EFFECT OF COMMERCIAL INOCULANTS ON FERMENTATION OF 1988 SILAGE CROPS

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Introduction

We have measured silage fermentation dynamics in over 50 crops since the development of a 4 x 14 inch PVC pipe, laboratory-scale silo in 1984. In many of these experiments, our objective was to determine how inoculants or inoculant/enzyme combinations affected the rate and efficiency of the ensiling process.

Twenty-five different inoculants have been tested over a wide range of ensiling conditions. Results show that the majority of silage inoculants available today are able to supply a high number of lactic acid bacteria (LAB) and to improve silage fermentation in most crops (KAES, Reports of Progress 494, 514, and 539).

Preliminary results of 17 experiments conducted in 1988 to determine the efficacy of 12 commercial inoculants are summarized here. An additional objective in six experiments was to study the effect of numbers of LAB supplied to the crop by inoculants on fermentation response. In two alfalfa experiments, combinations of inoculant and dextrose (fermentable sugar) and inoculant and enzyme (to increase fermentable sugar) were compared.

Experimental Procedures

The 12 inoculants evaluated and active ingredients as listed by the manufacturer or distributor are shown in Table 22.1. All silages were made from crops grown near Manhattan in 1988. A description of the crops used, harvest date, stage of maturity or cutting, chemical composition, and microorganism profile are presented in Tables 22.2, 22.3, and 22.4.

The laboratory silos were 4 x 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 to 125 lbs of fresh crop were placed on a plastic sheet, and the inoculant was applied and mixed thoroughly. All inoculants (and enzymes) were applied as liquids and used within 3 to 4 weeks after being received from the manufacturer. After all silage treatments 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 excluded air and filled all silos to similar densities. Silos were stored at approximately 85 F. Two or three silos per treatment were opened at various times post-filling during the first week, and end-product silages were evaluated at 90 days.

Chemical Analyses of the Pre-ensiled Crops and Silages. Pre-ensiled crops were analyzed for dry matter (DM), pH, total nitrogen, water soluble carbohydrates, acid detergent fiber,

neutral detergent fiber, and buffer capacity. Silages fermented from 3 hours to 7 days were analyzed for pH and lactic acid; end-product silages were analyzed for pH, lactic acid, volatile fatty acids, ethanol, and ammonia-nitrogen.

Microbiological Evaluations. Post-harvest, pre-ensiled samples of the crops and inoculants were weighed, mixed in a high-speed blender, and diluted in sterile buffer. The following microorganism counts were made after appropriate dilutions with sterile buffer:

Mesophilic count. That count provided an index of the number of aerobic and facultative anaerobic bacteria. Samples were added to Standard Plate Count agar (DIFCO) and incubated for 3 days at 32 C.

Yeast and mold count. Potato Dextrose agar was used with tetracycline and chloramphenicol (100 ug/ml each) to kill bacteria. The plates were incubated at 21 C for 3 days.

Lactic acid bacteria count. This measured the natural populations of lactic acid bacteria (LAB) present on the crops and the LAB provided by inoculants at the time they were applied to the crops. Samples were added to Bacto Lactobacilli MRS Broth to which 1.5% agar (Difco) was added and incubated 3 days at 32 C.

All counts were converted to colony-forming units per gram of crop or per gram or ml of inoculant.

Statistical Analyses. Mean responses of each inoculated crop were compared to the mean response of the untreated (control) crop by the analyses of variance procedure for a complete block design.

Summary of Results

Only a summary of the results from the 17 trials is presented here. Details from all 1987 and 1988 silage experiments will be included in a KAES bulletin to be published later this year.

Most of the commercial silage inoculants evaluated supplied a high number of lactic acid bacteria (LAB) per gram of crop--39 of 42 inoculants provided more than 10^5 (100,000) viable LAB per gram. Microorganism profiles showed that all six row crops (corn and sorghum) had at least 10^7 (10,000,000) naturally-occurring LAB per gram when ensiled. The forage crops (wheat, oats, alfalfa, and sudangrass) also had high numbers of indigenous LAB, ranging from 190,000 to 60,000,000 per gram.

As was observed in the 1987 silage crops, the whole-plant corns ensiled rapidly and all were at or below pH 4.0 by 48 hours post-filling. However all inoculants applied to corn and sorghum (Biomate, BioTal, Ecosyl, 1129, 1174, and TriLac) gave significantly lower pH values at several opening times during the first 4 days post-filling. Increasing the numbers of LAB supplied by the inoculants (BioTal or TriLac) to 1 million or more per gram, produced only a slight increase in the rate of pH decline.

Wheat, oats, and sudangrass were highly responsive to the inoculants--preliminary results showed higher lactic acid content and lower pH, acetic acid, ethanol, and ammonia-nitrogen values for inoculated vs. control silages. Increasing the numbers of LAB supplied by Biomate from 100,000 or 300,000 per gram to 500,000 or 1,000,000 did not improve the inoculant response in wheat (Trial 3) or oats and vetch (Trial 5).

In contrast to 1987 when all alfalfa silages were improved by inoculants, inoculants alone produced better silages in only two of the five 1988 alfalfa experiments (Trials 6 and 8). All eight silages in Trial 7 (both control and inoculated) were of very poor fermentation quality, with high pH values (5.3 to 5.8) and high acetic acid and ammonia-nitrogen contents (about 5.0% and .70%, respectively). Increasing the numbers of LAB supplied by the inoculants (Biomate, KemLac, Medipharm, or 1174), adding dextrose (fermentable sugar), or adding amylase enzymes in subsequent experiments (Trials 9 and 10) were much less effective in improving the fermentation than adding a combination of inoculant and dextrose. The results clearly showed the importance of having the inoculant present to achieve a rapid and efficient fermentation of the added substrate. The drought conditions likely contributed to the difficulties in successfully ensiling alfalfa in 1988.

Table 22.1. List of the 12 Inoculants Evaluated in Selected 1988 Trials, Their Manufacturer or Distributor, and Their Lactic Acid Bacteria Content

Inoculant	Manufacturer or Distributor	Lactic Acid Bacteria
AGMASTER ALFALFA SILAGE INOCULANT (AgMaster)	Marschall Products Division of Miles Laboratories, Madison, WI	*Lactobacillus plantarum* and *Pediococcus acidilactici*
SIL-ALL SILAGE INOCULANT (Sil-All)	Alltech, Nicholasville, KY	*L. plantarum, L. acidophilus,* and *Streptococcus faecium*
BIOMATE LAB CONCENTRATE (Biomate)	Chr. Hansen's Laboratory, Inc.,[1] Milwaukee, WI	*L. plantarum* and *P. cerevisiae*
BIOPOWER	BioTechniques Laboratories, Inc., Redmond, WA	*S. faecium* and *L. plantarum*
BIOTAL SILAGE INOCULANT (BioTal)	BioTal, Inc.[1] Minnetonka, MN	*L. plantarum* and *P. acidilactici*
ECOSYL	C-I-L Inc.[1], London Ontario, Canada	*L. plantarum*
KEMLAC	Kemin Industries, Inc.,[1] Des Moines, IA	*L. plantarum, L. bulgaricus,* and *L. acidophilus*
MEDIPHARM PF SOLUBLE (Medipharm)	Medipharm USA[1], Des Moines, IA	*S. faecium* M-74, *L. acidophilus, Pediococcus sp.,* and *L. plantarum*
PIONEER BRAND 1129 FORAGE SORGHUM SILAGE INOCULANT (1129)	Pioneer Hi-Bred International, Inc.,[1] Des Moines, IA	*L. plantarum* (multiple strains) and *S. faecium*
PIONEER BRAND 1174 WATER SOLUBLE SILAGE INOCULANT (1174)	Pioneer Hi-Bred International, Inc., Des Moines, IA	*L. plantarum* (multiple strains) and *S. faecium*
SI CONCENTRATE 40 A/F (SI Conc)	Great Lakes Biochemical Co., Inc., Milwaukee, WI	*L. plantarum, L. brevis, P. acidilactici, S. cremoris,* and *S. diacetylactis*
TRILAC	Quali Tech, Inc.,[1] Chaska, MN	*L. plantarum* and *P. cerevisiae*

[1]Indicates companies who provided partial financial assistance.

Table 22.2. Description, Harvest Date, Chemical Composition, and Microorganism Profile for the Crops Used in Trials 1 to 5

Item	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
	Wheat				
Variety	Siouxland	Siouxland	Arkan	Ogle Oats	Ogle & Vetch
Harvest Date, 1988	May 16	June 3	June 10	June 3	June 22
Stage of Maturity	Heading	Early-dough	Late-dough	Early-dough	Late-dough
Dry Matter, %	33.2	41.5	48.6	29.8	38.0
Water Soluble Carbohydrate, % of DM	6.0	9.4	3.9	9.1	7.2
Buffer Capacity[1]	---	18.3	34.5	28.8	23.2
Indigenous Microbes:	----------	CFU[2]/gram of crop	----------		
Mesophilic	1.1×10^8	3.0×10^8	8.2×10^7	1.5×10^8	2.5×10^8
Lactic Acid Bacteria	5.8×10^5	5.6×10^5	4.5×10^5	5.1×10^6	6.0×10^7
Yeast and Mold	$< 10^3$	1.0×10^5	4.1×10^4	3.6×10^3	5.5×10^4

[1]Milliequivalents of NaOH per 100 grams of crop DM required to raise the pH of the fresh material from 4.0 to 6.0.
[2]Colony-forming units.

Table 22.3. Harvest Date, Cutting, Chemical Composition, and Microorganism Profile for the Crops Used in Trials 6 to 11

Item	Alfalfa					Sudangrass
	Trial 6	Trial 7	Trial 8	Trial 9	Trial 10	Trial 11
Hybrid/Variety	Cody	Cody	Cody	Cody	Apollo II	Trudan 6
Harvest Date, 1988	May 17	June 8	June 10	July 13	August 2	July 29
Cutting No.	1st	2nd	2nd	3rd	4th	1st
Dry Matter, %	46.6	33.9	49.5	38.1	46.5	26.6
Water Soluble Carbohydrate, % of DM	7.8	3.9	3.6	4.5	5.3	18.7
Buffer Capacity[1]	---	54.0	51.5	46.8	---	34.8
Indigenous Microbes:	----------	CFU[2]/gram of Crop	----------			
Mesophilic	2.3×10^7	---	---	---	5.9×10^6	1.0×10^8
Lactic Acid Bacteria	2.3×10^6	2.7×10^6	8.0×10^4	1.9×10^5	6.7×10^5	2.6×10^7
Yeast and Mold	$<10^2$	---	1.2×10^4	---	1.1×10^4	2.6×10^4

[1]Milliequivalents of NaOH per 100 grams of crop DM required to raise the pH of the fresh material from 4.0 to 6.0.
[2]Colony-forming units.

Table 22.4. Description, Harvest Date, Chemical Composition, and Microorganism Profile for the Crops Used in Trials 11 to 17

Item	Trial 12	Trial 13	Trial 14	Trial 15	Trial 16	Trial 17
	Whole-plant Corn				Shelled Corn	Forage Sorghum
Hybrid	Pioneer 3377	Pioneer 3379	Funk's 1505	Pioneer 3379	------	DeKalb 25E
Harvest Date, 1988	August 11	August 15	August 16	August 18	Sept. 2	Oct. 13
Stage of Maturity	2/3 Milk Line	2/3 Milk Line	Black Layer	1/3 Milk Line	Black Layer	Late-dough
Dry Matter, %	38.4	35.2	40.0	32.5	76.0	28.4
Water Soluble Carbohydrate, % of DM	------	------	5.4	13.4	-----	6.1
Buffer Capacity[1]	31.5	26.7	-----	32.6	------	28.8
Indigenous Microbes: - - - - - - - - - - - - - - - - -CFU[2]/gram of Crop - - - - - - - - - - - -						
Mesophilic	2.4×10^8	2.9×10^8	5.1×10^8	2.7×10^8	2.7×10^8	3.9×10^7
Lactic Acid Bacteria	3.6×10^7	2.8×10^7	1.2×10^7	9.7×10^6	1.3×10^7	1.1×10^7
Yeast and Mold	2.5×10^4	8.0×10^3	1.5×10^5	2.6×10^4	2.7×10^4	2.5×10^5

[1]Milliequivalents of NaOH per 100 grams of crop DM required to raise the pH of the fresh material from 4.0 to 6.0.
[2]Colony-forming units.