant treatments (2, 3, 6, 7, and 10) did not result in a decrease in NDF-D with the addition of starch, and inoculant 7 even resulted in an increase in NDF-D.

Inoculant Effect. In the experiments with preinoculated wheat silages (Table 3), inoculants 6 to 10 resulted in increases in DM-D and NDF-D, as compared with the uninoculated control, in the S0 and S1 starch treatments, and inoculant 6 did so in S2 also. In S2, inoculants 7 to 10 resulted in significant decreases in NDF-D as compared with the uninoculated control. In the experiment with DFM wheat silages (Table 4), inoculants 1, 2, and 7 increased the NDF-D in S1, and inoculants 4, 5, and 7 to 10 increased the NDF-D in starch treatment S2. Inoculants 1 and 10 increased the DM-D in S1.

Table 3. Dry matter digestibility (DM-D) and NDF digestibility (NDF-D; g/kg) of the inoculated wheat silages¹ after 24 h of incubation

Silage treatment	S0		S1		S2	
	DM-D	NDF-D	DM-D	NDF-D	DM-D	NDF-D
Control	538 ^l	339 ^{efgh}	647 ^{gh}	318 ^{fghi}	765 ^{bcd}	239 ^{klm}
1. <i>Lactobacillus plantarum</i> MTD1 (Ecosyl)	441 ⁿ	328 ^{efghi}	642 ^{gh}	308 ^{fghi}	783 ^b	224 ^{lmn}
2. <i>Pediococcus pentosaceus</i> (Ecosyl)	483 ^m	349 ^{defg}	626 ^{hi}	291 ^{hijk}	775 ^b	218 ^{mno}
3. <i>L. plantarum</i> (Agri-King)	496 ^m	321 ^{fghi}	645 ^{gh}	279 ^{ijk}	781 ^b	252 ^{mno}
4. <i>Lactobacillus pentosus</i> (Agri-King)	492 ^m	356 ^{cdef}	639 ^{gh}	295 ^{ghij}	786 ^b	277 ^{ijklm}
5. <i>P. pentosaceus</i> (Agri-King)	500 ^m	339 ^{efgh}	660 ^{fg}	323 ^{fghi}	774 ^b	321 ^{ijkl}
6. <i>Enterococcus faecium</i> (C) (Agri-King)	583 ^k	398 ^{bcd}	731 ^e	396 ^{bcd}	887 ^a	395 ^{bcd}
7. <i>E. faecium</i> (Q) (Agri-King)	586 ^{jk}	404 ^{bc}	739 ^{de}	415 ^b	766 ^{bcd}	147 ^p
8. <i>Lactobacillus buchneri</i> (Biotol)	630 ^{hi}	407 ^{bc}	744 ^{cde}	425 ^{ab}	727 ^e	152 ^p
9. <i>L. buchneri</i> 11A44 (Pioneer)	612 ^{ij}	397 ^{bcd}	767 ^{bc}	470 ^a	727 ^e	191 ^{op}
10. <i>L. plantarum</i> and <i>E. faecium</i> 1188 (Pioneer)	571 ^k	381 ^{bcde}	727 ^e	395 ^{bcd}	742 ^{cde}	204 ^{mop}

^{a-p}For DM and NDF separately, means followed by different letters are different ($P < 0.05$).

¹S0 = without starch; S1 = starch:silage 1:2; S2 = starch:silage 2:1.

Table 4. Dry matter digestibility (DM-D) and NDF digestibility (NDF-D; g/kg) of the wheat silages¹ and directly fed microbials after 24 h of incubation

Silage treatment	S0		S1		S2	
	DM-D	NDF-D	DM-D	NDF-D	DM-D	NDF-D
Control	538 ^{mnop}	339 ^{abdefghijkl}	647 ^{ij}	318 ^{ijklmn}	798 ^{ab}	239 ^{op}
1. <i>Lactobacillus plantarum</i> MTD1 (Ecosyl)	538 ^{mnop}	365 ^{bdefghij}	692 ^{fg}	439 ^a	734 ^{de}	226 ^{op}
2. <i>Pediococcus pentosaceus</i> (Ecosyl)	551 ^{lmno}	368 ^{bdefghij}	630 ^j	391 ^{abcde}	716 ^{ef}	175 ^p
3. <i>L. plantarum</i> (Agri-King)	564 ^{lm}	388 ^{abdef}	669 ^{ghi}	349 ^{cdefghijk}	760 ^{cd}	272 ^{mno}
4. <i>Lactobacillus pentosus</i> (Agri-King)	553 ^{lmn}	381 ^{abdefg}	572 ^{kl}	289 ^{klmn}	778 ^{bc}	397 ^{abcd}
5. <i>P. pentosaceus</i> (Agri-King)	543 ^{mno}	355 ^{cdefghij}	593 ^k	269 ^{no}	825 ^a	378 ^{bcdefgh}
6. <i>Enterococcus faecium</i> (C) (Agri-King)	534 ^{nopq}	342 ^{cdefghijkl}	658 ^{hij}	322 ^{ghijklmn}	780 ^{ab}	272 ^{mno}
7. <i>E. faecium</i> (Q) (Agri-King)	511 ^{pq}	309 ^{ijklmn}	668 ^{ghi}	396 ^{abcde}	812 ^a	422 ^{ab}
8. <i>Lactobacillus buchneri</i> (Biotol)	523 ^{opq}	264 ^{no}	663 ^{hi}	288 ^{lmn}	813 ^a	343 ^{cdefghijkl}
9. <i>L. buchneri</i> 11A44 (Pioneer)	507 ^q	328 ^{ghijklm}	672 ^{ghi}	307 ^{jklm}	813 ^a	320 ^{ghijklmn}
10. <i>L. plantarum</i> and <i>E. faecium</i> 1188 (Pioneer)	539 ^{mno}	401 ^{abc}	679 ^{gh}	340 ^{defghijkl}	807 ^a	336 ^{efghijkl}

^{a-q}For DM and NDF separately, means followed by different letters are different ($P < 0.05$).

¹S0 = without starch; S1 = starch:silage 1:2; S2 = starch:silage 2:1.

In the experiments with preinoculated corn silages (Table 5), inoculants 1 to 4, 6 to 10, and 3 to 10 resulted in increases in DM-D in S1 and S2, respectively; inoculants 8 to 10 and 2 increased the NDF-D in S0 and S2, respectively, as compared with uninoculated controls. In the experiments with DFM-treated corn silages (Table 6), inoculants 1 to 5 increased DM-D in S1 as well as in S2; inoculants 5 and 8 to 10 increased NDF-D in S0, inoculants 2 to 4, 6 to 8, and 10 increased NDF-D in S1, and inoculants 3 and 6 to 10 increased NDF-D in S2.

Results of 48-h Incubation

The effects of LAB inoculants on the DM-D and NDF-D after 48 h of incubation of wheat and corn silages treated by DFM or preensiled with the inoculants are given in Tables 7 to 10.

Starch Effect. After 48 h of incubation, the DM-D and NDF-D were, as expected, higher than after 24 h

of incubation. Addition of starch resulted in increases in DM-D in both wheat and corn in both the preinoculated and the DFM experiments, as expected. Addition of starch resulted in increased NDF-D in wheat silages that were preinoculated with inoculants 6 to 10 in S1 and S2, as did inoculant 3 in S2 (Table 7).

In the experiments with corn (Tables 9 and 10), the response of NDF-D to starch addition varied among the various inoculant treatments. In the preinoculated corn silages (Table 9) in S1, inoculants 1, 2, and 6 resulted in increased NDF-D, whereas inoculants 3 to 5 and 10 resulted in decreases in NDF-D; with the other inoculants there was no change in NDF-D. In S2 there were sharp increases in NDF-D in treatments 3 to 5 as compared with those in S1. In the DFM corn silages (Table 10), starch addition caused the NDF-D to increase with inoculants 1 to 8 and 1 to 3 in S1 and S2, respectively; in the other inoculant treatments there was no change with starch addition.

Table 5. Dry matter digestibility (DM-D) and NDF digestibility (NDF-D; g/kg) of the inoculated corn silages¹ after 24 h of incubation

Silage treatment	S0		S1		S2	
	DM-D	NDF-D	DM-D	NDF-D	DM-D	NDF-D
Control	596 ^{ij}	422 ^{cde}	698 ^g	363 ^{ghijk}	810 ^d	352 ^{hijklmn}
1. <i>Lactobacillus plantarum</i> MTD1 (Ecosyl)	597 ^{jk}	364 ^{ghijk}	756 ^e	359 ^{ghijkl}	816 ^{cd}	366 ^{ghij}
2. <i>Pediococcus pentosaceus</i> (Ecosyl)	608 ^{ij}	377 ^{efghi}	756 ^e	324 ^{jklmno}	821 ^{bcd}	407 ^{cdefg}
3. <i>L. plantarum</i> (Agri-King)	568 ^{kl}	417 ^{cdef}	743 ^{ef}	309 ^{klmno}	844 ^{abc}	303 ^{mno}
4. <i>Lactobacillus pentosus</i> (Agri-King)	568 ^{kl}	371 ^{fghij}	745 ^{ef}	315 ^{klmno}	845 ^{abc}	304 ^{mno}
5. <i>P. pentosaceus</i> (Agri-King)	545 ^l	431 ^{bcd}	691 ^g	139 ^r	847 ^{abc}	232 ^q
6. <i>Enterococcus faecium</i> (C) (Agri-King)	547 ^l	391 ^{defgh}	752 ^{ef}	387 ^{defgh}	860 ^a	348 ^{ghijklmn}
7. <i>E. faecium</i> (Q) (Agri-King)	645 ^h	452 ^{abc}	742 ^{ef}	352 ^{hijklm}	851 ^{ab}	372 ^{efghij}
8. <i>Lactobacillus buchneri</i> (Biotol)	631 ^{hi}	498 ^a	737 ^{ef}	283 ^{op}	851 ^{ab}	292 ^p
9. <i>L. buchneri</i> 11A44 (Pioneer)	620 ^{hj}	496 ^a	730 ^{ef}	322 ^{ijklmno}	849 ^{ab}	257 ^{pq}
10. <i>L. plantarum</i> and <i>E. faecium</i> 1188 (Pioneer)	611 ^{ij}	473 ^{ab}	725 ^f	328 ^{ijklmno}	853 ^a	302 ^{op}

^{a-r}For DM and NDF separately, means followed by different letters are significantly different ($P < 0.05$).

¹S0 = without starch; S1 = starch:silage 1:2; S2 = starch:silage 2:1.

Table 6. Dry matter digestibility (DM-D) and NDF digestibility (NDF-D; g/kg) of the corn silages¹ and directly fed microbials after 24 h of incubation

Silage treatment	S0		S1		S2	
	DM-D	NDF-D	DM-D	NDF-D	DM-D	NDF-D
Control	596 ^{hij}	422 ^{hijklm}	698 ^{ef}	363 ⁿ	810 ^c	352 ⁿ
1. <i>Lactobacillus plantarum</i> MTD1 (Ecosyl)	612 ^{ghi}	453 ^{defghij}	751 ^d	384 ^{mn}	888 ^a	393 ^{klmn}
2. <i>Pediococcus pentosaceus</i> (Ecosyl)	595 ^{hij}	422 ^{hijklm}	750 ^d	450 ^{defghij}	880 ^{ab}	392 ^{lmn}
3. <i>L. plantarum</i> (Agri-King)	611 ^{ghi}	421 ^{hijklm}	743 ^d	420 ^{ijklm}	875 ^{ab}	486 ^{bcddefg}
4. <i>Lactobacillus pentosus</i> (Agri-King)	614 ^{gh}	440 ^{ghijkl}	764 ^d	495 ^{abcde}	857 ^b	350 ⁿ
5. <i>P. pentosaceus</i> (Agri-King)	625 ^g	496 ^{abcde}	749 ^d	369 ⁿ	884 ^{ab}	375 ^{mn}
6. <i>Enterococcus faecium</i> (C) (Agri-King)	576 ^{jk}	472 ^{bcddefgh}	695 ^{ef}	500 ^{abc}	813 ^c	486 ^{bcddefg}
7. <i>E. faecium</i> (Q) (Agri-King)	551 ^k	443 ^{ghij}	710 ^e	537 ^a	824 ^c	505 ^{ab}
8. <i>Lactobacillus buchneri</i> (Biotol)	592 ^{hij}	509 ^{ab}	687 ^{ef}	510 ^{ab}	799 ^c	454 ^{cdefghij}
9. <i>L. buchneri</i> 11A44 (Pioneer)	584 ^{ij}	498 ^{acd}	630 ^g	376 ^{mn}	823 ^c	508 ^{ab}
10. <i>L. plantarum</i> and <i>E. faecium</i> 1188 (Pioneer)	588 ^{hij}	493 ^{cdefg}	672 ^f	471 ^{bcddefghi}	806 ^c	448 ^{efghij}

^{a-n}For DM and NDF separately, means followed by different letters are different ($P < 0.05$).

¹S0 = without starch; S1 = starch:silage 1:2; S2 = starch:silage 2:1.

Inoculant Effect. In the experiments with inoculated wheat silages (Table 7), inoculants 6 to 10 and 7 to 10 resulted in increases in DM-D in S1 and S2, respectively, as compared with the uninoculated controls. Inoculants 1, 3, and 6 to 10 resulted in increases in NDF-D in S1; inoculants 3 and 5 to 10 resulted in increases in NDF-D in the S2 starch treatment, as compared with the uninoculated control. In the experiment with DFM (Table 8), inoculants 2, 6, and 8 to 10 resulted in increases in DM-D in S1. In S0 all inoculants but 3 resulted in decreases in NDF-D, whereas in S1 and S2 the effects of inoculants varied and were smaller.

In the experiments with inoculated corn silages (Table 9), most inoculants resulted in increases in DM-D in S0; inoculants 1, 2, and 6 to 10 resulted in increases in DM-D in S1; and all inoculants resulted in increases in DM-D in S2. With regard to NDF-D, only a few inoculants resulted in increases, which included inoculants 1, 2, 6, and 9 in S1 and inoculants 2 to 4

in S2, whereas with some inoculants a decrease was observed, as with inoculants 3 to 5 in S1. In the experiments with DFM corn silages (Table 10), inoculants 4 and 6 to 9 resulted in increases in DM-D of S1, whereas in the other inoculant treatments there was no change or a decrease. With regard to NDF-D, in S0 inoculants 9 and 10 resulted in increases and inoculants 1, 2, and 4 resulted in significant decreases. In S1 all inoculants resulted in increases in NDF-D, whereas in S2 the results were similar to or less than the control.

DISCUSSION

The present in vitro experiments were conducted as part of a broad research objective: to determine how LAB silage inoculants enhance ruminant performance. The first step in this project was to determine whether LAB included in silage inoculants could survive under rumen-like conditions. Previous results indicated that

Table 7. Dry matter digestibility (DM-D) and NDF digestibility (NDF-D; g/kg) of the inoculated wheat silages¹ after 48 h of incubation

Silage treatment	S0		S1		S2	
	DM-D	NDF-D	DM-D	NDF-D	DM-D	NDF-D
Control	617 ^{jk}	498 ^{lmn}	746 ^{hi}	500 ^{klmn}	843 ^{de}	490 ^{mn}
1. <i>Lactobacillus plantarum</i> MTD1 (Ecosyl)	601 ^{kl}	518 ^{ijklm}	742 ^{hi}	546 ^{ghi}	852 ^{cd}	519 ^{ijklm}
2. <i>Pediococcus pentosaceus</i> (Ecosyl)	601 ^{kl}	536 ^{ghijk}	734 ⁱ	523 ^{ijklm}	842 ^{de}	507 ^{ijklmn}
3. <i>L. plantarum</i> (Agri-King)	603 ^{kl}	527 ^{ijklm}	756 ^{hi}	539 ^{ghij}	851 ^{cd}	566 ^{fgh}
4. <i>Lactobacillus pentosus</i> (Agri-King)	570 ^m	510 ^{ijklm}	737 ^{hi}	495 ^{lmn}	840 ^{de}	503 ^{ijklmn}
5. <i>P. pentosaceus</i> (Agri-King)	591 ^{klm}	511 ^{ijklm}	749 ^{hi}	520 ^{ijklm}	847 ^{de}	531 ^{hijkl}
6. <i>Enterococcus faecium</i> (C) (Agri-King)	587 ^{lm}	518 ^{ijklm}	805 ^{fg}	660 ^{bc}	860 ^{bcd}	572 ^{fg}
7. <i>E. faecium</i> (Q) (Agri-King)	594 ^{klm}	509 ^{ijklm}	795 ^g	640 ^{cd}	875 ^{abc}	600 ^{ef}
8. <i>Lactobacillus buchneri</i> (Biotol)	607 ^{kl}	470 ⁿ	802 ^{fg}	617 ^{de}	879 ^{ab}	603 ^{ef}
9. <i>L. buchneri</i> 11A44 (Pioneer)	615 ^{jk}	509 ^{ijklm}	804 ^{fg}	682 ^{ab}	890 ^a	636 ^{cde}
10. <i>L. plantarum</i> and <i>E. faecium</i> 1188 (Pioneer)	597 ^{kl}	515 ^{ijklm}	823 ^{ef}	698 ^a	883 ^{ab}	602 ^{ef}

^{a-n}For DM and NDF separately, means followed by different letters are different ($P < 0.05$).

¹S0 = without starch; S1 = starch:silage 1:2; S2 = starch:silage 2:1.

Table 8. Dry matter digestibility (DM-D) and NDF digestibility (NDF-D; g/kg) of the wheat silages¹ and directly fed microbials after 48 h of incubation

Silage treatment	S0		S1		S2	
	DM-D	NDF-D	DM-D	NDF-D	DM-D	NDF-D
Control	617 ^{ghij}	498 ^{abcd}	746 ^{ef}	500 ^{abcd}	843 ^a	490 ^{bcdef}
1. <i>Lactobacillus plantarum</i> MTD1 (Ecosyl)	619 ^{ghi}	453 ^g	758 ^{de}	469 ^{defg}	858 ^a	471 ^{defg}
2. <i>Pediococcus pentosaceus</i> (Ecosyl)	616 ^{ghij}	446 ^{ghijk}	781 ^{bc}	533 ^a	861 ^a	492 ^{bcdef}
3. <i>L. plantarum</i> (Agri-King)	628 ^g	471 ^{defg}	751 ^{def}	476 ^{cdefg}	856 ^a	480 ^{cdefg}
4. <i>Lactobacillus pentosus</i> (Agri-King)	604 ^{ij}	422 ^{kl}	738 ^f	455 ^{ghij}	858 ^a	473 ^{cdefg}
5. <i>P. pentosaceus</i> (Agri-King)	619 ^{ghi}	457 ^{fghi}	757 ^{de}	459 ^{efghi}	849 ^a	449 ^{ghijk}
6. <i>Enterococcus faecium</i> (C) (Agri-King)	624 ^{gh}	463 ^{efgh}	766 ^{cd}	494 ^{bcde}	848 ^a	450 ^{ghijk}
7. <i>E. faecium</i> (Q) (Agri-King)	599 ^j	410 ^l	764 ^{cde}	491 ^{bcdef}	850 ^a	469 ^{defg}
8. <i>Lactobacillus buchneri</i> (Biotol)	605 ^{hij}	419 ^{kl}	780 ^{bc}	508 ^{abc}	850 ^a	446 ^{ghijk}
9. <i>L. buchneri</i> 11A44 (Pioneer)	603 ^{ij}	431 ^{hijkl}	777 ^c	493 ^{bcde}	850 ^a	452 ^{ghijk}
10. <i>L. plantarum</i> and <i>E. faecium</i> 1188 (Pioneer)	601 ^{ij}	425 ^{ijkl}	797 ^b	524 ^{ab}	845 ^a	461 ^{efgh}

^{a-l}For DM and NDF separately, means followed by different letters are different ($P < 0.05$).

¹S0 = without starch; S1 = starch:silage 1:2; S2 = starch:silage 2:1.

LAB silage inoculants survived in RF in vitro during 72 to 96 h of incubation and that LAB passed from silage samples to the RF (Weinberg et al., 2003, 2004a,b). The results of these experiments indicated that direct addition of LAB (DFM) to the RF resulted in an increase in the RF pH.

The purpose of the present experiments was to determine the effect of LAB, in various combinations with starch, on DM-D and NDF-D in vitro. The soluble starch represented a concentrate feed, whereas silage represented feeding only roughage. The starch levels used in our experiments were higher than are usually included in TMR for lactating cows. However, in the current model we used these high starch levels (1:2 and 2:1 of DM) to pick any interaction starch may have with the inoculant effect on DM and fiber digestibility. The DM-D and NDF-D levels were determined after 24 and 48 h of incubation to represent effective and potential digestibility. In intensive management as practiced in

Israel, the dairy cows are fed maintenance rations 4 times daily, and we assumed that under such conditions, most of the digested feeds have passed through the rumen within 24 h (Arieli et al., 1989); therefore, we considered that the digestibility results obtained after 24 h of incubation were indicative of the effective digestibility, whereas those obtained after 48 h of incubation represented the often-used digestibility values of a feed according to Tilley and Terry (1963), which we designated as the potential digestibility.

Other in vivo studies with silages of perennial ryegrass have shown that silages preinoculated with *L. plantarum* MTD1 enhanced the performance of various cattle. This was manifested in increased intake and live weight gain, and enhanced DM-D and NDF-D (Keady and Steen 1994, 1995; Keady et al., 1994; Rooke and Kafilzadeh, 1994). With regard to the effect of DFM, findings are variable: sometimes animal performance was enhanced (Wallace and Newbold, 1993; Khuntia

Table 9. Dry matter digestibility (DM-D) and NDF digestibility (NDF-D; g/kg) of the inoculated corn silages¹ after 48 h of incubation

Silage treatment	S0		S1		S2	
	DM-D	NDF-D	DM-D	NDF-D	DM-D	NDF-D
Control	666 ^p	537 ^{defgh}	740 ^j	495 ^{ijkl}	829 ^{de}	550 ^{defg}
1. <i>Lactobacillus plantarum</i> MTD1 (Ecosyl)	702 ^{klmn}	527 ^{efghi}	790 ^{gh}	631 ^{ab}	869 ^{ab}	484 ^{ijkl}
2. <i>Pediococcus pentosaceus</i> (Ecosyl)	720 ^{ijkl}	525 ^{efghi}	808 ^{efg}	630 ^{ab}	859 ^{bc}	628 ^{ab}
3. <i>L. plantarum</i> (Agri-King)	709 ^{klmn}	597 ^{bc}	681 ^{nop}	252 ^o	877 ^{ab}	627 ^{ab}
4. <i>Lactobacillus pentosus</i> (Agri-King)	694 ^{mno}	555 ^{def}	698 ^{lmn}	345 ⁿ	872 ^{ab}	635 ^a
5. <i>P. pentosaceus</i> (Agri-King)	676 ^{op}	571 ^{cd}	715 ^{klmn}	272 ^o	867 ^{ab}	565 ^{cd}
6. <i>Enterococcus faecium</i> (C) (Agri-King)	722 ^{jk}	515 ^{ghijk}	844 ^{cd}	625 ^{ab}	874 ^{ab}	482 ^{kl}
7. <i>E. faecium</i> (Q) (Agri-King)	729 ^j	552 ^{defg}	813 ^{ef}	509 ^{hijkl}	877 ^{ab}	498 ^{ijkl}
8. <i>Lactobacillus buchneri</i> (Biotol)	701 ^{klmn}	520 ^{fghij}	806 ^{fg}	505 ^{hijkl}	878 ^{ab}	522 ^{fghij}
9. <i>L. buchneri</i> 11A44 (Pioneer)	709 ^{klmn}	563 ^{cde}	807 ^{fg}	549 ^{defg}	886 ^a	541 ^{defgh}
10. <i>L. plantarum</i> and <i>E. faecium</i> 1188 (Pioneer)	705 ^{klm}	536 ^{defgh}	784 ^h	441 ^m	878 ^{ab}	473 ^{lm}

^{a-p}For DM and NDF separately, means followed by different letters are different ($P < 0.05$).

¹S0 = without starch; S1 = starch:silage 1:2; S2 = starch:silage 2:1.

Table 10. Dry matter digestibility (DM-D) and NDF digestibility (NDF-D; g/kg) of the corn silages¹ and directly fed microbials after 48 h of incubation

Silage treatment	S0		S1		S2	
	DM-D	NDF-D	DM-D	NDF-D	DM-D	NDF-D
Control	666 ^{mno}	537 ^{efghi}	740 ^l	495 ^{klm}	829 ^{fgh}	550 ^{def}
1. <i>Lactobacillus plantarum</i> MTD1 (Ecosyl)	603 ^a	371 ^p	758 ^{ijkl}	553 ^{def}	834 ^{efgh}	486 ^{lmn}
2. <i>Pediococcus pentosaceus</i> (Ecosyl)	633 ^p	466 ⁿ	746 ^{kl}	585 ^b	841 ^{efgh}	513 ^{hijk}
3. <i>L. plantarum</i> (Agri-King)	675 ^{mn}	505 ^{ijklm}	818 ^h	555 ^{cdef}	851 ^{defg}	539 ^{efgh}
4. <i>Lactobacillus pentosus</i> (Agri-King)	664 ^{mno}	493 ^{klm}	827 ^{gh}	621 ^a	858 ^{cde}	511 ^{ijkl}
5. <i>P. pentosaceus</i> (Agri-King)	667 ^{mno}	519 ^{ghijk}	830 ^{fgh}	568 ^{bcd}	847 ^{defg}	519 ^{ghijk}
6. <i>Enterococcus faecium</i> (C) (Agri-King)	658 ^{nop}	514 ^{hijk}	773 ^{ij}	563 ^{bode}	870 ^{bcd}	480 ^{mno}
7. <i>E. faecium</i> (Q) (Agri-King)	646 ^{op}	538 ^{efgh}	786 ⁱ	585 ^b	878 ^{abc}	493 ^{klm}
8. <i>Lactobacillus buchneri</i> (Biotal)	649 ^{op}	531 ^{fghij}	778 ^{ij}	582 ^{bc}	896 ^a	541 ^{efg}
9. <i>L. buchneri</i> 11A44 (Pioneer)	683 ^m	588 ^b	770 ^{ijk}	573 ^{bcd}	894 ^{ab}	540 ^{efgh}
10. <i>L. plantarum</i> and <i>E. faecium</i> 1188 (Pioneer)	663 ^{mno}	569 ^{bcd}	762 ^{ijkl}	561 ^{bode}	849 ^{defg}	414 ^o

^{a-p}For DM and NDF separately, means followed by different letters are different ($P < 0.05$).

¹S0 = without starch; S1 = starch:silage 1:2; S2 = starch:silage 2:1.

and Chaundhary, 2002; Nocek and Kautz, 2006), and sometimes not (Keady and Steen, 1996).

The present experiments were performed in vitro with wheat and corn silages. The experimental design of this study was actually factorial and included 2 modes of inoculant application (preinoculated silages vs. DFM), 2 crops, 2 incubation times, and 3 levels of starch (S0, S1, and S2). For practical reasons, we decided to analyze each crop, time point, and mode of application separately. The following discussion is based on appraisal of the results presented in Tables 1 to 10.

The results indicate that, overall, some LAB inoculants (6 to 10) applied at ensiling or added directly to the RF (DFM) have the potential to increase the DM-D and NDF-D, and perhaps thereby to enhance animal performance. Lactic acid bacteria do not possess any enzymatic capability to hydrolyze cell-wall constituents (Rooke and Hatfield, 2003), and in the present study we asked how they enhance the activity of ruminal cellulolytic bacteria.

The major significant inoculant effects on NDF-D were obtained after 24 h of incubation (Tables 3 to 6), whereas their effects after 48 h were mainly not significant (Tables 7 to 10). When the 24-h NDF-D levels elicited by the effective inoculants were compared with the levels in the respective uninoculated controls, the effects of the inoculants were larger in the wheat silages than in the corn silages. This was because, after 24 h, the NDF-D of the corn silages was higher than that of the wheat silages. It is not possible to point out consistent differences between inoculated silages and DFM with regard to in vitro digestibility. In the wheat silages the largest inoculant effect on NDF-D was observed in the low-starch treatment (S1), whereas in the corn silages the interaction with the starch was not so clear. It could well be that the differing responses of the 2

crops to soluble starch sprang from differences between wheat and corn silages with regard to the starch content in the forage, its chemistry, and solubility. As expected, starch depressed NDF-D because the rumen population of cellulolytic microorganisms prefers to consume the available soluble starch prior to development of mechanisms and enzymes needed for cell-wall degradation, which is a time-consuming process (Miron et al., 1996). In the corn silages after 48 h at S0, DM-D were improved with some inoculants, whereas NDF-D were not (Tables 9 and 10). This might be due to some solubilization of hemicellulose during ensiling, which improved DM-D but did not change or even decreased the digestibility of the residual NDF. The effective inoculants (mainly 6 to 10) seemed to minimize this inhibitory effect of the starch on NDF-D. We do not have a proper explanation for the mechanism of that effect, but we hypothesize that the effective LAB inoculants might have competed with the efficient lactate-producing rumen microorganisms (such as *Ruminobacter amylophilus* and *Streptococcus bovis*) on essential compounds such as mono- and disaccharides released from starch hydrolysis, ammonia, vitamins, and essential minerals. This competition might reduce the rate of lactate production by rumen bacteria and consequent pH decline in the rumen, which results in higher activity of cellulolytic rumen populations. This might explain the potential buffering effect of LAB silage inoculants in the rumen fluid, which resulted in higher pH values (Weinberg et al., 2003).

After 48 h of incubation, there was no major effect of the inoculants on DM-D and NDF-D (except for inoculants 6 to 10 in the preinoculated wheat silages at S1 and S2). The lack of effect after 48 h may be because the naturally amylolytic habitat of the rumen overcame the effects of the LAB inoculants. In this context, Filya

et al. (2007) also did not observe an inoculant effect on in vitro digestibility of alfalfa silages after 48 h.

The rumen is a very complex ecosystem, in which numerous microorganisms and factors play a role. More research is needed to elucidate the probiotic effect of LAB silage inoculants on ruminants. In future research, it would be worthwhile to study the interactions among added LAB and selected rumen bacterial strains. For this it will be possible to use novel molecular techniques, such as reverse transcription-PCR, which enable microorganisms to be traced without the need to culture LAB or rumen bacteria. Such techniques for both bacterial groups are already available (Shi et al., 1997a,b; Tajima et al., 2001; Stevenson et al., 2006).

CONCLUSIONS

Some LAB silage inoculants might have the potential to increase DM-D and NDF-D in the rumen after 24 h of in vitro incubation. This finding suggests that there are positive effects of these inoculants on the effective digestibility of TMR that contain wheat silages, and supports their further use for feeding high-yielding dairy cows. Further research is warranted to gain a better understanding of the beneficial (probiotic) effects of such inoculants on ruminants.

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