EFFECT OF A PROPIONIC ACID BACTERIAL INOCULANT ON FERMENTATION AND AEROBIC STABILITY OF WHOLE-PLANT CORN SILAGE [1]

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Summary

The effects of a strain of *Propionibacterium shermanii*, applied with and without lactic acid bacteria (LAB), on the ferment ation and aerobic stability of whole-plant corn silage was determined using laboratory-scale silos. The addition of LAB increased the rate of fermentation, and all inoculated silages underwent a more efficient ensiling process than control silage. Only silages made with *P. shermanii* had measurable levels of propionic acid in the 90-day silages. Corn silages made with *P. shermanii* were more stable when exposed to air than control or LAB-inoculated silages.

(Key Words: Silage, Aerobic Spoilage, Inoculant, Propionic Acid.)

Introduction

Aerobic instability during the feedout phase (i.e., short bunk life) is often a problem with whole-plant corn, sorghum, and winter cereal silages, particularly if the dry matter (DM) content exceeds 35%. When the silo is opened, oxygen has unrestricted access to the exposed feeding face. Aerobic microorganisms (i.e., yeast, mold, and bacteria) present in the silage can consume soluble nutrients, including lactic acid, which increases the te mperature and pH of the silage. If allowed to continue, the deterioration and spoilage can cause a silage to have virtually no nutritional value. A rapid removal of silage and correct sizing of the height and width of the feeding face can help minimize DM losses during the feedout phase.

Presently, there is considerable interest in using biological inoculants to overcome the problem of aerobic instability. These additives should produce substances in the silage that have antimycotic properties, which would inhibit the growth of yeast and mold in silage exposed to air. Propionic acid bacteria can ferment soluble carbo hydrates and lactic acid to propionic acid, which is an eff ective antimycotic agent. Although the production of propionic acid during the fermentation phase is a sound concept, results in a limited number of controlled experiments have been inconsistent.

Our objective was to deter mine the effect of a propionic acid bacterial inoculant on aerobic stability of whole-plant corn silage.

Experimental Procedures

A description of the whole-plant corn used in the trial, including harvest date, chemical composition, and epiphytic microflora, is presented in Table 1. The hybrid was Pioneer 3377, grown under irrigation and harvested at approximately 60 to 70% milk line stage of kernel maturity.

[1]Financial assistance and bacterial inoculants were provided by Lallemand S.A. Laboratorie Equipharm, Saint-Simon, France.

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Table 1. Chemical Composition and Epiphytic Microflora of the Chopped, Pre-Ensiled, Whole-Plant, Corn Forage

Item[1]	Value
Harvest date	August 21
Dry matter, %	33.5
pH	5.86
Buffering capacity, meq/100 g of DM	21.6
WSC[2]	11.4
CP[2]	8.2
NDF[2]	53.0
ADF[2]	24.8
LAB[3]	1.2 10 [7]
Yeast[3]	8.0 10 [6]
Mold[3]	1.2 10 [4]

[1]WSC = water-soluble carbohydrates; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; and LAB = lactic acid bacteria.
[2]Expressed as a percent of the forage DM.
[3]Colony-forming units per gram of forage.

The following six treatments were compared: 1) control (no additive), 2) Lallemand inoculant (to supply 700,000 cfu of LAB per gram of crop) (LAB), 3) *Propionibacterium shermanii* (to supply 1.4 10 [5] cfu per gram of crop) (PS 10^5), 4) treatment 2 + treatment 3, 5) *P. shermanii* (to supply 1 10 [6] cfu per gram of crop) (PS 10^6), and 6) treatment 2 + treatment 5. The Lallemand inoculant contained selected strains of *Lactobacillus plantarum* and *Pediococcus acidilactici* .

In preparation of the silages, 250 lb of chopped forage were placed on a polyethylene sheet, and the inoculants were applied and mixed thoroughly with the forage. All inoculants were applied as water solutions and used within 2 weeks after being received from the manufacturer. After all silages were prepared, the silos were filled on an alternating schedule, which distributed the time from harvest through silo filling equally across all treatments. The silos were packed with a hydraulic press, which exclude d air and filled all silos to similar densities.

There were 21 PVC laboratory-scale silos (4.5 lb capacity) and three polyethylene pail silos (35 lb capacity) per treatment. Three PVC silos per treatment were opened at 1/4, 1/2, 1, 2, 4, 7, and 90 days postfilling. The pail silos were opened at 90 days postfilling, and the silage from each treatment was composited. Aerobic stability was measured over a 10-day period using insulated, 4.5 lb capacity containers and thermocouple wires.

Chemical and Microbial Analyses of the Pre-ensiled Forage and Silages. Pre-ensiled forage was analyzed for DM; pH; total nitrogen; buffering capacity; water-solu ble carbohydrates; neutral detergent fibe r; acid detergent fiber; and total epiphytic LAB, yeast, and mold counts. Silage fermented from 6 hours to 7 days was analyzed for pH and lactic acid; end-product silages (90 days postfilling) were analyzed for pH, lactic acid, volatile fatty acids, ethanol, ammonia-nitrogen, and total yeast and mold counts. Silages in the aerobic stability measurements were analyzed for pH and total yeast and mold counts after 2, 4, 6, 8, and 10 days of exposure to air.

Results and Discussion

Although all six corn silages had very rapid rates of fermentation, those made with LAB (Treatments 2, 4, and 6) had lower ($P<.01$) pH values and higher ($P<.01$) lactic acid contents during the first 4 days postfilling than control silage (data not shown).

Fermentation characteristics and pH for the corn silages at 90 days postfilling are presented in Table 2. All five inoculated silages ensiled more efficiently than the control silage -- as evidenced by higher ($P<.05$) lactic acid contents and lactic to acetic acid ratios and lower ($P<.05$) acetic acid and ethanol contents. These results are consistent with several previous

studies on inoculated corn and sorghum silages (KAES Report of Progress 651, page 101). Only the four silages made with *P. shermanii* (with or without LAB) contained detectable amounts of propionic acid at 90 days. However, the level of this acid varied considerably among silages for each treatment and ranged from .06 to .33% of the silage DM.

Results of the aerobic stability measurements are shown in Tables 3 and 4. Corn silage treated with only PS 10^6 (Treatment 5) was clearly the most aerobically stable, and this silage also had the highest level of propionic acid (.22%). Corn silage made with only LAB (Treatment 2) had a slightly higher yeast count after the 90-day storage phase than the other five silages and also was the least stable in air. Its temperature exceeded the ambient by about 5°F after 94 hrs of exposure to air. Control and LAB + PS 10^5 silages (Treatments 1 and 4, respectively) began to heat and had yeast counts near $\log_{10}$ 8.00 after 118 to 122 hrs. PS 10^5 and LAB + PS 10^6 silages (Treatments 3 and 6, respectively) were the next to undergo aerobic spoilage after 146 to 148 hrs of exposure to air. When aerobic deterioration began in the four silages treated with *P. shermanii* it was at a slow rate and not the typical rapid increases in temperature and pH observed in aerobically unstable corn silage.

These results suggest that the strain of *P. shermanii* used was capable of competing with other microorganisms in the ensiling process; it produced propionic acid that was detectable in the 90-day corn silages. Silages inoculated with this organism were more stable during the feedout phase than control or LAB-inoculated silages. The *Propionibacterium* was more effective when applied at 10^6 cfu per gram of ensiled forage and when not in combination with LAB (i.e., *L. plantarum* and *P. acidilactici*).

Table 2. pH and Fermentation Characteristics for the Six Whole-Plant Corn Silages at 90 Days Postfilling

Treatment[1]	pH	Lactic Acid	Acetic Acid	Propionic Acid	Ethanol	NH_3-N	Lactic to Acetic
		% of the silage DM					
1. Control	3.67	4.75[d]	2.10[d]	ND[2]	.82[c]	.22[b]	2.3[b]
2. LAB	3.65	5.25[b]	1.64[ab]	ND	.50[a]	.16[a]	3.2[a]
3. PS 10^5	3.65	5.15[c]	1.88[c]	.17	.67[b]	.20[b]	3.1[a]
4. LAB + PS 10^5	3.66	5.10[c]	1.67[ab]	.08	.54[a]	.14[a]	3.1[a]
5. PS 10^6	3.66	5.12[c]	1.72[b]	.22	.64[b]	.21[b]	3.0[a]
6. LAB + PS 10^6	3.66	5.43[a]	1.56[a]	.17	.46[a]	.13[a]	3.5[a]

[1]LAB = lactic acid bacteria and PS = *P. shermanii*
[2]ND = not detected.
[a,b,c,d]Means in the same column with different superscripts differ ($P < .05$).

Table 3. Hour of Initial Temperature (Temp.) Rise, Peak Temperatures, and pH for the Six Whole-Plant Corn Silages during the 10-day Aerobic Stability Measurements

Treatment[1]	Initial Temp. Rise, hrs	First Peak Temp. hrs	First Peak Temp. °F	Second Peak Temp. hrs	Second Peak Temp. °F	Days Exposed to Air 4	6	8	10
						pH			
1. Control	118	146	101	210	122	3.67	4.00	5.37	6.11
2. LAB	94	140	106	238	121	4.03	5.20	6.00	5.93
3. PS 10^5	146	*	*	238	106	3.65	3.68	3.92	5.08
4. LAB + PS 10^5	122	*	*	204	98	3.66	4.00	4.49	5.13
5. PS 10^6	166	*	*	210	94	3.66	3.67	3.79	3.97
6. LAB + PS 10^6	148	*	*	210	98	3.66	3.69	3.92	4.27

[1]LAB = lactic acid bacteria and PS = *P. shermanii*.
*No distinguishable first peak. These si lages exhibited at gradual increase in temperature following the initial temperature rise.

Table 4. pH and Yeast and Mold Counts over Time for the Corn Silages during the 10-day Aerobic Stability Measurements

Treatment[1,2]		Days to Exposure to Air 0	2	4	6	8	10
		Log_{10} cfu/g of silage					
1. Control	Y	<2.0	4.62	7.08	8.38	8.84	8.36
	M	4.04	3.41	4.68	7.26	7.61	7.82
2. LAB	Y	4.30	6.53	8.43	9.34	9.34	9.08
	M	<2.0	<2.0	4.32	6.76	7.46	7.61
3. PS 10^5	Y	2.84	4.18	7.20	7.65	8.11	8.59
	M	3.28	3.83	4.57	6.49	7.57	8.18
4. LAB + PS 10^5	Y	2.74	5.54	6.75	8.88	9.23	9.40
	M	<2.0	<2.0	4.26	6.40	6.98	7.57
5. PS10^6	Y	<2.0	4.38	7.90	6.56	6.98	8.46
	M	<2.0	<2.0	4.34	6.32	7.40	8.18
6. LAB + PS 10^6	Y	2.95	4.48	6.18	7.92	8.18	9.04
	M	<2.0	<2.0	4.54	6.41	7.49	7.64

[1]LAB = lactic acid bacteria and PS = *P. shermanii*.
[2]Y = yeast count and M = mold count.