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ORIGINAL ARTICLE

Inoculants for ensiling low-dry matter corn crop: a midlactation cow perspective

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Summary

In many regions, optimum dry matter (DM) content of corn crop pre-ensilage cannot be ensured for management, agronomical and climatic reasons. Under such conditions, corn crops are harvested at low DM, and are easily exposed to unfavourable fermentation pathways and plant spoilage and wastage. Thus, it is a major question for dairy agriculturists whether certain microbial inoculants application to low-DM corn crop pre-ensilage affects silage quality and cow performance. The objective was to determine effects of adding microbial inoculants to low-DM corn crop at ensiling on silage quality, rumen fermentation and milk production of eight Holstein cows fed the treated silages. Whole corn plant was harvested at milk stage of maturity with 204 g DM/kg of fresh crop, cut to a theoretical particle length of 2 cm, filled in 60 t bunker silos, and treated layer by layer with either no inoculant (control), inoculant 'E' (100 000 cfu/g of fresh crop) containing mainly *Lactobacillus plantarum*, inoculant 'B' (100 000 cfu) containing mainly *Pediococcus pentosanus*, *Lactobacillus plantarum* and *Propionibacter freudenreichii* or a mixture of inoculants 'E' and 'B' (200 000 cfu). Inoculants were mixed with water and sprayed on thin layers of corn chops layer by layer followed by rolling to ensure proper oxygen outage and even microbial distribution throughout the plants. Eight multiparous lactating Holstein cows at 100 ± 20.5 days in milk were used in a replicated 4 × 4 Latin square design with four 20-day periods including 14 days of adaptation and 6 days of sampling. Dietary treatments were mixed rations containing corn silages with or without the inoculants. The basal diet contained 32.9% corn silage, 14.3% alfalfa hay and 52.8% concentrate on a DM basis. Inoculants did not affect silage pH or content of DM, CP, lactate, acetate, ash and total volatile fatty acids (VFA). Applying 'B' to corn crop resulted in higher water soluble carbohydrates (47.7 g/kg vs 29.8 g/kg) and lower neutral detergent fibre (494.1 g/kg vs 464.0 g/kg) compared with control. The combined inoculants increased silage butyrate relative to other treatments. The mixture of 'E + B' and 'B' moderately decreased rumen pH, when compared to 'E'. The 'E + B' increased rumen VFA concentrations relative to 'E' and control silage. Dry matter intake increased when corn crop was ensiled with 'E' than with control and 'E + B', but this had little impact on milk production or its energy concentrations. Milk energy yield tended to decrease when 'B' but not 'E' was applied alone, compared with control and 'E + B'. The estimated proportion of the

consumed energy secreted in milk increased when inoculants were applied together compared with when they were used separately. Results suggest positive effects of *Lactobacillus plantarum* containing inoculant on feed intake, some effects on corn silage water soluble carbohydrates, fibre and butyrate contents, rumen pH and VFA concentrations; but no significant effects on total tract nutrient digestibility or productivity of Holstein cows fed diets with 329 g corn silage/kg of diet DM.

Introduction

Corn silage is a palatable collective source of effective fibre and fermentable energy that makes up approximately half of the dietary forage on many commercial dairies (NRC, 2001; Kowsar et al., 2008; Soltani et al., 2009). Especially, if corn grown for ensilage is planted as a second crop in summer usually behind wheat and barley, sufficient heat and sunshine for optimum maturity at harvest may not occur. Thus, most corn crops are ensiled with <250 g/kg dry matter (DM) (NRC, 2001; Ranjbari et al., 2007). In addition, with ensiling low-DM whole plant in bunker silos (easily accessible to many farmers without a need for costly equipment and facilities), effective management strategies to maintain high quality preservation are a necessity (McDonald et al., 1991). Excessive moisture interferes with rapid establishment of lactic acid producing bacteria and delays a rapidly needed decline in pH, thus favouring clostridial growth that can subsequently increase seepage flow (Jonsson, 1991; Gordon et al., 1999). Moreover, ensilage results in solubilization of proteins and increases the ratio of rapidly degradable to slowly degradable crude protein (CP), which may reduce its nutritional value, palatability and N use efficiency by rumen microbes (Beever, 1993; Guo et al., 2007). Additives such as microbial inoculants are used to stabilize homo-fermentative reactions, limit proteolysis and acetate and butyrate production, thereby minimizing aerobic deterioration (Kung et al., 1993; McAllister et al., 1995). However, a paucity of global conclusive data exists on how several different inoculants influence low-DM corn crop ensilage and subsequent cow performance.

Ensilage quality and thus silage nutritional value is determined by the biochemical pathways and fermentation products during early fermentations and later as fermentation stabilizes. Separate and combined applications of lactic and propionic acid bacteria have previously been reported for high DM corn and non-corn forages (Kung et al., 1993; Charmley et al., 1996; Weinberg et al., 1999; Filya, 2003; Rizk et al., 2005). An ongoing scientific and commercial question is if normal or increased doses of lactic or propionic acid producing bacteria, as well as their mixtures, in low-DM corn crop will improve silage quality. The objective was to determine effects of applying inoculants containing either *Lactobacillus plantarum*; *Pediococcus pentosanus*, *Lactobacillus plantarum* and *Propionibacter freudenreichii*, or their additive combination, to low-DM corn crop (i.e. 200–220 g/kg) on silage biochemistry, *in vitro* digestion, feed intake, rumen fermentation, total tract nutrient digestibility and milk production of dairy cows fed diets with >30.0% corn silage in the diet DM.

Materials and methods

Microbial inoculants, corn crop ensilage and silage biochemistry

Corn crop (hybrid 700; Plant Breeding, Karaj, Iran) was harvested at milk stage of maturity with an average DM content of 20.04% and packed into four 60 t bunker silos, one per treatment. The corn crop was cut at a theoretical chop length of 2 cm and treated with either no inoculants (control), inoculant 'E' (100 000 cfu/g of fresh crop, Ecosyl®; Ecosyl Products, Stokesley, UK) mainly containing *Lactobacillus plantarum*, inoculant 'B' (100 000 cfu, Biotal®; Lallemand Animal Nutrition, Stokesley, UK) mainly containing *Pediococcus pentosanus*, *Lactobacillus plantarum* and *Propionibacter freudenreichii*, or the mixture of inoculants 'E + B' (200 000 cfu). Corn crop contained 90.1% organic matter (OM), 19.8% water soluble carbohydrates (WSC), 49.7% neutral detergent fibre (NDF) and 31.0% g acid detergent fibre (ADF) on a DM basis. The inoculants were applied to corn crops as crops were unloaded from silage wagons to silos. For even and homogeneous distribution of inoculants over the crop, inoculants (200 g/100 t) were mixed with water (150 L/silo) and then applied to the crops. For the control treatment, inoculants-free water was applied. For homogeneous microbial distribution throughout the silo, inoculants preparations were sprayed on thin layers of crops using a mobile machine, followed by heavy rolling during the packing process. The crops were put in the silo layer by

layer and little by little to ensure proper oxygen outage and consistent inoculant supply on corn crop chops. For silage sampling, first, six different sites on each silo were chosen, and about 15 kg silage was sampled from each site. Then, all subsamples were thoroughly mixed to obtain representative 1-kg samples for freezing until analysis. Later on for biochemical analysis, samples were thawed at room temperature and 30 g of silage was well mixed with 270 g of distilled water thrice, each time for 30 s, filtered through a two-layer cheesecloth, and the resulting extract was used for pH and volatile fatty acids (VFA) measurement. For WSC analysis (Dubios et al., 1956), the silage extract was centrifuged for 10 min at 2000 *g* at 4 °C. The supernatant was diluted 10 times, and 1 ml of diluted extract was added to 1 ml of distilled water, 0.15 ml of 0.8 wt/vol phenol, and 5 ml of concentrated sulfuric acid. The solution was cooled before it was read on a spectrophotometer at 470 nm wavelengths with glucose and xylose as standards. Silage ammonia was measured using a colorimetric procedure according to Filya (2003).

In vitro digestion

In vitro digestibility of the corn silages was determined using the method of Tilley and Terry (1963). Rumen fluid was obtained from two ruminally fistulated Naeini rams (Naein, Isfahan, Iran) fed corn silage for two adaptation weeks before rumen fluid collection. The rumen fluid from the two sheep were mixed and filtered through four-layer cheesecloth. Ten millilitres of rumen fluid and 30 ml of an artificial saliva (McDougall, 1948) were added to 0.5 g of corn silage in 100 ml prewarmed (39 °C) bottles. While adding saliva and rumen fluid to samples, bottles were maintained under CO_2 to limit oxygen exposure. The artificial saliva consisted of two solutions, with the first containing 10 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g NaCl, 0.1 g $CaCl_2.H_2O$ and 0.5 g urea/1000 ml of solution, and the second containing 15 g Na_2CO_3, and 1 g $Na_2S.9H_2O$ per 100 ml of solution. Twenty millilitres of the first solution was mixed with 1000 ml of the second solution, and an average pH of 6.9 to 7.1 was obtained. The bottles were capped and placed in a shaking incubator for 48 h. Four bottles with rumen fluid and saliva, but without silage, were incubated as the blank. Silage samples were dried and ground through a 1-mm screen before incubation, and there were three replicate bottles per treatment. After incubation, bottles were opened and the contents were centrifuged at 4700 *g* for 15 min at 4 °C to separate the supernatant and dry the solid portion at 55 °C. The *in vitro* digestibility coefficients were calculated as:

$$100-[(\text{post-incubation sample weight}/\text{original sample weight}) \times 100)].$$

In vivo lactation experiment and cow management

Eight multiparous lactating Holstein cows at 100 ± 20.5 days in milk producing 35 ± 2.2 kg milk at the commencement of the study were used in a replicated 4 × 4 Latin square design experiment with four periods. Each period had 14 days of adaptation and 6 days of sampling. As a result of the square being replicated with four experimental periods, and two cows per treatment per period, the experiment had eight replications per treatment for all animal response parameters. Cows were allocated to squares based on days in milk to take into account their different physiological states, and randomly assigned to treatments within squares. The periodical treatments arrangement was made in a particular way to balance for and thus minimize carry over effects (Cochran and Cox, 1992). Cows were offered a total mixed ration (TMR, Table 1) three times daily at 05:30, 13:30 and 21:30 hours to permit a maximum of 10% refusals. Clean wood shavings were used for

Table 1 Feed ingredients and chemical composition of the experimental control TMR

	g/kg DM
Corn silage	329
Alfalfa hay	143
Soybean meal (440 g/kg CP, solvent)	174
Ground barley grain	150
Ground corn grain	131
Whole cottonseeds	35
Palm oil long-chain fatty acids soap powder*	18
Calcium carbonate	4
Sodium bicarbonate	8
Mineral and vitamin supplement†	8
Chemical composition (mean ± standard error)	
DM, g/kg	468 ± 9.6
Organic matter, g/kg	924 ± 4.5
CP g/kg	168 ± 3.6
NDF, g/kg	417 ± 4.9
ADF, g/kg	231 ± 4.8
NE_L‡, Mcal/kg	1.6

*GP Feeds, Byley Industrial Estate, Middlewich, Cheshire, UK.
†Contained 250 000 IU vit. A, 50 000 IU vit. D, 1500 IU vit E, 120 g Ca, 7.7 g Zn, 20 g P, 20.5 g Mg, 186 g Na, 1.25 Fe, 3 g S, 14 mg Co, 1.25 g Cu, 56 mg I, and 10 mg Se per kg supplement (BehRoshd, Tehran, Iran).
‡Estimated by the NRC (2001).

bedding and refreshed twice daily. Diets were formulated for 35 kg daily milk yield using the V.5 CNCPS formulation program (Fox et al., 2004). Corn silage DM was monitored at the beginning of each period to make necessary adjustments in TMR preparation and delivery. To avoid post-fermentation nutrient malassimilation and silage palatability issues, at each feeding time, silage was harvested from the silo and used in TMR preparation just in sufficient amounts using the monitored daily feed intake data for individual cows. Cows were housed in individual boxes (4×4 m) with free access to automatic water troughs and salt blocks. Periodical body weight (BW) records were used to calculate the energy deposited in tissues. The energy content of daily BW differences was estimated as [(proportion empty body fat $\times$ 9.4) + (proportion empty body protein $\times$ 5.55)], with a presumption that empty BW gain was equal to 0.817 of live BW gain (NRC, 2001) (Table 5). Cows were cared for according to the guidelines of the Iranian Council of Animal Care (1995) at the Dairy Facilities of the Lavark Research Station (Isfahan University of Technology, Isfahan, Iran) from August to October 2007.

Feed and fecal sampling, processing, and analysis

Feeds and refusals from individual cows were sampled for 5 days during sampling days in each period and analysed for DM, ash (at 500 °C overnight), crude protein (CP; 1030 Micro Kjectel Auto Analyzer, method 984.13; AOAC, 1997), NDF (Van Soest et al., 1991; using heat-stable alpha-amylase and sodium sulphite), and ADF (method 973.18; AOAC, 1997). Fibre fractions are expressed without residual ash. Grab fecal samples were collected daily from individual cows for 5 days during sampling weeks at 11:00 and 15:00 hours and frozen at −20 °C until analysis. Fecal samples were thawed at room temperature (i.e. 25 °C) and dried at 55 °C for 72 h before DM, ash, CP, NDF and ADF analysis. Acid detergent insoluble ash (AIA) was measured in composited TMR and fecal samples, by period within cow, and used as an internal indigestible marker to calculate apparent total tract nutrient digestibility coefficients (Nikkhah et al., 2004).

Milking and milk sample processing, analysis, and energy calculations

Cows were milked three times daily in a milking parlour at 05:00, 13:00 and 21:00 hours. During the last 5 days of each period, milk yield was recorded for all cows at each milking. The amount of milk produced for each cow at each milking was obtained using graduated jars (Agri & SD, Frankfurt, Germany). Milk energy density (Mcal/kg) was estimated as: [(0.093 $\times$ fat content, g/kg) + (0.054 $\times$ crude protein content, g/kg) + (0.039 $\times$ lactose content, g/kg)]; according to NRC (2001), as shown in Table 5. Daily milk energy yield was obtained by multiplying milk volume by milk energy density. At each milking, cows were monitored for udder inflammation and presence of milk clots in the nipples to ensure that milk yield and composition were not affected by mastitis. All milk samples from individual cows in 50 ml plastic vials were preserved using potassium dichromate, and were analysed for fat, CP and lactose by Milk-O-Scan (134 BN Foss Electric, Hillerod, Denmark).

Rumen fluid sampling and processing

Rumen fluid from the ventral sac was sampled by rumenocentesis (Nordlund and Garrett, 1994) 4 h after the morning feeding on the last day of each period. A 16 cm square area caudoventral to the costochondral junction of the last rib on a line parallel with the top of the patella was clipped and washed with ethanol. The shaved area was scrubbed with Povidone-Iodine and Savlon, and sedated by injecting 8 ml of 0.02 wt/vol lidocaine-epinephrine to prevent bleeding. A stainless steel needle was inserted about 4 cm into the ventral sac of the rumen, and a 10 ml syringe was used to aspirate rumen fluid. For 3 days after each sampling, cows were injected intramuscularly with Penicillin-G-Procaine to minimize chances of infection. The pH of rumen fluid was measured immediately after sampling using a mobile pH meter (HANNA, instrument, S/N: 137243, Lisbon, Portugal). To stop fermentation, 20 μl of 0.5 wt/vol sulphuric acid was added to each millilitre of rumen fluid, and samples were kept at −20 °C until analysed for VFA using gas chromatography (0.25×0.32, id 0.3 μ WCOT Fused Silica Capillary, CHROMPACK CP. 9002, Model No. CP-9002 Serial No. 94 77 B, Delft, The Netherlands), as described by Bal et al. (2000).

Statistical analysis

In vitro silage biochemistry data were analysed as a completely randomized design with fixed effects of treatment or inoculants application. The *in vivo* Latin square design data were analysed as a mixed model using PROC MIXED of SAS (2003) with fixed effects

of treatment and period and random effect of cow. Restricted Maximum Likelihood was the method of estimating least square means and Between-Within was the method of calculating denominator degrees of freedom (SAS User's Guide, 2003). Pair-wise means comparisons were made using PDIFF option of the SAS program. Significant treatment differences were declared if $p \leq 0.05$ and trends were accepted if $p \leq 0.10$. Results are presented as least square means ± standard errors of differences.

Results

Silage biochemistry and rumen metabolites

Inoculants did not affect silage pH and content of DM, CP, lactate, acetate, ash and total VFA concentrations (Table 2). Applying 'B', but not 'E', to the corn crop resulted in higher WSC (47.7 g/kg vs. 29.8 g/kg) and lower NDF (494.1 g/kg vs. 464.0 g/kg) of the resulting silage. Silage *in vitro* DM digestibility was unaffected by treatments ($p > 0.10$). Silage butyrate concentrations were generally low; however, the combined inoculants increased ($p < 0.05$) silage butyrate content (0.73 g/kg) compared with control (0.26 g/kg), 'E' (0.24 g/kg) and 'B' (0.21 g/kg) treatments. The corn crop with lactic acid producing bacteria (E) tended ($p < 0.10$) to have higher silage ammonia concentrations compared to the corn crop with the combined dose or 'E + B' (2.36 g/kg vs. 1.90 g/kg).

The rumen pH at 4 h post-feeding in cows fed the control silage was not different from that of cows fed inoculants treated silages (Table 3). Rumen pH was, however, lower ($p < 0.05$) in cows fed silages treated with 'B' and 'E + B' vs. 'E' alone (5.94 and 5.85 vs. 6.24). As such, rumen total VFA concentrations were higher ($p < 0.05$) in cows fed 'E + B' treated silages than in cows fed 'E' treated and control silages. Rumen acetate concentrations were lower in cows fed control (61.1 mmol/l) or 'E' treated (61.8 mmol/l) silages than in cows fed 'B' treated (68.8 mmol/l) or 'E + B' treated (72.3 mmol/l) silages. Cows fed the silage with both inoculants tended ($p \leq 0.10$) to have higher propionate concentrations compared with other groups. The 'acetate + butyrate to propionate' ratio and butyrate and branched-chain fatty acids concentrations were unaffected by treatments (Table 3).

Feed intake, milk production and energy partitioning

Total tract nutrient digestibility coefficients were comparable amongst treatments (Table 4). Control

Table 2 Biochemical properties of control and inoculant corn silages in g/kg of DM except for pH

	Corn silages with or without microbial additives*				
Item	Control	Ecosyle (E)	Biotal (B)	E + B	SE
DM	216.8	221.6	213.6	219.9	3.9
CP	74.3	74.9	74.4	72.9	1.7
pH	3.65	3.67	3.67	3.62	0.04
Ash	89.9	91.5	90.0	91.1	2.7
Water soluble carbohydrates	29.8^{b}	38.0^{ab}	47.7^{a}	40.2^{ab}	5.5
NDF	494.1^{a}	485.8^{ab}	464.0^{b}	475.9^{ab}	9.7
ADF	318.0^{a}	306.4^{ab}	293.8^{ab}	282.3^{b}	11.4
Ammonia†	1.95	2.36	2.09	1.90	0.2
Lactate	76.6	85.8	74.8	78.1	6.3
Acetate	31.1	24.9	27.6	24.7	3.3
Propionate	0.25	1.04	0.93	0.59	0.47
Butyrate	0.26^{b}	0.24^{b}	0.21^{b}	0.73^{a}	0.10
Total VFA	108.0	109.3	103.8	103.5	6.3
In vitro DM digestibility	574.0	562.0	563.1	598.9	15.9

Means with different superscripts in the same row differ at $p < 0.05$.
*Control = no inoculant, 'E' = Ecosyl (100 000 cfu, Ecosyl®) containing *Lactobacillus plantarum*, 'B' = Biotal (100 000 cfu, Biotal®) containing *Pediococcus pentosanus*, *Lactobacillus plantarum* and *Propionibacter freudenreichii*. 'E + B', 200 000 cfu. Three replicate samples were analysed per treatment.
†For 'E' vs. 'E + B', $p = 0.10$.

Table 3 Rumen pH and metabolite concentrations in mmol/l

	Corn silages with or without microbial additives*				
Item	Control	Ecosyle (E)	Biotal (B)	'E + B'	SE
pH	6.04^{ab}	6.24^{a}	5.94^{b}	5.85^{b}	0.13
Total VFA	104.6^{b}	108.3^{b}	111.7^{ab}	123.7^{a}	6.65
Acetate	61.1^{b}	61.8^{b}	68.8^{a}	72.3^{a}	2.77
Propionate†	22.8	22.9	22.9	27.5	2.56
Butyrate	14.6	14.5	13.9	16.5	2.15
Valerate	2.2	3.7	1.8	2.8	0.95
Isovalerate	1.9	2.0	2.1	1.9	0.42
Isobutyrate	0.9	1.2	0.9	0.7	0.30
Caproate	1.2	2.2	1.2	1.3	0.64
Acetate + butyrate:propionate	3.3	3.3	3.7	3.3	0.30

Means with different superscripts in the same row differ at $p < 0.05$.
*Control = no inoculant, 'E' = Ecosyl (100 000 cfu, Ecosyl®) containing *Lactobacillus plantarum*, 'B' = Biotal (100 000 cfu, Biotal®) containing *Pediococcus pentosanus*, *Lactobacillus plantarum* and *Propionibacter freudenreichii*. 'E + B', 200 000 cfu.
†For 'E + B' vs. other treatments, $p \leq 0.10$.

Table 4 Total tract nutrient digestibility ($p > 0.10$)

	Corn silages with or without microbial additives*				
Item	Control	Ecosyle (E)	Biotal (B)	'E + B'	SE
DM %	65.3	69.2	66.7	66.9	2.3
OM %	66.6	70.5	68.1	68.7	2.1
CP %	65.3	69.3	65.2	67.4	2.2
NDF %	58.1	61.3	60.0	61.6	2.5
ADF %	54.7	57.7	56.7	56.5	2.8

*Control = no inoculant, 'E' = Ecosyl (100 000 cfu, Ecosyl®) containing *Lactobacillus plantarum*, 'B' = Biotal (100 000 cfu, Biotal®) containing *Pediococcus pentosanus*, *Lactobacillus plantarum* and *Propionibacter freudenreichii*. 'E + B', 200 000 cfu.

and 'E + B' fed cows had lower DMI ($p < 0.05$) than 'E' fed cows (Table 5). Refusals amounts were similar among treatments. Milk energy density and its 'fat to protein ratio' did not differ, but milk fat yield was lower for 'B' cows compared with that for control cows ($p < 0.05$). Milk protein was unaffected by inoculants. Milk energy yield were similar for control and 'E + B', while both resulted in numerically greater ($p \leq 0.10$) milk energy yield vs. 'B'. Either of 'E' and 'B' alone decreased ($p < 0.05$) the ratio of milk NE_L to consumed NE_L compared with when both were applied together. The estimated maintenance energy expenditure (i.e. NE_L intake – [Milk NE_L + BW gain NE_L]) tended to increase ($p \leq 0.10$) when the two inoculants were used separately, but not together (Table 5).

Discussion

The current study is the first to determine effects of particular doses and combinations of certain lactic and propionic acid bacteria type inoculants on silage biochemistry and consequently on mid-lactation cow performance. In addition, the study utilized diets consisting of reasonable and statistically practical levels of corn silage that would allow biological effects to be detected if truly existed, thus avoiding type 2 statistical error. Moreover, an emphasis on mid-lactation cows highlights wide applications of the data to on-farm scenarios, considering an overwhelming majority of mid-lactation cows in commercial settings.

Table 5 Milk production and composition, BW gains and energy dynamics calculations

	Corn silages with or without microbial additives*				
	Control	Ecosyle (E)	Biotal (B)	'E + B'	SE
DMI, kg/d	21.5^{b}	22.8^{a}	22.4^{ab}	21.7^{b}	0.50
Refusals, kg/d	1.6	1.4	1.5	1.5	0.37
NE_L intake (DMI × 1.6), Mcal/day	34.4^{b}	36.5^{a}	35.8^{ab}	34.7^{b}	0.80
Milk yield, kg/day	33.7	32.9	32.9	34.1	0.86
Milk fat, g/kg	40.2	39.9	39.0	39.7	1.18
Fat yield, kg/day	1.35^{a}	1.31^{ab}	1.27^{b}	1.33^{ab}	0.034
Milk crude protein, g/kg	30.5	30.8	30.6	30.8	0.27
Crude protein yield, kg/day	1.02	1.01	1.02	1.04	0.024
Milk lactose, g/kg	54.4	54.5	54.7	54.4	0.42
Lactose yield, kg/day	1.84	1.80	1.81	1.86	0.050
Milk energy†, Mcal/kg	0.75	0.75	0.75	0.75	0.011
Milk energy yield‡, Mcal/day	25.4	24.8	24.5	25.6	2.18
Milk fat : protein	1.32	1.30	1.28	1.28	0.035
Body weight (BW), kg	649.5	653.8	650.5	652.0	3.00
BW gain, g/d	63	356	275	144	196.7
BW gain NE_L§, Mcal/day	0.15	0.89	0.69	0.35	0.48
Milk NE_L : total NE_L intake	0.74^{a}	0.68^{b}	0.69^{b}	0.74^{a}	0.025
BW gain NE_L : total NE_L intake	0.004	0.024	0.019	0.010	0.0142
NE_L intake – (Milk NE_L + BW gain NE_L)¶, Mcal	8.82	10.81	10.58	8.69	1.00

Means with different superscripts in the same row differ at $p < 0.05$.

*Control = no inoculant, 'E' = Ecosyl (100 000 cfu, Ecosyl®) containing *Lactobacillus plantarum*, 'B' = Biotal (100 000 cfu, Biotal®) containing *Pediococcus pentosanus*, *Lactobacillus plantarum* and *Propionibacter freudenreichii*. 'E + B', 200 000 cfu.

†[(0.093 × fat content) + (0.054 × crude protein content) + (0.039 × lactose content)] g/kg; NRC (2001).

‡For Control vs. 'B', $p = 0.10$; 'B' vs. 'E + B', $p < 0.10$.

§Estimated as [(proportion empty body fat × 9.4) + (proportion empty body protein × 5.55)]; empty BW gain = 0.817 × live BW gain; NRC (2001).

¶For Control vs. 'E', 'E' vs. 'E + B', Control vs. 'B', and 'B' vs. 'E + B', $p \leq 0.10$.

Although ensiling reduces plant and nutrient wastage and allows continuous access to palatable forage sources of energy and effective NDF (Muck and Pitt, 1993; Kowsar et al., 2008), it solubilizes nutrients of mainly proteins, thereby challenging efficient microbial assimilation of N and ATP into microbial mass in the rumen (Beever, 1993). It is also known that ensiling less nitrogenous plants with more easily fermentable carbohydrates (e.g. corn crop vs. alfalfa) is less difficult to optimize in bunker silos (McDonald et al., 1991). As such, excessive moisture and altered chemical composition at harvest and 'between harvest until ensiling' are rather the major challenges affecting corn crop preservation quality and silage nutritional value. Thus, what remains unanswered for the prime agriculturists is whether certain doses or combinations of microbial inoculants will affect silage quality and cow performance when applied to low-DM corn crop easily exposed to adverse fermentation conditions. Such answers will provide bio-scientific grounds for economical assessment of using such inoculants for low-DM corn crop.

The similar silage pH and concentrations of DM, CP, lactate, acetate, ash and total VFA with low-DM corn crop agree with earlier studies using high-DM crops (Bergen et al., 1991; Kung et al., 1993; Merry et al., 1995), suggesting that these microbial inoculants did not alter fermentation of low DM corn crop. The much lower WSC content of silage vs. fresh crop (e.g. 29.8 g/kg vs. 198 g/kg) indicates some microbial use of easily fermentable carbohydrates during ensilage. The higher silage WSC and lower NDF suggest that the enzymatic activities of *Pediococcus pentosanus* and *Propionibacter freudenreichii* may have increased fibre fermentation. However, the reason for the increased silage butyrate concentrations by the combined inoculants is not entirely known. As both inoculants alone did not affect silage butyrate levels, only an imaginary interaction of lactic and propionic acid bacteria could have increased silage butyrate with both inoculants together. The optimally low butyrate concentrations were indicative of fine quality control silo fermentation, thus partly explaining minor treatment responses. The similar silage ammonia concentrations suggest no major effects or only little impact of lactic and propionic acid producing bacteria on proteolysis and N use during ensiling. In addition, unaffected ammonia levels suggest no or minor effects on establishment of favourable bacteria for a rapid decline of silage pH in the absence of inoculants. These data, alongside the similar *in vitro* digestibility values (Table 2) provide novel insights into practicality of using microbial inoculants in low-DM corn crop ensilage, suggesting limited influence of facultative lactic and propionic acid producing microbials on low-DM corn silage quality parameters.

Inoculants may hasten establishment of lactic acid producing bacteria, increase energy efficiency and minimize spoilage (McDonald et al., 1991; NRC, 2001; Guo et al., 2007). These mean a more controlled release of substrates during early fermentation that will maintain silage nutritional value and palatability later on. Such goals are more challenging to accomplish when fresh forage is lower in WSC and higher in degradable CP. Compared with legume forages, corn crops have much lower CP, and higher WSC and starch, making a high quality silage less difficult to achieve. However, the current study suggests that low-DM corn crops do not significantly benefit from inoculants, at least during ensiling. In addition, the data suggest that the relatively high WSC and low CP contents of corn crop already suffice and facilitate optimum silage preservation in the absence of these microbials.

Increased rumen VFA concentrations do not necessarily suggest increased daily rumen VFA production. The reasoning stands because a multitude of factors including DM and effective NDF intakes, chewing activity, rumen buffering capacity and outflow rate, and diurnal variation in microbial activity and VFA production and absorption collectively contribute to varying VFA concentrations and absorption rates (Robinson et al., 1997; Kowsar et al., 2008; Nikkhah et al., 2008). As a result, altered VFA concentrations do not necessarily represent altered nutrient metabolism, and thus, milk solids precursors bioavailability. The relatively higher rumen VFA concentrations in 'E + B' fed is consistent with their lower rumen pH. To enlighten the current results, future early lactation studies would benefit from multiple rumen fluid sampling that could additionally determine post-feeding patterns in rumen fermentation. Lack of treatment effects on total tract nutrient digestibility concurs with no effects on *in vitro* DM digestibility, suggesting that treatment differences in silo and rumen fermentation properties, as well as DMI were insufficient to cause differences in total tract digestion.

Increased DMI for 'E' vs. 'E + B' suggests an improvement in silage palatability by mainly lactic acid bacteria, and not necessarily by a combination of lactic and propionic acids bacteria. The limited and similar amounts of refusals indicate that cow selection for or against particular feed particles did

not affect treatment responses in DMI, rumen fermentation, and milk production. The reasonably high milk fat levels and fat to protein ratios across treatments are suggestive of healthy rumen conditions and adequate eNDF intake and digestion (Kowsar et al., 2008). The finding that 'E' and 'B', when used alone, reduced the ratio of milk NE_L to consumed NE_L, may suggests that a combination of lactic and propionic acid producing bacteria that maintains DMI (vs. control) also maintains cow productivity; while increased DMI by 'E' or 'B' did not increase productivity. As such, reduced proportional partitioning of NE_L intake toward milk for 'E' and 'B' vs. control and 'E + B' was partly due to some increase in tissue deposition, and to a larger degree was due to trends for increased maintenance energy expenditure (Table 4).

In summary, inoculants containing either 'E' or *Lactobacillus plantarum* (100 000 cfu); 'B' or *Pediococcus pentosanus*, *Lactobacillus plantarum,* and *Propionibacter freudenreichii* (100 000 cfu); and 'E + B' (200 000 cfu) were applied to low-DM corn crop (204 g/kg of fresh crop at harvest) to determine whether and how inoculants affect low-DM corn crop silage quality and cow performance. Inoculant 'B' resulted in higher and lower silage content of WSC and NDF respectively. The combined doses affected silage butyrate, rumen pH and rumen VFA concentrations only to some extent. Inoculants had no effects on silage pH and content of DM, CP, lactate, acetate, propionate, and ammonia levels or *in vitro* DM digestibility. Despite increased DMI by 'E', total tract nutrient digestibility, milk production and milk energy yield did not increase. Results suggest that using bacterial inoculants to accelerate lactate and propionate synthesis and improve silage quality during low-DM corn crop in bunker type silos did not improve mid-lactation cow productivity despite effects on silage composition and DMI. The data are of practical importance, as a majority of cows in commercial dairies are in mid lactation. In light of the moderately increased DMI, future studies during early lactation, when DMI is usually insufficient, and measuring other silage parameters (e.g. aerobic stability) will further conclusions on the biological magnitude of altered DMI on energy balance and milk secretion.

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References

AOAC, 1997: *Official Methods of Analysis*, 15th edn. Association of Official Analytical Chemists, Arlington, VA, USA.

Bal, M. A.; Shaver, R. D.; Jirovec, A. G.; Shinners, K. J.; Coors, J. G., 2000: Crop processing and chop length of corn silage: effects on intake, digestion, and milk production by dairy cows. *Journal of Dairy Science* **83**, 1264–1273.

Beever, D. E., 1993: Rumen function. In: J. M. Forbes, J. France (eds), *Quantitative Aspects of Ruminant Digestion and Metabolism*, 1st edn. CABI Publishing, Wallingford, UK, pp. 187–215.

Bergen, W. G.; Byrem, T. M.; Grant, A. L., 1991: Ensiling characteristics of whole-crop small grains harvested at milk and dough stages. *Journal of Animal Science* **69**, 1766–1774.

Charmley, E.; Winter, K. A.; McRae, K. B.; Fillmore, S. A. E., 1996: Effect of inoculation on silage quality and performance of steers fed grass and cereal silages either alone or in combination. *Canadian Journal of Animal Science* **76**, 571–577.

Cochran, W. G.; Cox, G. M., 1992: *Experimental Designs*, 2nd edn. Wiley-Interscience Publications, New York, USA.

Dubios, M.; Giles, K. A.; Hamilton, J. K.; Ronerts, P. A.; Smith, F., 1956: Colorometric method for determination of sugars and related substances. *Annual Chemistry* **28**, 350.

Filya, I., 2003: The Effect of Lactobacillus buchneri and Lactobacillus plantarum on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. *Journal of Dairy Science* **86**, 3575–3581.

Fox, D. G.; Tedeschi, L. O.; Tylutki, T. P.; Russell, J. B.; Van Amburgh, M. E.; Chase, L. E.; Pell, A. N.; Overton, T. R., 2004: The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. *Animal Feed Science and Technology* **112**, 29–78.

Gordon, F. J.; Dawson, L. E. R.; Ferris, C. P.; Steen, R. W. J.; Kilpatrick, D. J., 1999: The influence of wilting and forage additive type on the energy utilization of grass silage by growing cattle. *Animal Feed Science and Technology* **79**, 15–27.

Guo, X. S.; Ding, W. R.; Han, J. G.; Zhou, H., 2007: Characterization of protein fractions and amino acids in ensiled alfalfa treated with different chemical additives. *Animal Feed Science and Technology* **142**, 89–98.

Iranian Council of Animal Care, 1995: *Guide to the Care and Use of Experimental Animals*, Vol. 1. Isfahan University of Technology, Isfahan, Iran.

Jonsson, A., 1991: Growth of clostridium tyrobutiricum during fermentation and aerobic deterioration of grass silage. *Journal of Science, Food and Agriculture* **54**, 557–568.

Kowsar, R.; Ghorbani, G. R.; Alikhani, M.; Khorvash, M.; Nikkhah, A., 2008: Corn silage partially replacing short alfalfa hay to optimize forage use in total mixed rations for lactating cows. *Journal of Dairy Science* **91**, 4755–4764.

Kung, L. Jr; Chen, J. H.; Knutsen, E. M., 1993: Effect of microbial inoculants on the nutritive value of corn silage for lactating dairy cows. *Journal of Dairy Science* **76**, 3763–3770.

McAllister, T. A.; Selinger, L. B.; McMahon, L. R.; Bae, H. D.; Lysyk, T. J.; Oosting, S. J.; Cheng, K. J., 1995: Intake, digestibility and aerobic stability of barley silage inoculated with mixture of Lactobacillus Plantarum and Enterococcus faecium. *Canadian Journal of Animal Science* **75**, 425–432.

McDonald, P.; Henderson, A. R.; Hesron, S. J. E., 1991: *The Biochemistry of Silage*, 2nd edn. Chalcombe Publications, Marlow, UK.

McDougall, E. I., 1948: Studies on ruminant saliva. The composition and ourput of sheep's saliva. *Biochemistry Journal* **43**, 99–109.

Merry, R. J.; Winters, A. L.; Thomas, P. L.; Muller, M.; Muller, T., 1995: Degradation of fructans by epiphytic and inoculated lactic acid bacteria and plant enzymes during in silage of normal and sterile hybrid ryegrass. *Journal of Applied Bacteriology* **79**, 583–591.

Muck, R. E.; Pitt, R. E., 1993: *Ensiling and its Effect on Crop Quality*. in Proc. Natl. Silage Prod. Conf., Syracuse, NY. NRAES-67. Northeast Reg. Agric. Ext. Serv., Ithaca, NY, pp. 57–66.

Nikkhah, A.; Alikhani, M.; Amanlou, H., 2004: Effects of feeding ground or steam-flaked broom sorghum and ground barley on performance of dairy cows in mid-lactation. *Journal of Dairy Science* **87**, 122–130.

Nikkhah, A.; Furedi, C. J.; Kennedy, A. D.; Crow, G. H.; Plaizier, J. C. P., 2008: Effects of feed delivery time on feed intake, rumen fermentation, blood metabolites and productivity of lactating cows. *Journal of Dairy Science* **91**, 1–12.

Nordlund, K. V.; Garrett, E. F., 1994: Rumenocentesis: a technique for collecting rumen fluid for the diagnosis of subacute rumen acidosis in dairy herds. *Bovine Practitioner* **28**, 109–112.

NRC, 2001: *Nutrient Requirements of Dairy Cattle*, 7th rev edn. Nat. Acad. Sci., Washington, DC.

Ranjbari, M.; Ghorbani, G. R.; Nikkhah, A.; Khorvash, M., 2007: Chemical composition, crude protein fractions, and ruminal degradation of maize silage produced in Isfahan. *International Journal of Dairy Science* **2**, 66–72.

Rizk, C.; Mustafa, A. F.; Phillip, L. E., 2005: Effects of inoculation of high dry matter alfalfa silage on ensiling characteristics, ruminal nutrient degradability and dairy cow performance. *Journal of Science, Food and Agriculture* **85**, 743–750.

Robinson, P. H.; Gill, M.; Kennelly, J. J., 1997: Influence of time of feeding a protein meal on ruminal fermentation and forestomach digestion in dairy cows. *Journal of Dairy Science* **80**, 1366–1373.

SAS User's Guide, 2003. *Version 9.1. Edition*. SAS Institute Inc., Cary, NC, USA.

Soltani, A.; Ghorbani, G. R.; Alikhani, M.; Samie, A. H.; Nikkhah, A., 2009: Ground versus steam-rolled barley grain for lactating cows: a clarification into conventional beliefs. *Journal of Dairy Science* **92**, 3299–3305.

Tilley, J. M. A.; Terry, R. A., 1963: A two stage technique for the in vitro digestion of forage crops. *Journal of British Grass Society* **18**, 104–111.

Van Soest, P. J.; Robertson, J. B.; Lewis, B. A., 1991: Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597.

Weinberg, Z. G.; Ashbell, G.; Hen, Y., 1999: The effect of Lactobacillus buchneri and L. plantarum, applied at ensiling, on the ensiling fermentation and aerobic stability of wheat and sorghum silages. *Journal of Industrial Microbiology* **23**, 218–222.