Effect of Microbial Inoculants on the Nutritive Value of Corn Silage for Lactating Dairy Cows[1]

L. KUNG, JR., J. H. CHEN, E. M. KRECK, and K. KNUTSEN
Delaware Agricultural Experiment Station
Department of Animal Science and Agricultural Biochemistry
College of Agricultural Sciences
University of Delaware
Newark 19717-1303

ABSTRACT

Two experiments were conducted to evaluate the effect of microbial inoculation on the composition and nutritive value of corn silage for lactating cows. In Experiment 1, forage was untreated or treated at ensiling with Pioneer® 1174 or Ecosyl® silage inoculants. Forage was offered for free choice consumption, and concentrate was fed by a computerized feeder. Treatment with 1174 inoculant had little effect on silage composition and no effect on cow performance. Silage treated with Ecosyl® inoculant had greater lactic acid content, but also greater acetic acid and ammonia N contents. Production of 3.5% FCM was greatest from cows fed silage treated with Ecosyl®. In Experiment 2, silage was untreated or treated with Ecosyl® and fed in a TMR. Inoculation had no effect on silage composition but increased 3.5% FCM production and DMI as length of time on treatment increased. Microbial inoculation can improve the nutritive value of corn silage for lactating cows even if changes in fermentation end products are minimal.
(**Key words**: silage, microbial inoculant, fermentation, lactating cows)

INTRODUCTION

Microbial inoculation has improved the fermentation of grass and legume silages (7, 9). Microbial inoculation appears to have minimal effects on the fermentation of corn silage (2, 5, 19). However, changes in silage fermentation have not always been related to improved animal performance (17). In two studies (7, 8), cows fed inoculated grass silage produced more milk than those fed untreated silage, despite minor effects on silage composition. In addition, only one other study (20) described the nutritive value of inoculated corn silage for lactating dairy cows. Wohlt (20) reported that inoculated silages appeared to be more stable upon exposure to air and, when fed to cows, increased FCM by .7 kg/d.

The objective of this study was to determine the effect of microbial inoculation on the fermentation of corn silage treated with two bacterial inoculants and the subsequent effect on nutritive value and performance by lactating dairy cattle.

MATERIALS AND METHODS

Experiment 1

Corn silage was harvested randomly in the medium-dough stage of maturity from a uniform field and stored in bag silos. The field was sectioned randomly to minimize bias. Treatments were made in the following order: 1) untreated silage, 2) silage treated with Ecosyl® inoculant (*Lactobacillus plantarum*; Zeneca Bio Products, Billingham, England), or 3) silage treated with Pioneer® 1174 inoculant (*L. plantarum* and *Streptococcus faecium*; Pioneer Hybrid International Inc., West Des Moines, IA). The alternate load method was not used to fill silos because of logistics. Inoculants were mixed in water and applied according to the label instructions to supply 1 × 10^5 cfu of lactic acid bacteria/g of silage. Inoculation was performed at the bagger with a sprayer (Nevtro Sales Ltd., London, ON, Canada). To void contamination, the last load of

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forage placed in the Ecosyl® bag was left untreated and was not used in the study. In addition, the previous inoculant mix was drained from the tank, which subsequently was flushed with water for 5 min before being refilled with the 1174 inoculant. Approximately 45 to 55 tonnes of forage were made for each treatment. All forages were treated and sealed in their respective bag silos within 6 h on a clear day.

During silo filling, grab samples of forage were collected from each wagon load (treated samples were collected after inoculation). Forage was chilled on ice immediately until processing. Forage (25 g) was homogenized for 1 min in 250 ml of deionized water, and the pH was recorded. The homogenate was filtered through Whatman filter paper (number 54; Clifton, NJ), acidified, and frozen until further analyses. Water extracts were analyzed for ammonia N (13) and for acetic and lactic acids by gas chromatography (model 5890A; Hewlett-Packard Co., Avondale, PA) using a 80/20 Carbopack B-DA/4% Carbowax® 20M column (Supelco, Inc., Bellefonte, PA). Representative samples were air dried before being ground through a 1-mm screen. Ground samples were analyzed for DM (60°C for 48 h in a forced-air oven), ADF (6), NDF (18), and N (1). Mixtures of inoculant and water and samples of forage were plated for viable lactic acid bacteria on Rogosa SL agar (Difco, Detroit, MI) with a single overlay and incubated for 48 h at 30°C.

Three nylon bags containing 500 g of representative forage for each treatment were buried in each silo. Buried bags were removed during feeding, and DM recovery was calculated by difference after correction for DM content.

After 130 d of ensiling, silages were fed to multiparous lactating Holstein cows in two experimental periods. In each period, cows averaged 70 ± 33 DIM and were housed in a barn with 20 Calan gates (American Calan, Northwood, NH) and a computerized grain feeder. Based on pretreatment milk production, cows were blocked in groups of three and assigned randomly to one of three experimental diets varying in corn silage treatment: control silage, silage treated with Ecosyl®, and silage treated with 1174. Cows were fed silage for ad libitum intake twice daily (0800 and 1700 h) and received 1.36 kg of long alfalfa hay once daily (0800 h) through the Calan gate system. A pelleted concentrate was fed through a computerized grain feeder and limited to 1 kg/3 kg of milk. The concentrate was formulated to contain 1.5% sodium bicarbonate, 1.5% iodized trace-mineralized salt (with .6 ppm of sodium selenite), and .6% magnesium oxide. The concentrate also contained 8000 IU of vitamin A, 2000 IU of vitamin D, and 30 IU of vitamin E/454 g of feed. A 3-wk preliminary period preceded a 6-wk treatment period. During the pretreatment period, cows were fed a mixture of the three silages. Fresh water was available at all times. Weights of silage offered and refused (once daily) were recorded. The grain feeder was calibrated twice within each period, and grain consumption was recorded each day by computer.

Cows were weighed on 2 consecutive d at the start and end of each period and at weekly intervals during treatment. Composite milk samples from consecutive p.m. and a.m. milkings were collected and analyzed weekly by the Maryland DHIA Laboratory for fat and protein (Milk-O-Scan; Foss Technology, Hillerød, Denmark). Silage, grain, and ort samples were collected on alternate days and composited on a weekly basis. All feeds were analyzed for DM, NDF, ADF, and N as described. Silages also were analyzed for fermentation end-products as previously described.

Experiment 2

Corn silage was harvested in the medium-dough stage of maturity from the same field and stored in bag silos. Treatments were untreated silage or silage treated with Ecosyl® inoculant (*L. plantarum*). Application was as described in Experiment 1. All forages were treated and sealed in their respective bag silos within 4 h on a clear day.

After at least 120 d of ensiling, corn silage was fed to 20 multiparous Holstein cows (average 73 ± 25 DIM) that had access to Calan gates. On the basis of pretreatment milk production, cows were blocked in groups of two and assigned randomly to one of the two treatments. The diet was fed as a TMR that consisted of 50% corn silage, 45% concentrate, and 5% alfalfa hay on a DM basis. The concentrate and hay were similar in composition to feeds used in Experiment 1. Cows were fed the TMR for ad libitum intake twice daily (0800 and 1700 h). A 10-d preliminary period preceded a 9-wk pretreatment period. During the pretreatment period, cows were fed a mixture of the two silages. Fresh water was available at all times. Cows were milked twice daily, and milk production was recorded by computer.

Cows were weighed on 2 consecutive d at the start and end of the treatment period and at weekly intervals during treatment. Composite milk samples from consecutive p.m. and a.m. milkings were collected and analyzed weekly for fat and protein. Silage and TMR samples were collected on alternate days, composited, and analyzed on a weekly basis. Feed refusals were collected once daily, weighed, and analyzed for DM content (60°C for 48 h). The DMI was calculated by the difference of feed offered and feed refused. Feeds were analyzed as previously described.

Statistical Analyses

Experiment 1. Data were analyzed by ANOVA by the general linear models procedure of SAS (14). Model effects for the lactation data included period, block(period), treatment, period × treatment, block × treatment(period), week, period × week, block × week (period), treatment × week, and period × treatment × week. Data from the pretreatment period were used as covariate for all variables. Treatment was tested using the type III mean squares for period × treatment as an error term. Significance was declared at $P < .05$ unless otherwise noted.

Experiment 2. Model effects for the lactation data included block, treatment, treatment × block, week, and treatment × week. Data from the pretreatment period were used as covariate for all variables. Treatment was tested using the type III mean squares for treatment × block as an error term. Significance was declared at $P < .05$ unless otherwise noted.

RESULTS

The composition of hay and concentrate fed during Experiment 1 is shown in Table 1. Alfalfa hay was of average quality, and the concentrate DM averaged 26.3% CP. The mixtures of water and inoculant applied to forages at ensiling contained 73×10^6 cfu (Ecosyl® inoculant) and 32×10^6 cfu (Pioneer® 1174)/ml of lactic acid-producing bacteria. Accurate counts of colonies of lactic acid bacteria from forage samples were not possible because of growth of bacilli organisms. Thus, lactic acid bacteria on all forage samples (untreated and treated) were roughly estimated to be 1×10^5 cfu. All corn silages fermented well, and their compositions during feeding are shown in Table 2. The DM of control silage was higher than that of inoculated silages, but the difference was small. The CP, ADF, NDF, and mineral contents were similar for all silages. The pH of silage treated with 1174 was greater than that of control and silage treated with Ecosyl®. Lactic acid ($P < .10$), acetic acid, and ammonia N content were greater in silage treated with Ecosyl®. Dry matter recovery was similar among treatments.

Milk production and composition, feed intake, and BW changes from Experiment 1 are shown in Table 3. Pretreatment milk production was about 37 kg/d. Milk production and milk protein content were similar among treatments during the treatment periods. Although not statistically different, milk fat content tended to be greater from cows fed silage treated with Ecosyl® (3.60%) than from those

TABLE 1. Composition of alfalfa hay and grain fed in Experiment 1.

Item	DM	CP	ADF	NDF	Ca	P	Mg	K
	(% of DM)							
Alfalfa hay[1]	90.5	17.9	34.9	48.8	.94	.30	.21	3.00
Grain mix[2]	89.8	26.3	12.1	29.1	1.37	.74	.67	1.34

[1]n = 11.

[2]n = 12.

TABLE 2. Composition of silages (DM basis) offered during Experiment 1.

Item	Control	Ecosyl®[1]	1174[2]	SE[3]
DM, %	34.7[a]	32.9[b]	33.3[b]	.5
CP, %	7.71	7.60	7.57	.14
ADF, %	22.7	24.0	24.5	.5
NDF, %	44.1	43.1	43.8	.8
Ca, %	.25	.23	.22	. . .
P, %	.26	.26	.25	. . .
Mg, %	.20	.19	.18	. . .
Mg, %	.20	.19	.18	. . .
K, %	1.01	1.19	1.11	. . .
pH	3.70[b]	3.69[b]	3.81[a]	.03
Lactate, %	4.71[d]	5.28[c]	4.48[d]	.27
Acetate, %	1.82[b]	2.36[a]	1.65[b]	.18
Ammonia N, %	.062[b]	.072[a]	.056[b]	.004
DM Recovery, %	94.3	92.8	90.7	1.7

[a,b]Means in columns with unlike superscripts differ ($P < .05$).

[c,d]Means in columns with unlike superscripts differ ($P < .10$).

[1]Zeneca Bio Products, Billingham, England.

[2]Pioneer Hybrid International, West Des Moines, IA.

[3]n = 12 for all components, except n = 3 for DM recovery, and n = 1 for minerals.

fed control silage (3.46%) or silage treated with 1174 (3.48%). As a result, cows fed silage treated with Ecosyl® produced 1.5 and 1.4 kg more 3.5% FCM than cows fed control silage and silage treated with 1174, respectively. Silage, hay, and concentrate intakes were similar among treatments. Similarly, BW, BW changes, and feed efficiencies were not different among treatments, but cows fed silage treated with Ecosyl® tended to gain more weight and have greater feed efficiencies.

TABLE 3. Milk production, intake and BW weights from cows fed inoculated corn silages in Experiment 1.

	Control	Ecosyl®[1]	1174[2]	SE[3]
Milk				
kg/d	35.5	35.6	35.2	.8
Fat, %	3.46	3.60	3.48	.08
Protein, %	3.08	3.05	3.02	.03
3.5% FCM, kg/d	34.8	36.3	34.9	.9
DMI, kg/d				
Silage	11.9	10.8	11.7	.3
Hay	1.2	1.2	1.2	
Concentrate	10.3	10.6	10.2	.2
Total	23.4	22.6	23.1	.4
BW, kg	623	636	633	7
BW Change per d, kg	.29	.51	.30	
Feed efficiency				
Milk/feed, kg/kg	1.51	1.57	1.52	.03
Feed costs,[4] $/d	3.05	3.06	3.03	. . .
Milk income[5]	9.20	9.59	9.22	. . .
Income over feed costs,[6] $/d	6.15	6.53	6.19	. . .

[1]Zeneca Bio Products, Billingham, England.

[2]Pioneer Hybrid International, West Des Moines, IA.

[3]All values are least square means; n = 11 or 12; largest standard errors reported.

[4]Based on intakes and the following costs: cost to inoculate 909 kg of silage (wet basis) as $.80. Feed costs per kilogram of DM: corn silage, $.075, inoculated silages, $.078; hay, $.122, and concentrate, $.196.

[5]Milk was valued at $.264/kg of 3.5% FCM.

[6]Milk income minus feed costs.

TABLE 4. Composition (DM basis) of TMR in Experiment 2.[1,2]

Item	DM	CP	ADF	Ca	P	Mg	K
	(%)						
Control	55.8	16.5	21.2	.81	.42	.34	1.59
Ecosyl®[3]	55.7	16.4	19.4	.83	.42	.35	1.49
SE	.5	.2	.8	. . .	. . .	. . .	. . .

[1]DM, CP, and ADF: average values from eight weekly composite samples.

[2]Minerals: one analysis from a composite of weekly samples.

[3]Zeneca Bio Products, Billingham, England.

Cows fed the diet containing silage treated with Ecosyl® had income over feed costs in excess of $.30/d more per cow.

Experiment 2

The mixture of Ecosyl® inoculant and water applied to forage containing 55×10^6 cfu/ml of lactic acid-producing bacteria. Inoculation increased lactic acid bacteria from 53×10^4 to 200×10^4 cfu/g in treated silage.

The compositions of the TMR for control and treated groups were similar and are shown in Table 4. The DM, ADF, and CP contents of the TMR were constant throughout the study. Diets averaged about 16.5% CP and 20% ADF and met or exceeded requirements for macrominerals (12). The quality of corn silages fed during the study was good and did not differ between treatments (Table 5).

Milk production and composition, feed intake, and BW changes are shown in Table 6. Although values were not significantly different for the 9-wk treatment period, cows fed diets containing silage treated with Ecosyl® produced milk that tended to have more fat (+.19%; $P < .20$) and more 3.5% FCM (+1.8 kg; $P < .19$). The DMI was greater for cows fed silage treated with Ecosyl® and increased with the onset of treatment (Figure 1). Cows fed silage treated with Ecosyl® produced more 3.5% FCM during the last 3 wk of treatment than did cows fed diets with control silage (Figure 2). Milk protein content was unaffected by treatment. Changes in BW and feed efficiencies were unaffected by treatment. Cows fed silage treated with Ecosyl® had income over feed costs that were $.14/d greater per cow.

DISCUSSION

Microbial inoculation has improved the fermentation of legume silages by causing a more rapid decrease in pH, increasing lactic acid content, and decreasing ammonia and acetic acid contents (9). In Experiment 1, inoculation with 1174 silage inoculant had no effect on corn silage composition except for higher pH. Although inoculation with Ecosyl® silage inoculant had no effect on pH, lactic acid content was greater than in other treatments. However, in contrast to most findings with inoculants, acetic acid and ammonia N contents also were

TABLE 5. Composition (DM basis) of silages in Experiment 2.

Treatment	DM	CP	ADF	pH	Lactic acid	Acetic acid	NH_3 N
	(%)				(%)		
Control	39.8	8.68	23.3	3.79	4.03	.97	.056
Ecosyl®[1]	39.8	8.51	23.0	3.78	3.67	.94	.069
SE[2]	.5	.31	.5	.05	.32	.06	.003

[1]Zeneca Bio Products, Billingham, England.

[2]n = 8.

TABLE 6. Milk production, DMI, and BW from cows fed control and corn silage treated with Ecosyl® in Experiment 2.

	Control	Ecosyl®[1]	SE[2]
Milk, kg/d	36.9	37.5	.6
Fat, %	3.43	3.62	.07
Protein, %	3.05	3.07	.02
3.5% FCM, kg/d	36.5	38.3	.7
DMI, kg/d	22.8[b]	25.1[a]	.4
BW, kg	676	689	29
BW Change per d, kg	.20	.31	.17
Feed efficiency			
Milk/feed, kg/kg	1.61	1.54	.04
Feed costs,[3] $/d	3.01	3.34	. . .
Milk income[4]	9.65	10.12	. . .
Income over feed costs,[5] $/d	6.64	6.78	. . .

[a,b]Means with unlike superscripts differ ($P < .02$).

[1]Zeneca Bio Products, Billingham, England.

[2]n = 10.

[3]Based on intakes and the following costs: cost to inoculate 909 kg of silage (wet basis) was $.80. Feed costs per kilogram of DM: corn silage, $.075, inoculated silages, $.078; hay, $.122, and concentrate, $.196.

[4]Milk valued at $.264/kg of 3.5% FCM.

[5]Milk income minus feed costs.

increased by this treatment, which suggests poorer quality silage. Using the same inoculant, we reported (10) a marked decrease in acetic acid and ammonia N contents in wilted alfalfa silage. In Experiment 2, treatment with Ecosyl® silage inoculant had no effect on silage composition. Recently, Bolsen et al. (2) also reported that inoculation of corn silage with 1174 inoculant had no effect on the fermentation of corn silage. These findings are similar to other data (3, 4, 15, 16), which showed that inoculation had little effect on commonly measured fermentation characteristics of corn silage. High numbers of epiphytic

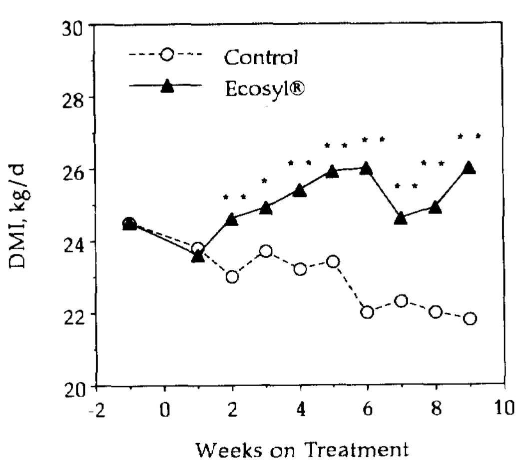

Figure 1. Effect of a microbial inoculant (Ecosyl®; Zeneca Bio Products, Billingham, England) on the total DMI of cows during 9 wk of treatment. * Different from control ($P < .07$); ** different from control ($P < .05$). Experiment 2.

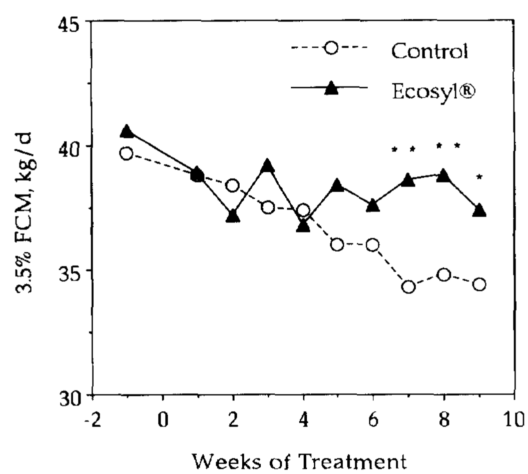

Figure 2. Effect of a microbial inoculant (Ecosyl®; Zeneca Bio Products, Billingham, England) on 3.5% FCM production of cows during 9 wk of treatment. * Different from control ($P < .07$); ** different from control ($P < .05$). Experiment 2.

lactic acid bacteria and good ensiling characteristics of the crop (e.g., low buffering capacity, high DM, and fermentable substrate) may be responsible for these findings (2).

In Experiment 1, we were unable to explain why cows fed silage treated with Ecosyl® tended to consume less silage and more concentrate than cows fed control silage or silage treated with 1174. Overall, silage treated with 1174 silage inoculant was similar to control silage and had no effect on cow performance. In Experiment 2, DMI increased with time on treatment for cows fed silage treated with Ecosyl® (Figure 1). In both experiments, cows fed silage treated with Ecosyl® tended to produce more 3.5% FCM than those fed control silage, resulting in increased return over feed costs of $.14 to .34/d per cow. However, milk production was increased the most in the later weeks of treatment for cows fed silage treated with Ecosyl® (Figure 2) in Experiment 2. No trend was found for improved performance with time on treatment in Experiment 1. The increase in FCM and intake for cows fed silage treated with Ecosyl® cannot be explained from the nutrient content of the TMR and silages because the two were similar for both treatments and varied little over weeks (data not shown). However, common measurements of silage fermentation (for example, lactic acid and ammonia N) cannot always be equated with cow performance. These findings suggest that factors other than commonly measured end products of fermentation may improve the nutritive value of silages and be responsible for improved performance. For example, Luther (11) reported that microbial inoculation had no effect on corn silage fermentation, but DM digestion and N retention in steers were improved. Wohlt (20) reported little difference in silage composition, but cows fed inoculated corn silage produced .7 kg/d more FCM than did cows fed control silage. Similarly, compositions of control and inoculated silages were similar, but cows fed grass silage treated with Ecosyl® produced 2.1 kg (7) and 1.3 kg (8) more milk than cows fed untreated grass silage.

In both experiments, income over feed costs were greater with silages inoculated with Ecosyl®. In Experiment 1, return on investment was >10, and, in Experiment 2, the return on investment was >3 for cows fed silages treated with Ecosyl® inoculant.

CONCLUSIONS

Addition of microbial inoculants to corn silage caused minor changes in silage fermentation. Inoculation of silage with 1174 silage inoculant had minimal effects on silage fermentation and no effect on cow performance. Inoculation with Ecosyl® silage inoculant tended to increase production of 3.5% FCM in two experiments and DMI in one experiment. Factors other than traditionally measured silage fermentation end products may be responsible for improved cow performance.

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