

EFFECTS OF BIOMATE® INOCULANT AND DEXTROSE ON THE FERMENTATION OF ALFALFA SILAGES¹

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Summary

This study documented once again that ensiling alfalfa is difficult and unpredictable. Adding 2% dextrose or Biomate® inoculant alone or in combination had little influence on the ensiling process but did improve fermentation efficiency somewhat. The pre-ensiling characteristics (i.e., dry matter (DM) and water soluble carbohydrate (WSC) values, buffering capacity, and epiphytic microflora) at the different cuttings and stages of maturity undoubtedly influenced the effectiveness of the two additives. Apparently, alfalfa often has too little WSC and too much buffering capacity to produce adequately preserved silage, especially when ensiled at a low DM content (less than 30 to 34%).

(Key Words: Silage, Alfalfa, Bacterial, Inoculant, Dextrose.)

Introduction

The goal of silage fermentation is to produce enough lactic acid and to inhibit plant catabolic enzymes and growth of undesirable epiphytic microorganisms. The most numerous undesirable microflora are the Enterobac-

teriaceae and yeasts and molds; they compete with the lactic acid bacteria (LAB) for fermentable sugars. Clostridial spores (obligate anaerobes) can also multiply rapidly as soon as oxygen is depleted and can lead to extensive deterioration.

Alfalfa is generally recognized as difficult to ensile, because of its high buffering capacity, wide range in moisture contents, and low level of water soluble carbohydrates (WSC). Typically, multiple cuttings are ensiled at numerous stages of maturity throughout the growing season, which further contributes to the variability seen in alfalfa silage. Stimulating fermentation by adding bacterial cultures has become common. These products are safe to handle and help establish a homolactic fermentation (fermentations producing only lactic acid). Our objective was to determine the effects of a commercial bacterial inoculant and WSC additions on the ensiling process of two alfalfa cuttings, each harvested at three maturity stages. The effect of these additives on microbial succession was presented last year (KAES Report of Progress 623).

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Experimental Procedures

In 1989, a second-year stand of Cody alfalfa was mowed and swathed at the 2nd and 4th cuttings each at late-bud, 10% bloom, and 50% bloom and wilted in the windrow for 5 to 6 h prior to chopping. The chopped alfalfa received no additive (control), dextrose at 2% of the forage DM, Biomate inoculant (*Lactobacillus plantarum* and *Pediococcus cerevisiae*; from Chr. Hansen's Bio Systems, Milwaukee, Wisconsin) to provide 1.5×10^5 colony-forming units (cfu)/g of fresh forage, or a combination of dextrose and Biomate. All material was ensiled in 4×14 in. PVC laboratory silos and packed to the same density using a hydraulic press. Each silo was equipped with a Bunsen valve at one end, which excluded air but enabled gases to escape. Silos were stored at 80 ± 5 F. Three silos per treatment were opened at various times during the 90-day ensiling period. Silage samples were taken aseptically for microbiological and chemical analyses at each opening.

Each alfalfa cutting was analyzed separately as a split-plot design, in which the whole-plot was a randomized complete block and opening times were the sub-plots. The general linear models procedure of SAS® was used to analyze the data, and a probability of $P < .05$ was used to denote significance, unless otherwise indicated.

Results and Discussion

Presented in Table 1 are the chemical compositions and epiphytic microflora count of the chopped, pre-ensiled alfalfas. Even though wilting times were the same, the DM in the chopped material averaged 37.4% at the second cutting vs. 26.1% at the fourth. Temperature was higher and relative humidity was lower when the second cutting was wilted. The 10 and 50% bloom, second cutting alfalfas had the lowest buffering capacities, and the late-bud, second cutting had the lowest WSC content. Both buffering capacity and WSC content were relatively high for the fourth

cutting alfalfas. All five categories of epiphytic microorganisms were found on the pre-ensiled material and Enterobacteriaceae were predominant (10^6 cfu/g). The lactobacilli, pediococci, and leuconostoc group of LAB was only a small and variable proportion of the total population; 10^2 to 10^6 cfu/g.

Fermentation results are presented in Table 2. pH decreased ($P < .05$) as stage of maturity advanced. It was lowered ($P < .05$) by dextrose and the combination treatments in the fourth cutting silages, but only by the combination in the second cutting silages. Lactic acid increased ($P < .05$) and acetic acid, ethanol, and ammonia-nitrogen decreased ($P < .05$) as maturity advanced for the second cutting silages, but not in the fourth cutting silages. The combination-treated silages had the best fermentation profiles; more lactic acid and less acetic acid, ethanol, and ammonia-nitrogen. Adding dextrose improved the fermentation of the fourth cutting silages compared to the controls. Lactic acid content in the second cutting silages increased ($P < .05$) from day 1 to 3 but did not change ($P > .05$) during the remainder of the ensiling period. For the fourth cutting silages, lactic acid contents were similar ($P > .05$) during the first 7 days, but decreased sharply ($P < .05$) thereafter. Acetic acid, ethanol, and ammonia-nitrogen increased in both second and fourth cutting silages throughout the 90-day ensiling period.

Shown in Tables 3 and 4 are the changes that occurred in the fermentation characteristics during the ensiling period. Silages treated with Biomate alone had lower ($P < .05$) pH values only at 12 h compared to the control silages, but the combination-treated silages had lower pHs all the way to day 90. In general, adding dextrose alone to the fourth cutting silages had the same effect on the rate of pH decline as combining dextrose with inoculant. At the end of the 90-day ensiling period, all silages had similar pH values ($P > .05$), regardless of treatment.

Although lactic acid was slightly higher in the second cutting, late-bud alfalfa silages than in either the 10 or 50% bloom silages at days 1 and 3, it was lower ($P < .05$) in the late-bud silages thereafter. In the fourth cutting silages, lactic acid was higher in the 50% bloom than in either the late-bud or 10% bloom silages at each time period. In contrast to the second cutting silages, stage of maturity did not consistently influence acetic acid levels; silages from each maturity stage had the highest value at some time during the ensiling period. Ammonia-nitrogen content was highest ($P < .05$) in the late-bud silages from days 3 to 90, but ethanol levels were not affected by stage of maturity.

In second cutting silages, those treated with Biomate alone had the highest lactic acid at day 1 of fermentation. After day 1, the combination-treated silages had higher ($P < .05$) lactic acid levels than the control and Biomate-treated silages. The combination and dextrose-treated silages had similar ($P > .05$) lactic acid values after day 3. In the fourth cutting alfalfa, dextrose-treated silages had higher ($P < .05$) lactic acid during the first 7 days than controls, but only the combination silages maintained these higher levels at the end of 90 days. Biomate inoculant alone did not affect lactic acid content at any time during the ensiling period.

At 90 days, all treated silages had lower acetic acid levels than control silages, but only Biomate-treated and combination silages produced lower ($P < .05$) levels of ethanol. Ammonia-nitrogen content was not affected ($P > .05$) by the additive treatments. Butyric acid was detected in only two of the 24 silages; .15 and .87% in second cutting, late-bud control and Biomate-treated silages, respectively. Propionic acid was present in a few of the silages, but always less than .2% of the dry matter.

The difficulties encountered in successfully ensiling alfalfa were similar to those in several previous studies (KAES Report of Progress 567). Among the six control alfalfa silages, only two (second cutting, 10 and 50% bloom) were well preserved, as evidenced by a low and stable pH; relatively high lactic acid; and low acetic acid, ethanol, and ammonia-nitrogen. Those two alfalfas also had higher pre-ensiled DM and lower buffering capacities than the other four alfalfas. The addition of Biomate inoculant to the alfalfa silages improved the fermentation profile at the end of the 90-day ensiling period compared to the controls, but did not increase the number of well preserved silages. We have previously observed that inoculants improved the fermentation characteristics in numerous crops even when control silages were also satisfactorily preserved, but inoculants have not consistently improved silages that might not be capable of adequate fermentation.

All alfalfa silages benefitted from dextrose addition alone, especially in the first few days of fermentation, as evidenced by higher lactic acid and lower pH. However, by the end of 90 days, the only improvement from adding dextrose alone was a modest reduction in acetic acid in the second cutting silages. The increased acetic acid and decreased lactic acid in the latter stages of fermentation in all alfalfa silages, especially the fourth cutting, probably demonstrates WSC depletion and subsequent fermentation of lactic acid to acetic acid by lactic acid bacteria.

Table 1. Chemical Composition and Epiphytic Microflora Count of the Chopped, Pre-ensiled Alfalfas

Item	2nd cutting			4th cutting		
	Late-bud	10% bloom	50% bloom	Late-bud	10% bloom	50% bloom
Dry matter, %	31.2	36.7	44.4	25.3	27.6	25.5
Buffering capacity, meq/100 g of DM	55.7	44.5	39.3	55.4	49.2	46.3
Water soluble carbohydrates, % of the DM	6.8	8.9	9.7	10.2	10.4	10.8
pH	5.8	5.7	5.7	5.9	5.9	5.8
)))))) log ₁₀ cfu/g of fresh forage)))))))					
Lactobacilli, pediococci, and leuconostocs	1.78	5.78	4.09	4.72	5.03	5.81
Enterobacteriaceae	6.76	6.45	5.83	6.80	6.63	6.49
Yeasts and molds	5.34	5.37	5.85	5.30	5.57	5.66
Lactate-assimilating yeasts	2.00	4.41	4.84	3.78	4.63	5.04
Lactate-fermenting clostridial spores	0	0	0	2.17	0	1.60

Table 2. Effects of Stage of Maturity, Additive Treatment, and Time during the Ensiling Period on the Fermentation of Second and Fourth Cutting Alfalfa Silages

Item	Maturity ¹			Additive ²				Time, days				Statistical significance for comparison ¹			
	LB	10	50	C	D	B	D+B	1	3	7	90	M	A	T	A×T
)))))) Second cutting)))))))														
pH	5.38 ^a	4.80 ^b	4.72 ^b	5.19 ^a	4.99 ^a	4.98 ^a	4.72 ^b	5.03 ^a	4.82 ^b	5.01 ^a	4.78 ^b	**	*	**	NS
)))))) % of the silage DM)))))))														
Lactic acid	4.33 ^b	5.38 ^{ab}	5.92 ^a	3.98 ^b	5.20 ^{ab}	5.00 ^b	6.65 ^a	2.25 ^b	5.42 ^a	5.79 ^a	6.07 ^a	†	*	**	NS
Acetic acid	4.90 ^a	3.07 ^b	1.87 ^c	3.52 ^a	3.32 ^{ab}	3.34 ^{ab}	2.93 ^b	1.61 ^d	2.42 ^c	3.18 ^b	5.02 ^a	**	†	**	NS
Ethanol	.43 ^a	.44 ^a	.23 ^b	.38 ^a	.42 ^a	.34 ^b	.33 ^b	.36	.42	.39	.42	**	**	*	NS
NH ₄ -N	.65 ^a	.29 ^b	.14 ^c	.41	.33	.42	.29	.07 ^c	.17 ^{bc}	.33 ^b	.64 ^a	**	NS	**	NS
)))))) Fourth cutting)))))))														
pH	5.34 ^a	5.08 ^b	4.99 ^b	5.35 ^a	4.97 ^b	5.31 ^a	4.91 ^b	4.91 ^c	5.27 ^a	5.23 ^a	5.09 ^b	**	**	**	**
)))))) % of the silage DM)))))))														
Lactic acid	3.88 ^b	3.72 ^b	5.57 ^a	3.53 ^b	4.89 ^a	3.63 ^b	5.52 ^a	4.61 ^a	5.32 ^a	5.31 ^a	2.46 ^b	**	†	**	NS
Acetic acid	2.46 ^c	4.34 ^a	3.46 ^b	3.61 ^a	3.38 ^{ab}	3.50 ^{ab}	3.20 ^b	1.87 ^b	1.93 ^b	2.17 ^b	5.38 ^a	**	NS	**	NS
Ethanol	.20 ^c	.41 ^a	.28 ^b	.30	.27	.32	.30	.20 ^b	.11 ^b	.14 ^b	.48 ^a	**	*	**	NS
NH ₄ -N	.66 ^a	.43 ^c	.54 ^b	.60 ^a	.51 ^{bc}	.58 ^{ab}	.48 ^c	.22 ^d	.48 ^c	.58 ^b	.74 ^a	**	*	**	NS

¹LB = late-bud; 10 = 10% bloom; and 50 = 50% bloom.

²C = control; D = dextrose; and B = Biomate.

³M = stage of maturity; A = additive treatment; T = time in the ensiling period; and A×T = interaction between additive and time.

^{a,b,c,d,e}Means in the same row within maturity, additive, and time with different superscripts differ (P < .05).

†P < .10. *P < .05. **P < .01.

Table 3. Effects of Stage of Maturity and Additive Treatment on the Fermentation Characteristics at Different Times during the Ensiling Period of Second Cutting Silages

Time in the ensiling period, days	Item ¹	Maturity (M)			Additive (A)				Statistical significance for comparison	
		LB ²	10	50	C ³	D	B	D+ B	M	A
.5	pH	5.58 ^a	5.18 ^b	5.50 ^a	5.59 ^a	5.48 ^{ab}	5.26 ^b	5.33 ^b	*	†
1	pH	5.24	4.89	4.95	5.32 ^a	5.26 ^a	4.93 ^{ab}	4.61 ^b	NS	*
3	pH	5.20 ^a	4.70 ^b	4.57 ^b	5.12 ^a	4.81 ^{ab}	4.85 ^a	4.50 ^b	**	*
7	pH	5.64 ^a	4.86 ^b	4.54 ^b	5.28 ^a	4.97 ^{ab}	5.13 ^a	4.68 ^b	**	*
90	pH	5.37 ^a	4.62 ^b	4.35 ^c	4.89 ^{ab}	4.68 ^{ab}	4.91 ^a	4.64 ^b	**	†
))))))))) % of the silage DM)))))))										
1	LA	3.51	1.76	1.47	1.14 ^b	1.11 ^b	3.07 ^a	3.67 ^a	NS	*
	AC	2.37 ^a	1.72 ^b	.74 ^c	1.62	1.66	1.67	1.49	**	NS
	ETOH	.43 ^a	.45 ^a	.20 ^b	.38	.38	.33	.34	*	NS
	NH ₃ -N	.10 ^a	.06 ^b	.04 ^b	.05	.07	.08	.08	**	NS
3	LA	5.76	5.17	5.33	4.05 ^b	4.98 ^b	5.19 ^b	7.46 ^a	NS	*
	AC	3.29 ^a	2.58 ^b	1.38 ^c	2.27	2.47	2.68	2.24	**	NS
	ETOH	.43 ^b	.62 ^a	.22 ^c	.41 ^b	.47 ^a	.43 ^{ab}	.38 ^b	**	*
	NH ₃ -N	.26 ^a	.14 ^b	.10 ^b	.20	.16	.17	.13	**	NS
7	LA	4.45 ^b	5.85 ^a	7.05 ^a	4.46 ^b	6.07 ^{ab}	5.07 ^b	7.54 ^a	*	*
	AC	4.61 ^a	3.01 ^b	1.92 ^c	3.46	3.31	3.27	2.68	**	NS
	ETOH	.39 ^b	.55 ^a	.22 ^c	.40 ^{ab}	.46 ^a	.34 ^b	.34 ^b	**	*
	NH ₃ -N	.56 ^a	.31 ^b	.13 ^c	.38	.33	.37	.24	**	NS
90	LA	3.32 ^b	6.87 ^a	8.01 ^a	4.77 ^b	6.64 ^{ab}	5.72 ^b	7.13 ^a	**	†
	AC	7.72 ^a	4.56 ^b	2.76 ^c	5.71 ^a	4.95 ^b	4.98 ^b	4.42 ^c	**	**
	ETOH	.46 ^b	.56 ^a	.26 ^c	.46 ^a	.48 ^a	.37 ^b	.38 ^b	**	*
	NH ₃ -N	1.28 ^a	.43 ^b	.21 ^b	.76	.54	.76	.50	**	NS

¹LA = lactic acid; AC = acetic acid; NH₃-N = ammonia-nitrogen; and ETOH = ethanol.

²LB = late-bud; 10 = 10% bloom; and 50 = 50% bloom.

³C = control; D = dextrose; and B = Biomate.

^{a,b,c}Means in the same row within maturity and additive with different superscripts differ (P < .05).

†P < .10.

*P < .05.

**P < .01.

Table 4. Effects of Stage of Maturity and Additive Treatment on the Fermentation Characteristics at Different Times during the Ensiling Period of Fourth Cutting Silages

Time in the ensiling period, days	Item ¹	Maturity (M)			Additive (A)				Statistical significance for comparison	
		LB ²	10	50	C ³	D	B	D+ B	M	A
0.5	pH	5.20 ^a	5.21 ^a	4.95 ^b	5.28 ^a	5.12 ^b	5.15 ^b	4.93 ^c	**	**
1	pH	5.10 ^a	4.84 ^b	4.77 ^b	5.13 ^a	4.75 ^b	5.08 ^a	4.66 ^c	**	**
3	pH	5.53 ^a	5.18 ^b	5.10 ^b	5.54 ^a	4.99 ^b	5.56 ^a	4.99 ^b	**	**
7	pH	5.47 ^a	5.12 ^b	5.10 ^b	5.46 ^a	5.00 ^b	5.46 ^a	4.98 ^b	**	**
90	pH	5.34 ^a	4.95 ^b	4.97 ^b	5.21	5.02	5.14	4.98	**	NS
)))))) % of the silage DM)))))))										
1	LA	5.23 ^a	3.12 ^b	5.49 ^a	3.41 ^c	4.71 ^b	4.27 ^{bc}	6.06 ^a	**	**
	AC	1.18 ^b	3.33 ^a	1.09 ^b	1.95	1.75	1.94	1.82	**	NS
	ETOH	0 ^b	.43 ^a	.18 ^b	.21	.12	.27	.21	**	NS
	NH ₃ -N	.27 ^a	.11 ^b	.28 ^a	.21	.23	.22	.21	**	NS
3	LA	4.81 ^b	4.20 ^c	6.96 ^a	3.85 ^b	6.60 ^a	4.16 ^b	6.69 ^a	**	**
	AC	1.06 ^b	3.46 ^a	1.27 ^b	2.14	1.81	2.10	1.68	**	NS
	ETOH	0 ^b	.35 ^a	0 ^b	.14	.11	.12	.10	**	NS
	NH ₃ -N	.62 ^a	.34 ^c	.48 ^b	.54 ^a	.44 ^b	.53 ^a	.41 ^b	**	**
7	LA	4.28 ^b	4.92 ^b	6.74 ^a	4.10 ^b	6.32 ^a	4.43 ^b	6.40 ^a	**	**
	AC	1.53 ^b	3.59 ^a	1.38 ^b	2.31	2.05	2.30	2.00	**	NS
	ETOH	0 ^b	.41 ^a	0 ^b	.13	.14	.13	.14	**	NS
	NH ₃ -N	.72 ^a	.45 ^c	.58 ^b	.66 ^a	.51 ^b	.66 ^a	.51 ^b	**	**
90	LA	1.81	2.63	2.90	1.74 ^b	2.64 ^{ab}	1.99 ^{ab}	3.42 ^a	NS	†
	AC	2.26 ^c	6.28 ^b	7.61 ^a	5.63	5.58	5.44	4.90	**	NS
	ETOH	.51 ^a	.37 ^b	.55 ^a	.50	.43	.50	.48	**	NS
	NH ₃ -N	.88 ^a	.63 ^b	.72 ^b	.83 ^a	.71 ^{ab}	.77 ^{ab}	.66 ^b	*	*

¹LA = lactic acid; AC = acetic acid; NH₃-N = ammonia-nitrogen; and ETOH = ethanol.

²LB = late-bud; 10 = 10% bloom; and 50 = 50% bloom.

³C = control; D = dextrose; and B = Biomate.

^{a,b,c}Means in the same row within maturity and additive with different superscripts differ (P < .05).

†P < .10.

*P < .05.

**P < .01.