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EFFECT OF INOCULANT AND ENZYME ADDITIVES ON PRESERVATION AND NUTRITIVE VALUE OF ALFALFA SILAGE[1,2,3,4,5]

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Summary

Lactic acid bacteria (LAB) inoculants[2,5] and several enzyme additives[3,4] were evaluated in various combinations using fifth cutting alfalfa. The field-wilted crop was characterized by a high buffer capacity (63.0 meq/100 g of DM), low fermentable carbohydrate (5.4% of the DM), and a high number of indigenous LAB (over one million per g). In contrast to several previous studies, the inoculants and enzymes had very little effect on rate and efficiency of fermentation. The 90-d treated silages had similar fiber and digestibility values, compared to the control. Treated silages tended to have higher lactic acid values, but all silages had relatively high acetic acid and ethanol contents, which indicate an inefficient ensiling process.

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

Introduction

Alfalfa is one of the most difficult crops to successfully preserve as silage because of its high buffering capacity, wide range in moisture contents, and often low level of water soluble carbohydrates. Inoculants and, to a lesser degree, enzymes have improved silage fermentation efficiency and nutritive value in several previous studies. Our objective was to further document the effect of various inoculant and enzyme treatments on alfalfa silage quality.

Experimental Procedures

The eight treatments are shown in Table 37.1. Silages were made from fifth cutting alfalfa on September 9, 1988. The laboratory silos used were 4 × 14 in. PVC pipes closed with Jim-Caps on each end; the Cap on the top was fitted with a Bunsen valve to allow CO_2

[1]Partial financial assistance was provided by Chr. Hansen's Bio Systems, Milwaukee, Wisconsin; Genencor, South San Francisco, California; Finnsugar Bioproducts, Inc., Schaumburg, Illinois; and Medipharm USA, Des Moines, Iowa.

[2]Biomate® was provided by Chr. Hansen's Bio Systems and contains *Lactobacillus plantarum* and *Pediococcus cerevisiae*.

[3]Cytolase® enzyme-containing product was provided by Genencor.

[4]Clampzyme® was provided by Finnsugar Bioproducts and contains cellulases, hemicellulases, and glucose oxidase. Amylase and pectinase enzymes were provided by Finnsugar.

[5]Medipharm PF Soluble® was provided by Medipharm USA and contains *Lactobacillus plantarum, L. acidophilus, Pediococcus sp.,* and *Streptococcus faecium* M-74.

to escape. For filling, 100 lb of chopped alfalfa was placed on a plastic sheet, and the treatments were applied and mixed thoroughly. All enzymes were added according to the manufacturers' recommendations. After all treatments were prepared, the silos were filled as rapidly as possible on an alternating schedule, which distributed the time from harvest to silo filling equally across treatments and replications. The silos were filled with a hydraulic press, which compacted them to similar densities and excluded air. The silos were stored at ambient temperature, and three silos from each treatment were opened at .5, 1, 3, 7, 14, and 90 d post-ensiling for evaluation.

Table 37.1. Description of the Eight Treatments Compared

Treatment no.	Description
1	Control (untreated)
2	Medipharm PF (Med PF)[1]
3	Amylase (Amyl)
4	Amyl + Clampzyme (Clamp) + Pectinase (Pect)
5	Med PF + Amyl + Clamp + Pect
6	Cytolase (Cyto)
7	Biomate[2]
8	Biomate + Cyto

[1]Med PF provided 1.9×10^5 CFU of LAB per g of crop.
[2]Biomate provided 1.5×10^5 CFU of LAB per g of crop.

Microbial profiles were determined for the alfalfa and inoculants. Post-harvested, pre-ensiled samples were aseptically weighed, macerated in a Waring blender, and diluted with sterile phosphate buffer. The indigenous (naturally occurring) lactic acid bacteria on the crop were determined. Samples were added to MRS broth, plated, and incubated for 3 d at 37 C. Mesophilic bacteria (aerobes and facultative anaerobes) were plated on Standard Plate Count agar (Difco) and incubated for 3 d at 37 C. Yeast and mold counts were done by using potato dextrose agar with tetracycline and chloramphenicol added to kill bacteria. Plates were incubated for 3 d at 21 C. All results were converted to colony-forming units (CFU) per g of crop.

Analyses pre-ensiling were forage dry matter (DM), pH, water soluble carbohydrates (WSC), buffer capacity (BC), crude protein, acid detergent fiber (ADF), and neutral detergent fiber (NDF). Silages fermented for .5 to 14 d were analyzed for pH, lactic acid, volatile fatty acids (VFAs), and ethanol. Ninety-day end point silages were analyzed for crude protein, ADF, NDF, WSC, pH, lactic acid, VFAs, ethanol, ammonia-nitrogen, and DM content.

Silage aerobic stability was determined by placing about 2 lb of silage into expanded, plastic-lined polystyrene buckets, putting the buckets in a temperature controlled room (65 F), and monitoring the temperature of the silage mass twice daily for 10 d. If a silage's temperature rose 10 F over ambient, it was considered aerobically unstable. Aerobic stability was the number of hours between the initial exposure to air and the onset of heating. Silage digestibility was measured using the *in vitro* DM disappearance, two-stage, Terry and Tilley technique.

Statistical analyses were done for a random complete block design with three replications. Mean responses for each treatment were compared by analysis of variance, and means were separated by Least Significant Difference. No interactions are reported.

Results and Discussion

Presented in Table 37.2 are the microbial and chemical profiles of the pre-ensiled alfalfa. The field-wilted crop contained 37.2% DM and was characterized by a high buffer capacity, low water soluble carbohydrates, and a high number of lactic acid bacteria.

Presented in Tables 37.3 and 37.4 are results for the fermentation dynamics and the 90-d chemical composition and in vitro DM digestibility of the silages.

All silages fermented rather quickly, reaching pH values between 4.9 and 5.0 and lactic acid contents between 4.5 and 5.0% by the third day post-filling. However, the silages were not stable, with acetic acid and ethanol levels increasing nearly two- to threefold from d 3 to 90. The inoculant and enzyme combination silages (treatments 5 and 8) were the most efficiently preserved, as evidenced by the lowest pHs, highest lactic to acetic acid ratios, and lowest ethanol values. Chemical composition, in vitro DM digestibility, and DM recovery were not affected by silage treatment. All eight silages were highly stable in air and did not heat or deteriorate during the 10 d of exposure.

Table 37.2. Microbial and Chemical Profile of the Pre-ensiled, Pre-treated Alfalfa

Item	Profile
	-- CFU/g of alfalfa --
Lactic acid bacteria	3.3×10^6
Mesophilic bacteria	7.2×10^7
Yeasts	2.5×10^4
Molds	5.7×10^4
Dry matter, %	37.2
pH	5.98
Buffer capacity[1]	63.9
	--% of the alfalfa DM--
Crude protein	19.1
Neutral detergent fiber	34.1
Acid detergent fiber	26.8
Water soluble carbohydrates	5.4

[1]Milliequivalents of NaOH per 100 g of crop DM required to raise the pH from 4.0 to 6.0.

These results are inconsistent with the majority of our alfalfa silage studies in 1986 and 1987 (KAES Reports of Progress 514, 539, and 568), when silages were improved by inoculant and/or inoculant + enzyme additions. However, in three of five other studies conducted in 1988, inoculants alone failed to produce better alfalfa silages. The drought conditions could have contributed to the difficulties in successfully ensiling alfalfa in 1988.

Several studies are currently in progress to better understand the many crop and environmental factors that influence the "ensileability" of alfalfa (see pages 102 and 105 of this report).

Table 37.3. pH and Chemical Composition over Time for the Eight Alfalfa Silages

Time post-filling, days	Item[1]	Control	Med PF	Amyl	Amyl+ Clamp +Pect	Med PF+ Amyl+ Clamp +Pect	Cyto	Bio-mate	Cyto+ Bio-mate	LSD[2]
.5	pH	5.50	5.45	5.47	5.43	5.47	5.49	5.44	5.42	.01
	LA	.52	.63	.58	.62	.70	.64	.71	.69	.04
	AA	.47	.52	.49	.48	.50	.51	.56	.54	.04
	ETOH	.123	.134	.124	.136	.130	.129	.144	.126	.04
	NH_3-N	.023	.024	.024	.024	.024	.022	.024	.024	.002
1	pH	5.03	5.00	5.04	5.02	5.02	5.04	4.93	4.92	.01
	LA	1.85	2.22	1.86	1.83	2.08	2.12	2.40	2.29	.06
	AA	.72	.73	.63	.80	.74	.71	.61	.71	.04
	ETOH	.294	.279	.294	.291	.290	.288	.254	.272	.07
	NH_3-N	.053	.052	.052	.048	.049	.047	.050	.050	.003
3	pH	4.99	4.95	4.94	4.89	4.87	4.92	4.89	4.90	.01
	LA	4.84	4.98	4.67	5.04	5.28	5.44	4.57	4.69	.11
	AA	1.84	1.76	1.84	1.88	1.81	2.22	1.90	1.74	.08
	ETOH	.353	.326	.354	.352	.328	.410	.324	.302	.03
	NH_3-N	.172	.168	.171	.171	.167	.175	.159	.159	.008
7	pH	4.97	4.95	4.92	4.90	4.87	4.92	4.92	4.87	.01
	LA	4.75	4.36	5.26	5.12	5.44	5.50	5.59	6.17	.39
	AA	2.46	2.30	2.32	2.33	2.21	2.47	2.33	2.38	.34
	ETOH	.411	.387	.411	.373	.330	.468	.340	.358	.04
	NH_3-N	.226	.228	.233	.230	.229	.240	.235	.231	.020
14	pH	4.95	4.94	4.91	4.90	4.82	4.91	4.92	4.86	.01
	LA	5.33	5.31	5.71	5.75	5.37	5.68	5.69	6.03	.44
	AA	2.81	2.63	2.64	2.85	2.44	2.67	2.76	2.54	.11
	ETOH	.492	.468	.480	.518	.376	.482	.400	.370	.03
	NH_3-N	.293	.291	.285	.285	.260	.284	.293	.265	.019
90	pH	4.91	4.88	4.90	4.86	4.80	4.76	4.90	4.72	.01
	LA	4.26	4.70	3.62	5.36	5.92	5.69	4.66	5.71	.25
	AA	4.35	4.20	5.22	4.32	4.67	4.18	4.66	4.14	.63
	ETOH	.511	.464	.500	.514	.435	.531	.421	.390	.07
	NH_3-N	.340	.324	.330	.315	.332	.322	.332	.321	.029

[1]LA = lactic acid, AA = acetic acid, ETOH = ethanol, and NH_3-N = ammonia nitrogen. All values are reported as a percent of the silage dry matter.
[2]Least significant difference.

Table 37.4. Chemical Composition, Digestibility, and DM Recovery for the Eight Alfalfa Silages

Item[1,2]	Control	Med PF	Amyl	Amyl+ Clamp +Pect	Med PF+ Amyl+ Clamp +Pect	Cyto	Bio-mate	Cyto+ Bio-mate	LSD[3]
Dry matter, %	35.4	35.4	35.5	35.1	35.9	35.1	35.1	34.4	—
	---------- % of the silage DM ----------								
CP	19.3	19.5	19.9	19.4	19.5	19.4	19.2	19.3	.5
NDF	34.8	36.0	35.4	35.7	35.4	35.3	37.9	35.5	1.9
ADF	27.1	27.4	27.8	29.2	28.0	29.2	30.3	30.2	1.6
IVDMD	67.4	68.0	69.7	68.2	67.4	68.6	69.1	67.3	2.8
DM recovery	94.5	94.7	94.3	93.1	94.9	94.0	95.7	93.8	1.2

[1]CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, and IVDMD = in vitro dry matter disappearance.
[2]DM recovery is expressed as a percent of the dry matter ensiled.
[3]Least significant difference.