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Abstract

The efficacy of 13 commercial bacterial silage inoculants was evaluated on 3rd and 4th cutting alfalfa. All inoculants supplied at least 100,000 colony-forming units (cfu) of lactic acid bacteria (LAB) per gram of ensiled crop, and each inoculant increased the rate and efficiency of the ensiling process. Inoculated alfalfa silages had lower pH values; higher lactic acid contents; and lower acetic acid, ethanol, and ammonia-nitrogen contents than control (untreated) silages. The addition of dextrose (fermentable substrate) in combination with a bacterial inoculant improved the quality of the fermentation phase in both cuttings of alfalfa.

Keywords

Cattlemen's Day, 1996; Kansas Agricultural Experiment Station contribution; no. 96-334-S; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 756; Beef; Silage; Innoculant; Fermentation; Alfalfa

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EFFECT OF BACTERIAL INOCULANTS ON THE FERMENTATION OF ALFALFA SILAGES [1]

K. K. Bolsen, D. R. Bonilla [2], M. A. Young, and R. A. Hart-Thakur

Summary

The efficacy of 13 commercial bacterial silage inoculants was evaluated on 3rd and 4th cutting alfalfa. All inoculants supplied at least 100,000 colony-forming units (cfu) of lactic acid bacteria (LAB) per gram of ensiled crop, and each inoculant increased the rate and efficiency of the ensiling process. Inoculated alfalfa silages had lower pH v alues; higher lactic acid contents; and lower acetic acid, ethanol, and ammonia-nitrogen contents than control (untreated) silages. The addition of dextrose (fermentable substrate) in combination with a bacterial inoculant improved the quality of the fermentation phase in both cuttings of alfalfa.

(Key Words: Silage, Inoculant, Fermentation, Alfalfa.)

Introduction

The effect of silage additives on fermentation dynamics has been documented in over 100 experiments using laboratory-scale silos at Kansas State University in the past 10 years. Results showed that the vast majority of inoculants supplied a high number of LAB (at least 100,000 cfu per gram of forage) and improved silage fermentation efficiency in all silage crops. Our objective study was to measure the efficacy of 13 silage inoculants available in 1992, using third and fourth cutting alfalfa. Because alfalfa is often a sugar-limited crop when ensiled below 35% dry matter (DM), dextrose and a combination of dextrose and inoculant were included as additional treatments.

Experimental Procedures

The 13 inoculants evaluated and their LAB content as listed by the manufacturer or distributor are shown in Table 1. Two trials were conducted using alfalfa grown near Manhattan, Kansas. A description of each alfalfa, including harvest date, chemical composition, and epiphytic microflora, is presented in Table 2.

The laboratory silos used were 4 14 inch PVC pipes closed with Jim-caps on each end. One Jim-cap was fitted with a Bunsen valve to allow gases to escape. For filling, 100 lb of chopped alfalfa 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 to 3 weeks after being received from the manufacturer or distributor. The colony-forming units (cfu) of LAB supplied per gram of pre-ensiled alfalfa by the inoculants is shown in Tables 3 and 4. Dextrose was applied at 2% of the forage DM. After all treatments were prepared, the silos were filled on an alternating schedule, which distributed the time from harvest (chopping) through silo filling equally across all treatments. The silos were packed with a hydraulic press, which excluded air and filled all silos to similar densities. Silos were stored at approximately 76 to 80°F. Three silos per treatment were opened at 1/2, 1, 3, 7, and 90 days postfilling.

[1]Financial assistance was provided by Lallemand S.A. Laboratorie Equipharm, Saint-Simon, France.

[2]Former graduate student. Current address: San Juan, Puerto Rico.

Table 1. List of the 13 Inoculants Evaluated in the Two Trials, Their Manufacturer or Distributor, and Their Lactic Acid Bacteria (LAB) Content

Inoculant	Manufacturer or Distributor	LAB[1]
Lallemand	Lallemand S.A. Laboratorie Equipharm, Saint-Simon, France	*L.*[2] *plantarum and P. acidilactici*
Biomate	Chr. Hansen's BioSystems, A Division of Chr. Hansen's, Inc., Milwaukee, WI	*L. plantarum and P. cerevisiae*
Ecosyl	ICI, Inc., Wilmington, DE	*L. plantarum*
Sil All	Alltech, Inc., Nicholasville, KY	*L. plantarum, P. acidilactici, and S. faecium*
Biotal	Biotal, Inc., Eden Prairie, MN	*L. plantarum and P. pentosaceus*
Bio Power	BioTechniques Laboratories, Inc., Redmond, WA	*S. faecium and L. plantarum*
Quest	Quest International, Hoffman Estates, IL	*L. plantarum*
Kem Lac	Kemin Industries, Inc., Des Moines, IA	*L. plantarum, L. bulgaricus, and L. acidophilus*
AgMaster	Marshall Products, A Division of Rhone-Poulenc, Inc., Madison, WI	*L. plantarum and P. acidilactici*
1174	Pioneer Hi-Bred International, Inc., Des Moines, IA	*L. plantarum and S. faecium*
Trilac	Quali Tech, Inc., Chaska, MN	*L. plantarum and P. acidilactici*
H/MF	Medipharm USA, Des Moines, IA	*L. plantarum, S. faecium, and Pediococcus* sp.
SI Concentrate	Laporte Biochem, Inc., Milwaukee, WI	*L. plantarum, L. brevis, P. acidilactici, S. cremoris, and S. diacetylactis*

[1]None of the additives contained enzymes.
[2]*L = Lactobacillus*; *P = Pediococcius*; *S = Streptococcus*.

Table 2. Chemical Composition and Epiphytic Microflora of the Chopped, Pre-ensiled Forages Used in Trials 1 and 2

Item[1]	Trial 1: 3rd Cutting Alfalfa	Trial 2: 4th Cutting Alfalfa
Harvest date[2]	July 21	August 6
Dry matter, %	32.4	40.5
pH	5.95	5.82
Buffering capacity, meq/100 g of DM	56.8	43.6
	——% of the forage DM——	
WSC	5.6	6.8
CP	21.2	20.4
NDF	38.8	40.6
ADF	27.4	31.2
	——cfu/g of forage——	
LAB	$1.2\ 10^{5}$	$6.7\ 10^{6}$
Yeast and mold	$1.8\ 10^{5}$	$2.6\ 10^{4}$

[1]WSC = water-soluble carbohydrates; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; and LAB = lactic acid bacteria. [2]Alfalfa

Chemical and Microbial Analyses of the Pre-ensiled Alfalfas and Silages. Pre-ensiled alfalfa was analyzed for DM; pH; total nitrogen; buffering capacity; water-soluble carbohydrates (WSC); neutral detergent and acid detergent fiber contents; and total epiphytic LAB, yeast, and mold counts. Silages fermented from 12 hours to 7 days were analyzed for pH and lactic acid; end-product silages (90 days postfilling) were analyzed for pH, lactic acid, volatile fatty acids, ethanol, and ammonia-nitrogen.

Statistical Analyses. Mean responses of each inoculant- and dextrose- treated silage were compared to the mean response of the control silage by the analysis of variance procedure for a complete block design.

Results and Discussion

Shown in Tables 3 and 4 are pH and lactic acid over time for the alfalfa silages in Trails 1 and 2, respectively. Presented in Tables 5 and 6 are pH and fermentation characteristics of the alfalfa silages at 90 days postfilling in Trials 1 and 2, respectively.

Trial 1. The pre-ensiled alfalfa had a relatively high buffering capacity and low WSC content. As a result, the 90-day pH values were relatively high (4.78 to 4.91), except for the two dextrose-treated silages (4.57 and 4.54). All of the nine inoculants supplied at least 100,000 cfu of LAB per gram of crop, but the rate of fermentation was fastest for the inoculant that supplied the highest number of LAB (Trilac). All inoculated silages had lower pH's ($P<.01$) and higher lactic acid contents ($P<.01$) than the control silages at 3, 7, and 90 days postfilling. The nine inoculated, 90-day silages were characterized by having significantly higher lactic acid contents and lactic to acetic acid ratios and lower acetic acid, ethanol, and ammonia-nitrogen contents than controls. The dextrose + inoculant silage underwent a more homofermentative ensiling process than its dextrose-treated counterpart.

Trial 2. The pre-ensiled alfalfa had a lower buffering capacity (43.6 vs. 56.8 meq per 100 g of DM), higher WSC content (6.8 vs. 5.6% of the forage DM), and a higher epiphytic LAB population ($6.7\ 10^{6}$ vs. $1.2\ 10^{5}$/g) than the alfalfa used in Trial 1. As a result, the fermentation phase began within the first 12 hours postfilling for all silages, including the control silage. Also, the 90-day pH values were relatively low (4.17 to 4.34) for all inoculant- and dextrose-treated silages.

All 12 inoculants supplied at least 100,000 cfu of LAB per gram of crop, and all inoculated silages had a faster rate of fermentation than the control. Only the control silage had a pH value above 4.90 (5.13) at 3 days postfilling. All inoculated silages had a lower pH ($P<.01$) and higher lactic acid content ($P<.01$) than the control silages at 12 and 24 hr and 3, 7, and 90 days postfilling. All 12 inoculated, 90-day silages underwent a more efficient ensiling process than the control silage. The fermentation characteristics indicated that the dextrose + inoculant treatment gave the most favorable 90-day silage.

Table 3. pH and Lactic Acid over Time for the 12 Alfalfa Silages in Trial 1

		Time Postfilling[1]				
Treatment[2,3]		12 hrs	24 hrs	3 days	7 days	90 days
Control	pH	5.90	5.84	5.43	5.24	4.91
	LA	.2	.3	1.4	2.1	4.0
Lallemand	pH	5.86^x	5.39	5.10	4.88	4.78
(5.4 1 0^5)	LA	.2^x	1.6	3.8	4.6	5.4
Dextrose	pH	5.92^x	5.85^x	4.99	4.74	4.57
	LA	.2^x	.3^x	3.9	5.2	5.9
Lallemand	pH	5.85^x	5.29	4.67	4.68	4.54
+ Dextrose	LA	.3^x	1.6	4.9	5.5	6.0
ICI	pH	5.84	5.81^x	5.11	4.98	4.82
(1.0 1 0^5)	LA	.3^x	.3^x	3.7	3.9	4.9
Alltech	pH	5.88^x	5.77^x	5.02	4.92	4.78
(2.4 1 0^5)	LA	.2^x	.4^x	3.7	4.5	5.2
Biotal	pH	5.84	5.38	4.97	4.90	4.83
(1.0 1 0^5)	LA	.3^x	1.7	4.2	4.6	5.1
BioTechniques	pH	5.87^x	5.70	5.01	4.95	4.83
(1.8 1 0^5)	LA	.2^x	.5	3.6	4.5	4.9
Kemin	pH	5.86^x	5.77^x	5.20	5.09	4.84
(1.0 1 0^5)	LA	.2^x	.5	3.3	4.1	5.1
Marschall	pH	5.90^x	5.73	5.21	5.10	4.85
(1.8 1 0^5)	LA	.2^x	.4^x	3.4	4.1	4.9
Pioneer	pH	5.88^x	5.79^x	5.11	5.05	4.78
(1.4 1 0^5)	LA	.2^x	.3^x	3.4	4.2	5.3
Quali Tech	pH	5.40	4.82	4.78	4.69	4.78
(8.1 1 0^5)	LA	1.3	3.2	4.6	5.2	5.3

[1]LA = lactic acid expressed as a % of the silage dry matter.
[2]LAB supplied per gram of pre-ensiled crop is shown in parentheses.
[3]Inoculant- and dextrose-treated means differ (P<.01) from control means, unless the treated mean has a superscript (x).

Table 4. pH and Lactic Acid over Time for the 15 Alfalfa Silages in Trial 2

		Time Postfilling[1]				
Treatment[2,3]		12 hrs	24 hrs	3 days	7 days	90 days
Control	pH	5.52	5.35	5.13	4.94	4.56
	LA	1.0	1.6	2.4	4.2	4.4
Lallemand	pH	5.29	5.17	4.75	4.68	4.30
(5.0 1 0^5)	LA	1.7	3.0	4.9	5.7	6.1
Dextrose	pH	5.18	5.10	4.72	4.67	4.28
	LA	2.0	3.1	4.5	5.1	5.9
Lallemand	pH	5.13	5.03	4.47	4.43	4.17
+ Dextrose	LA	2.3	3.5	5.8	6.4	6.7
Chr. Hansen's	pH	5.26	5.11	4.75	4.66	4.28
(1.3 1 0^5)	LA	1.8	3.0	4.8	5.6	6.1
ICI	pH	5.29	5.22	4.81	4.72	4.32
(1.0 1 0^5)	LA	1.6	2.5	4.6	5.3	6.1
Alltech	pH	5.29	5.21	4.77	4.66	4.30
(3.1 1 0^5)	LA	1.7	2.8	4.7	5.5	6.0
Biotal	pH	5.27	5.16	4.78	4.71	4.31
(1.1 1 0^5)	LA	1.8	2.9	4.8	5.5	6.2
BioTechniques	pH	5.30	5.25[x]	4.85	4.72	5.34
(1.3 1 0^5)	LA	1.7	2.4	4.3	5.3	6.1
Quest	pH	5.29	5.24[x]	4.81	4.78	4.33
(1.7 1 0^5)	LA	1.6	2.5	4.5	4.9	5.8
Kemin	pH	5.29	5.24[x]	4.81	4.71	4.33
(1.4 1 0^5)	LA	1.7	2.6	4.7	5.7	5.9
Marschall	pH	5.28	5.18	4.88	4.67	4.31
(3.0 1 0^5)	LA	1.8	3.0	4.5	5.4	6.0
Pioneer	pH	5.29	5.21	4.77	4.65	4.32
(1.5 1 0^5)	LA	1.7	2.9	4.8	5.7	6.1
Medipharm	pH	5.21	5.04	4.73	4.66	4.28
(3.4 1 0^5)	LA	2.0	3.1	4.9	5.7	6.5
Laporte	pH	5.28	5.23[x]	4.79	4.69	4.34
(1.7 1 0^5)	LA	1.5	2.4	4.6	5.0	5.7

[1]LA = lactic acid expressed as a % of the silage dry matter.
[2]LAB supplied per gram of pre-ensiled crop is shown in parentheses.
[3]Inoculant- and dextrose-treated means differ ($P<.05$) from control means, unless the treated mean has a superscript (x).

Table 5. pH and Fermentation Characteristics for the 12 Alfalfa Silages at 90 Days Postfilling in Trial 1

Treatment[1]	pH	Lactic Acid	Acetic Acid	Ethanol	NH_3-N	Lactic to Acetic
		—— % of the silage DM ——				
Control	4.91	4.0	2.9	.44	.36	1.4
Lallemand	4.78	5.4	2.0	.18	.21	2.7
Dextrose	4.57	5.9	2.8[x]	.38[x]	.21	2.1
Lallemand + Dextrose	4.54	6.0	1.8	.18	.20	3.4
ICI	4.82	4.9	2.1	.26	.23	2.3
Alltech	4.79	5.2	2.1	.23	.21	2.4
Biotal	4.83	5.1	2.2	.21	.20	2.2
BioTechniques	4.83	4.9	2.3	.25	.24	2.1
Kemin	4.84	5.1	2.1	.25	.21	2.4
Marschall	4.85	4.9	2.2	.24	.24	2.2
Pioneer	4.79	5.3	2.1	.19	.20	2.5
Quali Tech	4.78	5.3	1.9	.17	.20	2.7

[1]Inoculant- and dextrose-treated means differ ($P<.01$) from control means, unless the treated mean has a superscript (x).

Table 6. pH and Fermentation Characteristics for the 15 Alfalfa Silages at 90 Days Postfilling in Trial 2

Treatment[1]	pH	Lactic Acid	Acetic Acid	Ethanol	NH_3-N	Lactic to Acetic
		—— % of the silage DM ——				
Control	4.56	4.4	2.3	.28	.33	2.0
Lallemand	4.30	6.1	1.6	.13	.21	3.4
Dextrose	4.28	5.9	2.1[x]	.26[x]	.20	2.8
Lallemand + Dextrose	4.17	6.7	1.5	.08	.18	4.5
Chr. Hansen's	4.28	6.1	1.7	.14	.20	3.6
ICI	4.32	6.1	1.7	.15	.22	3.5
Alltech	4.30	6.0	1.6	.11	.19	3.7
Biotal	4.31	6.2	1.6	.16	.20	3.9
BioTechniques	4.34	6.1	1.6	.13	.21	3.7
Quest	4.33	5.8	1.7	.14	.20	3.3
Kemin	4.33	5.9	1.6	.10	.20	3.8
Marschall	4.31	6.0	1.7	.16	.21	3.5
Pioneer	4.32	6.1	1.6	.14	.18	3.9
Medipharm	4.28	6.5	1.7	.10	.18	3.8
Laporte	4.34	5.7	1.9	.18	.24	3.0

[1]Inoculant- and dextrose-treated means differ ($P<.01$) from control means, unless the treated mean has a superscript (x).