A Final Report on

An Evaluation of Silaferm and a Microbial Inoculant on the Fermentation and Aerobic Stability of Corn Silage

UD Study Number: 05and 06 Aji

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INTRODUCTION

Various nitrogenous compounds have been added to silages to improve their nutritive value (Muck and Kung, 1997). The added benefits from such additives specifically fall into two categories: 1) increased concentration of nitrogenous compounds that can be used by rumen microbes for synthesis of microbial protein and 2) an antifungal effect that inhibits the growth of yeasts and molds thereby improving the stability of silages when they are exposed to air. The objective of this study was to determine the effect of various nitrogenous additives and a microbial inoculant on the fermentation and aerobic stability of corn silage.

MATERIALS AND METHODS

Corn crop: Forage corn grown in the 2005 (05 Aji) and 2006 (06 Aji) seasons were harvested at a whole plant DM of about 35% DM. For 05 Aji, six 50-kg piles of chopped whole plant corn were prepared. One pile of forage was treated with either: 1) nothing, control (**C**), 2) Ajinomoto Silaferm added at 50 lb/ton of wet forage (**A1**) (about 3.5% added CP/ton DM), 4) Ajinomoto Silaferm (**A2**) (about 7% added CP/ton DM), or 4) *L. buchneri* and *P. pentosaceus* (**LBC**) (400,000 and 100,000 cfu/g of wet forage, respectively). Silaferm was supplied by Ajinomoto, USA, Inc, Eddyville, IA. The microbial inoculant was supplied by Lallemand Animal Nutrition, Milwaukee, WI.

For Aji 06, four 50-kg piles of chopped whole plant corn were weighed out. Forage was treated with either: **C** = untreated silage, **P** = Proteferm (AAFCO 36.1 condensed extracted glutamic acid fermentation product) added at 50 lb/ton, **So** = Silaferm (old formulation) added at 50 lb/ton, **Sn** = Silaferm (new formulation) added at 50 lb/ton. Active products were supplied by Ajinomoto.

Silos: All additives were applied uniformly using a hand sprayer or via manual application onto the forage while constantly mixing. After thorough mixing forages were packed in 20 l silos that were sealed at the top with two layers of plastic (3 mil) and duct tape. All silos had a packing density of about 200-220 kg of DM/m^3. All silos were

stored between 23 to 26ºC. Weights of empty and full macro silos were recorded at filling and at opening.

Analytical procedures: Analytical procedures were as outlined by Kung and Ranjit (2001). Before ensiling, samples were obtained after the addition of each treatment and stored on ice until they were returned to the lab for processing. The DM content of fresh forages and silages were determined by drying at 60°C for 48 h in a forced-draft oven. Samples of forages and silages (25 g) were homogenized in 225 ml of sterile quarter-strength Ringer's solution for 1 min and then, filtered through Whatman 54 filter paper (Whatman, Inc., Clifton, NJ). The pH of the homogenized solutions were determined with a Corning pH meter (model no. 220, Corning Science Products, Corning, NY). The filtered water extract were acidified with 15 µl of 50% (wt/vol) H_2SO_4 to reduce the pH of the extract to < 2.0 before freezing. Water extracts were analyzed for ammonia-N by using the phenol-hypochlorite procedure described by Weatherburn (1967). Water-soluble carbohydrates (WSC) were analyzed by the procedure described by Nelson (1944). After oven drying, feed samples were ground through a 1-mm screen using a Cyclone Sample Mill (Udy Corp., Fort Collins, CO). Samples were analyzed for laboratory DM by placing 0.5 g into a 100°C oven for 24 h. Samples were also analyzed for NDF by using sulfite and amylase (Van Soest et al., 1991) and ADF (Robertson and Van Soest, 1981) using an Ankom^{200} Fiber Analyzer (Ankom Technology, Fairport, NY). Total N was determined by combustion using a Leco CNS 2000 Analyzer (St. Joseph, MI) and CP was calculated by multiplying total N by 6.25. Total N was analyzed only for d0 and d 120 samples. The ash content was determined by placing 0.5 g of sample into a 600°C furnace for 4 h. Silages were also analyzed for fermentation end products as previously described. For analysis of lactic acid, acetic acid, propionic acid, butyric acid, and ethanol, water extracts were prepared and analyzed by HPLC. In vitro DMD was conducted in 50 ml polycarbonate tubes using the method described by Goering and Van Soest, 1970).

The numbers of lactic acid bacteria on fresh forage and silages were determined on water extracts by pour plating in ¼ strength Ringer's solution (made with Oxoid BR0052,

Unipath, Basingstoke, Hampshire, UK) were pour plated in De Man-Rogosa-Sharpe (**MRS**) agar (Oxoid CM361). Plates were incubated at 32°C for 48 h. Yeast and molds were determined by pour plating serial dilutions in malt extract agar (Oxoid CM59) that had been acidified, after autoclaving, by the addition of 85% lactic acid at a concentration of 0.5% vol/vol. Plates for lactic acid bacteria and yeasts and molds were incubated at 32°C for 48 h.

Dry matter recoveries were calculated from measuring the weight of the empty silos, and knowing the initial and final silo weights and DM concentrations of the fresh and ensiled material. Aerobic stability was determined by returning 5 kg of each replicate to a clean 20-L silo without packing. Thermocouple probes were placed in the geometric center of each sample mass and a double layer of cheesecloth was placed over each silo to prevent drying and contamination, but allowing exposure to air. Ambient temperature as well as the temperature from each bucket were recorded every min and averaged after 2 h by a data logger (model number CR10X, Campbell Scientific, Inc., Logan, UT). The samples were allowed to aerobically deteriorate at room temperature (22 to 24°C). Aerobic stability was defined as the number of h before the temperature of the mass increased 2°C above the ambient temperature (Moran et al., 1996).

Statistical analysis: All microbial data were transformed to $\log_{10}$ and are presented on a wet weight basis. Chemical data are presented on a DM basis. Data were analyzed using the general linear models procedure of SAS (1998) for a completely randomized design. Differences among means were tested using Tukey's Test (Snedecor and Cochran, 1980). An α level of $P < 0.05$ were deemed significant.

RESULTS AND DISCUSSION

The chemical and microbial composition of freshly chopped corn before treatment for 05Aji is shown in Table 1. These compositions were typical for forage harvested at our site with the exception that the numbers of LAB were quite high. In past studies from our group the numbers of LAB have usually been around 5 to 6 log cfu/g. Data after 120 d of ensiling is shown in Table 2. Dry matter recovery was numerically greater for

A1 and A2 than for C or LBC but the differences were not statistically different. Numbers of LAB were low in A1 and greatest in A2 compared to C and LBC. In many past studies, addition of *L. buchneri* has resulted in increased residual numbers of LAB most likely due to their dominance of the fermentation (Schmidt et al., 2007). Numbers of yeasts were also numerically lower (but not statistically) for A1 and A2 compared to other treatments. Some differences in silage pH were detected among treatments but these differences were not substantial. Lactic acid was unaffected by treatment but A2 had lower acetic acid than did C and LBC. Treatments A1 and A2 had the highest lactic:acetic acid ratios followed by C and then LBC. The concentration of ethanol was not different between C and LBC but it was lower in A1. As expected, the CP and NH3-N contents of silages were not affected by LBC relative to C but they increased with the additions of A1 and A2. Treatments did not affect the ADF and NDF content of silages. The residual WSC content of silages was greatest in C followed by LBC and lowest in A1 and A2 suggesting a more extensive fermentation for the later two treatments. The aerobic stability of A1 and A2 averaged 85 h and was numerically better than C (51 h) and LBC (51.5) and corresponds to the higher ammonia-N probably resulting in the lower numbers of yeasts found with these treatments (Kung et al., 2000). In this experiment, addition of LBC did not result in changes in silage fermentation as usually found. Typically, addition of inoculants with *L. buchneri* results in increased concentrations of acetic acid, less yeasts and improved aerobic stability (Kleinschmit and Kung, 2006) and these trends were observed in a previous experiment (Schmidt et al., 2006). The exact reason(s) for this lack of response is(are) unknown but the extremely high numbers of LAB on the fresh forage may have presented excessive competition for *L. buchneri*. The in vitro DM digestibility of corn silages after 24 h of incubation is shown in Figure 1. Dry matter digestibility was not different among treatments although it was numerically highest for A1 and A2.

The composition of fresh forage for 06Aji is shown in Table 3. As in 05Aji, the nutrient and chemical composition was as expected. The chemical and microbial composition of corn silages after 150 d of ensiling is shown in Table 4. All treatments improved the recovery of DM when compared to C. Relative to C, treatments did not affect the numbers of final LAB or yeasts. Treatment with So and Sn, but not P resulted

in silage with a higher pH than C which was a related to generally lower amounts of lactic and acetic acids for these treatments. Treatments So and Sn also had lower levels of ethanol than did C. Similar effects were observed in a previous study (04 Aji) and in the first experiment of this study (05Aji). As expected, treatments P, So and Sn resulted in silages with higher concentrations of CP and NH3-N than C. These treatments also resulted in lower ADF, NDF and WSC concentrations than C. Ash content was unaffected by treatments. Aerobic stability was not different among treatments most likely because numbers of yeasts did not differ. The addition of nitrogenous additives increased the in vitro DM digestibility of the corn silages when compared to C (Figure 2). A similar trend was reported in a previous study (Schmidt el al., 2006).

CONCLUSIONS

In two studies, the addition Silaferm resulted in some general trends in silage fermentation that were similar to that found in a previous study (Schmidt el al., 2006). Specifically, this included lower concentrations of ethanol and WSC but higher concentrations of CP and NH3-N. In one of two studies, Silaferm increased in vitro DM digestion. Effects on numbers of yeasts and aerobic stability were not apparent. The addition of Silaferm to corn silage increases the CP content and thus improves nutritive value. Potential improvements in DM digestion are an added benefit. Future studies should be conducted to evaluate the potential effects of the amino acid/peptide content of Silaferm on microbial protein synthesis in the rumen and animal performance.

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Table 1. Chemical and microbial composition of fresh corn forage 05Aji.

Item	
LAB[1], log cfu/g	8.09
Yeasts, log cfu/g	5.92
Molds, log cfu/g	5.03
DM, %	31.37
pH	5.47
CP, %	8.60
NH3-N, %	0.06
ADF, %	23.39
NDF, %	43.49
WSC, %	7.57
Ash, %	3.76

[1] Lactic acid bacteria.

Table 2. Chemical and microbial composition of corn silage after 120 d of ensiling 05Aji.

Item	C[1]	LBC	A1	A2	S.E.
DM, %	33.17	33.07	34.58	34.83	0.44
DM recovery, %	93.98	94.27	98.52	98.04	1.22
LAB[2], log cfu/g	5.25^{b}	5.09^{b}	< 4.00^{c}	7.65^{a}	0.19
Yeasts, log cfu/g	4.59	4.48	3.73	3.54	0.56
pH	3.57^{c}	3.59^{bc}	3.63^{ab}	3.66^{a}	0.01
Lactic acid, %DM	6.44	5.77	6.08	6.37	0.18
Acetic acid, %DM	1.29^{ab}	1.30^{a}	1.07^{bc}	0.97^{c}	0.05
Lactate:acetate ratio	5.02^{c}	4.48^{c}	5.67^{b}	6.56^{a}	0.14
Ethanol, %DM	1.74^{a}	1.70^{a}	1.38^{b}	1.48^{ab}	0.07
CP, %	9.28^{c}	9.06^{c}	11.63^{b}	14.05^{c}	0.15
NH_3-N, %	0.09^{c}	0.10^{c}	0.40^{b}	0.76^{a}	<0.01
ADF, %	20.32	22.52	21.28	21.54	0.70
NDF, %	40.70	43.18	39.10	40.03	1.22
WSC[3], %	1.05^{a}	0.89^{b}	0.70^{c}	0.67^{c}	<0.01
Ash, %	3.57^{b}	3.53^{b}	3.66^{ab}	4.21^{a}	0.14
Aerobic stability, h	51.0	57.5	81.5	88.5	12.69

[1] C - nothing, control; LBC - *Lactobacillus buchneri* and *Pediococcus pentosaceus* (400,000 and 100,000 cfu/g of wet forage, respectively); A1 - Ajinomoto Silaferm (42% CP), at 50 lb/ton of wet forage; A2 - Ajinomoto Silaferm, at 100 lbs/ton of wet forage.

[2] Lactic acid bacteria.

[a, b, c]Means within columns with unlike superscript differ ($P < 0.05$).

Table 3. Chemical (DM basis) and microbial composition (wet basis) of fresh whole-plant corn 06 Aji.

Item	
LAB[1], log_{10} cfu/g	6.82
Yeasts, log_{10} cfu/g	5.85
Molds, log_{10} cfu/g	5.54
DM, %	36.83
pH	5.57
CP, %	8.41
NH_3-N, %	0.03
ADF, %	23.26
NDF, %	39.41
WSC[2], %	8.64
Ash, %	3.06

[1]Lactic acid bacteria.

[2]Water soluble carbohydrates.

Table 4. Chemical (DM basis) and microbial composition (wet basis) of corn silage after 150 d of ensiling 06 Aji.

Items	C[1]	P	So	Sn	S.E
DM recovery, %	87.04[b]	98.16[a]	95.21[a]	93.42[a]	1.23
DM, %	32.57[b]	36.71[a]	35.62[a]	34.95[a]	0.46
LAB[2], log_{10} cfu/g	6.68	6.55	7.79	6.19	0.48
Yeasts, log_{10} cfu/g	6.54[ab]	6.44[ab]	6.77[a]	5.91[b]	0.19
pH	3.61[b]	3.67[ab]	3.70[a]	3.70[a]	0.01
Lactic acid, %	6.28[a]	5.12[b]	5.05[b]	5.22[ab]	0.26
Acetic acid, %	1.35[a]	0.98[b]	1.02[b]	1.06[b]	0.04
Lactate:acetate ratio	4.63	5.21	4.95	4.95	0.24
Ethanol, %	1.54[a]	1.11[b]	1.14[b]	1.26[b]	0.05
CP, %	8.92[b]	11.97[a]	11.77[a]	12.68[a]	0.23
NH_3-N, %	0.104[c]	0.460[b]	0.548[a]	0.573[a]	0.02
ADF, %	26.80[a]	22.71[b]	23.69[b]	22.29[b]	0.60
NDF, %	44.67[a]	39.87[b]	39.70[b]	38.19[b]	1.02
WSC[3], %	1.31[a]	0.98[b]	0.92[b]	0.99[b]	0.04
Ash, %	4.10	4.27	4.33	4.43	0.11
Aerobic stability, h	35	33	30	37	2.87

[1]C = untreated silage, P = Proteferm (AAFCO 36.1 condensed extracted glutamic acid fermentation product) added at 50 lb/ton, So = Silaferm (old formulation) added at 50 lb/ton, Sn = Silaferm (new formulation) added at 50 lb/ton.

[2] Lactic acid bacteria.

[3] Water soluble carbohydrates.

[a, b, c]Means within columns with unlike superscript differ ($P < 0.05$).

Figure 1. Effect of nitrogenous additives or a microbial inoculant on the in vitro dry-matter digestibility of corn silage after 120 d of storage. C = untreated, control A1 = Ajinomoto Silaferm added at 50 lb/ton of wet forage A2 = Ajinomoto Silaferm added at 100 lbs/ton of wet forage (about 7% added CP/ton DM), LBC = *L. buchneri* and *P. pentosaceus* (400,000 and 100,000 cfu/g of wet forage, respectively).

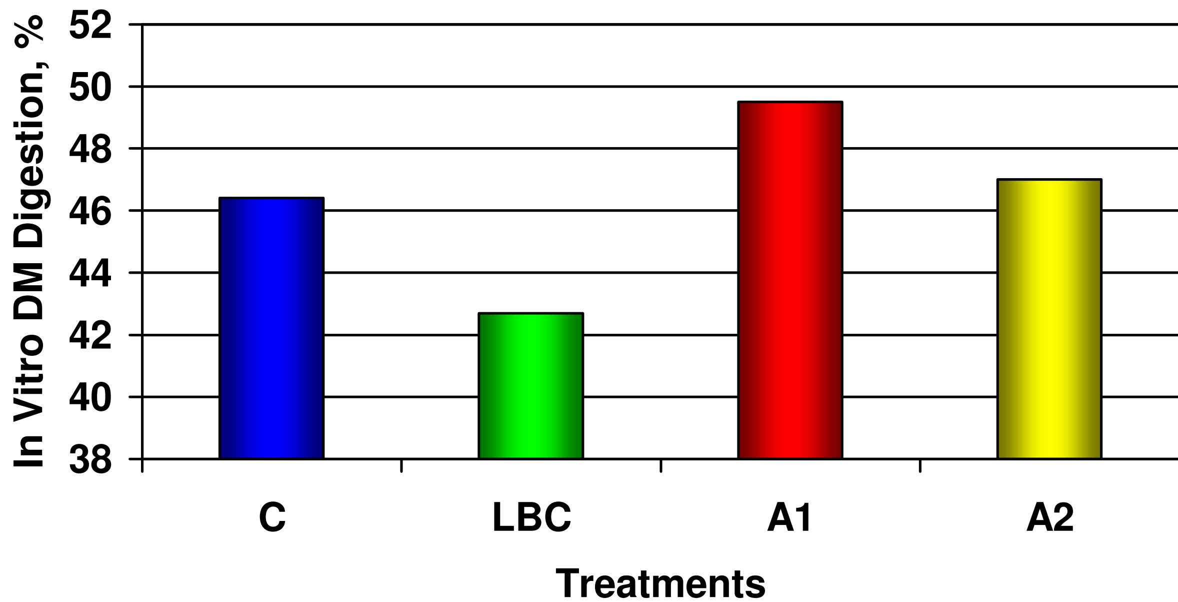

Figure 2. Effect of nitrogenous additives on the in vitro dry-matter digestibility of corn silage after 150 d of storage. C = untreated silage, P = Proteferm (AAFCO 36.1 condensed extracted glutamic acid fermentation product) added at 50 lb/ton, **So** = Silaferm (old formulation) added at 50 lb/ton, **Sn** = Silaferm (new formulation) added at 50 lb/ton. 06Aji. Bars with unlike superscripts differ $P < 0.05$.