

Evaluation of Silaferm or ammonium chloride as additives in grass silage.

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Abstract

The effects of silage additives Silaferm or ammonium chloride upon the ensiling of grass silage were studied in an experiment. Silaferm or ammonium chloride alone or with added monosodium glutamate or lysed bacterial cell mass was mixed with fresh cut triticale prior to ensiling and compared with a non-treated control. After 35 days of ensiling, Silaferm had lower ADF and NDF ($P < 0.05$), higher insoluble true protein compared with Control ($P < 0.05$), and tended to have better aerobic stability compared with all others. Organic acid production was not different among treatments with the exception that Silaferm tended to lower acetic acid and ammonium chloride tended to increase acetic acid concentration compared with Control. These data show that Silaferm is an effective silage additive for grass silage that appears to decrease proteolysis and improve aerobic stability. Ammonium salts are a large constituent of Silaferm but they are not as effective alone compared with Silaferm.

Introduction

In a previous experiment, adding Silaferm (condensed extracted amino acid fermentation solubles) to corn plant at the time of ensiling resulted in corn silage with a greater concentration of true protein nitrogen as well as increased resistance to aerobic deterioration. Silaferm is a mixture of several components, including ammonium chloride, glutamic acid and bacterial cell mass along with small amounts of organic acids, peptides, magnesium, potassium and other minerals. Forms of non-protein nitrogen such as urea or ammonia have been studied extensively as corn silage additives. Addition of ammonia has resulted in greater production of organic acids, decreased enzymatic degradation of corn protein during ensiling and improved aerobic stability. However, the mode of action for ammonium chloride or sulfate as an additive is likely different from

that of ammonia due to large differences of pH and the presence of a strong, negative ion in ammonium salts of mineral acids.

Objectives

The objectives of this experiment were to evaluate the effectiveness of Silaferm as an additive in production of grass silage and to determine the individual effects of Silaferm components ammonium chloride, bacterial cell mass or monosodium glutamate upon ensiling of grass and its subsequent aerobic stability.

Materials and methods

Silaferm, ammonium chloride or ammonium chloride plus either monosodium glutamate (MSG) or bacterial cell cream (BCC) was added to freshly cut triticale (*Triticosecale spp.*) to provide 3 kg added nitrogen per ton of fresh forage weight and compared with a non-treated control. Table 1 gives the amount of each additive used in treatments. The additives were thoroughly incorporated with approximately 10 kg fresh forage in replicates of four. Each replicate was divided into two silos (5.5 kg each) and one was opened after 3 days and the other after 35 days of ensiling. The laboratory silos were 23cm X 33cm cryovac bags. Air was evacuated from bags at the time of ensiling using a vacuum pump.

Table 1. Composition of treatments mixed with silage. Expressed as a percentage of forage weight, as is basis.

Item	Treatment				
	Control	Silaferm	NH ₄ Cl	NH ₄ CL+MSG ¹	NH ₄ CL+BCC
Silaferm		5.0			
NH ₄ Cl			1.0	1.0	1.0
MSG				0.84	
Cell Cream					0.66
Water			10.0	10.0	10.0

Upon opening a silo-bag, the contents were thoroughly mixed by hand before sampling. For dry matter determination, 200grams was dried in a forced air oven at 60°C for 48 hours. Silage pH was determined after 50 grams was added to 400ml of water. The water-silage mixture, used to extract organic acids and ammonia, was placed in a

Table 2. Fiber and ash content of silages on different days.

Day	Treatment					SEM
	Control	Silaferm	NH4Cl	NH4Cl + MSG	NH4Cl + Cell Cream	
<i>neutral detergent fiber, % of DM</i>						
0	64.13 ^a	60.52 ^b	61.86 ^{ab}	62.05 ^{ab}	61.96 ^b	0.75
3	65.24 ^a	60.35 ^c	62.77 ^b	62.00 ^b	63.42 ^b	0.35
35	64.39 ^a	59.62 ^b	63.14 ^c	63.47 ^{ac}	63.50 ^{ac}	0.27
7 days exp.	65.49 ^a	60.03 ^b	62.30 ^{ab}	63.12 ^{ab}	64.20 ^a	0.91
<i>acid detergent fiber, % of DM</i>						
0	44.45 ^a	41.54 ^b	42.43 ^{ab}	42.78 ^{ab}	42.29 ^b	0.49
3	46.50 ^a	43.05 ^b	44.30 ^{bc}	44.62 ^c	44.17 ^{bc}	0.35
35	45.13 ^a	42.28 ^b	44.65 ^a	44.42 ^a	45.10 ^a	0.34
7 days exp.	45.45 ^a	42.15 ^b	43.37 ^{ab}	44.79 ^{ab}	44.32 ^{ab}	0.68
<i>ash, % of DM</i>						
0	10.92	11.60	10.83	11.24	10.76	0.21
3	10.34 ^a	11.55 ^b	10.60 ^a	10.80 ^a	10.57 ^a	0.16
35	10.80 ^a	12.25 ^b	10.74 ^a	11.25 ^a	10.92 ^a	0.13
7 days exp.	11.62	12.23	11.37	11.68	11.51	0.24

^{abc}Means within rows with unlike superscripts differ. P < 0.05.

refrigerator overnight. The following morning, a portion of the extract was filtered using Whatman 54 paper, acidified with 50% sulfuric acid and frozen for later analysis. Fresh forage samples and aerobically exposed silage were processed in the same manner.

For evaluation of silage aerobic stability, sixteen 2mm diameter holes were made at the top of each silo-bag after sampling on day 35. The silo-bags were weighed before and after aerobic exposure. Silage temperature was monitored daily by inserting a digital thermometer into the center of each silo. After 7 days of aerobic exposure, the silages were thoroughly mixed and sampled.

Nitrogen

Kjeldahl nitrogen procedure was used to determine total nitrogen and insoluble nitrogen fractions described by Licitra (1996) in all dried samples. Ammonia nitrogen was determined in the water extracts using a phenol – hypochlorite procedure (Russell 1944).

Lactic acid content was analyzed with HPLC. Volatile fatty acids were analyzed using a Varian gas chromatograph equipped with a Chrompack FFAP 25m column. Run conditions were: injector 200°C, column initial temperature 60°C final temperature 200°C with a temperature rise of 20°C per minute, detector 280°C. Water extract samples were thawed and 4ml was combined with 1ml of 25% metaphosphoric acid containing 2g / liter of 2-ethyl butyric acid as an internal standard.

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed non-sequentially using an Ankom fiber analyzer.

Ash was determined as residue remaining after 3 hours at 600°C.

Results

Treated silages generally had lower NDF and ADF contents compared with Control (table 2), primarily due to a diluting effect by treatment. No additive contained a measurable amount of fiber using the method described above. However, calculating the expected NDF content in treated silage using the measured NDF of Control and its percentage of the total dry matter in treated groups results in 52.8, 58.6, 57.2 and 58.8% expected NDF for Silaferm, NH₄Cl, NH₄Cl+MSG and NH₄Cl+BCC, respectively. All the measured values were higher than the expected values, especially for Silaferm. The same trend was evident in measured versus expected ADF values.

Silaferm had greater ash content compared with all others (Silaferm = about 20% ash, dry basis) on day 35.

Table 3. Organic acid content of silages on different days.

Day	Treatment					SEM
	Control	Silaferm	NH ₄ Cl	NH ₄ Cl + MSG	NH ₄ Cl + Cell Cream	
<i>lactic acid, % of DM</i>						
0	0.38	0.35	0.81	1.18	0.31	0.20
3	3.59	3.63	2.93	3.41	2.84	0.27
35	5.13	4.41	5.05	3.77	4.33	0.37
7 day exp.	3.77	4.58	3.65	3.96	3.03	0.53
<i>acetic acid, % of DM</i>						
0	0.12	0.19	0.18	0.17	0.13	0.03
3	0.80	0.98	1.03	1.14	1.34	0.11
35	1.12 ^{ab}	0.90 ^a	1.72 ^{bc}	1.99 ^c	1.99 ^c	0.18
7 day exp.	0.21 ^a	0.52 ^b	0.18 ^a	0.19 ^a	0.16 ^a	0.07
<i>propionic acid, % of DM</i>						
0	ND	ND	ND	ND	0.02	
3	0.03	0.03	0.06	0.06	0.06	0.01
35	0.03	0.04	0.01	0.01	0.02	0.01
7 day exp.	0.04	0.03	0.03	0.03	0.03	0.01
<i>butyric acid, % of DM</i>						
0	ND	ND	ND	ND	ND	
3	0.05	0.04	0.03	0.01	ND	0.02
35	0.07 ^a	0.01 ^b	ND	0.01 ^b	0.01 ^b	0.01
7 day exp.	0.06	0.01	ND	0.01	0.01	0.01

^{abc}Means within rows with unlike superscripts differ. P < 0.05.

ND: Not detected at or above .01% of dry matter.

On day 35, lactic acid was numerically lower in all treated silages compared with Control (table 3) but only NH₄Cl+MSG neared a level of significance (P=0.11). After 7 days of exposure, lactic acid declined in all silages except Silaferm and NH₄Cl+MSG. Treatments NH₄Cl+MSG and NH₄Cl+BCC had higher concentrations of acetic acid compared with Control on day 35. Adding NH₄Cl alone only tended to increase acetic acid (P=0.19). Silaferm was not different compared with Control and had a significantly lower concentration of acetic acid compared with the other treatments.

However, after 7 days of aerobic exposure, Silaferm had a significantly higher concentration of acetic acid compared with all others due to less degradation. Propionic and butyric acid were both very low in all silages and no differences existed except Control had higher butyric acid level on day 35 compared with treated silages.

Total nitrogen, ammonia nitrogen and non-protein nitrogen was higher in treated silages, by design, compared with Control (table 4). Ammonia nitrogen was determined with water extracts of wet silages while all other nitrogen fractions, including non-protein nitrogen (fraction A), were determined using the oven dried silage samples. Non-protein nitrogen includes ammonia nitrogen as well as nitrogen of free amino acids, peptides smaller than 8 amino acids and urea. Therefore, non-protein nitrogen (fraction A) must be equal to or greater than ammonia nitrogen; however, on day 0, measured ammonia nitrogen was greater than non-protein nitrogen in treated samples. Huber et. al. (1980) reported that 28% of ¹⁵N-labeled ammonia-N added to corn silage was recovered in the insoluble N fraction when water was used as the solute. This could have lead to an over-estimation of true protein N and a concurrent under-estimation of non-protein N (fraction A) in the dried samples used for N fractionation while ammonia N in the fresh samples used for ammonia determination remained soluble and high. However, true protein N, expressed as a percent of dry matter, did not differ between Control and treatment groups on day 0 so it is unlikely that any ammonia N was incorporated into insoluble fractions. In addition, the increase in percent non-protein N for all treatment groups is equal to the increase in total N within in each treatment within sample day, indicating that all added nitrogen was recovered in the soluble non-protein N fraction, as expected. The unexpected, high ammonia N values are most likely due to an increase in ammonia N in the samples during storage. Desrochers et. al. (1998) found that ammonia concentrations in both blood and plasma samples increased 38% and 15%, respectively, after freezing. During the ensiling period, non-protein nitrogen increased in all silage. Ammonia nitrogen increased in Control and numerically decreased in treated silages. True protein nitrogen consists of fractions B1 + B2 + B3 + C (or total N – npn fraction A). It is common for true protein N to decrease during ensiling due to proteolysis. Ammonia addition to forage has been shown to decrease proteolysis, probably by inactivating enzymes with high pH. The additives used in this trial did not increase pH (table 5);

however, Silaferm had more true protein remaining at day 35 compared with Control (figure 1). Further analysis of the nitrogen content shows that the higher true protein N in Silaferm silage was primarily in the B2 fraction. This fraction is degraded in the rumen at an intermediate rate. No difference existed between treatments in insoluble N fraction C. This protein is completely non-digestible by animals. Interestingly, this fraction also decreased during ensiling for all silages.

Table 4. Nitrogen content and fractions of silages on different days.

Day	Treatment					SEM
	Control	Silaferm	NH4Cl	NH4Cl + MSG	NH4Cl + Cell Cream	
<i>total nitrogen, % of DM</i>						
0	1.29 ^a	2.36 ^b	2.12 ^b	2.19 ^b	2.15 ^b	0.06
3	1.23 ^a	2.38 ^b	2.19 ^b	2.32 ^b	2.16 ^b	0.06
35	1.53 ^a	2.65 ^b	2.37 ^b	2.30 ^b	2.42 ^b	0.09
7 days exp.	1.60 ^a	3.07 ^b	2.39 ^a	1.94 ^b	2.66 ^a	0.27
<i>ammonia nitrogen, % of DM</i>						
0	0.16 ^a	1.63 ^b	1.42 ^b	1.53 ^b	1.51 ^b	0.06
3	0.26 ^a	1.56 ^b	1.39 ^{bc}	1.42 ^{bc}	1.21 ^c	0.08
35	0.22 ^a	1.51 ^b	1.32 ^b	1.30 ^b	1.23 ^b	0.10
7 days exp.	0.43 ^a	0.95 ^b	0.74 ^b	0.78 ^b	0.91 ^b	0.06
<i>non-protein nitrogen – A, % of DM</i>						
0	0.40 ^a	1.47 ^b	1.25 ^b	1.35 ^b	1.28 ^b	0.07
3	0.61 ^a	1.67 ^b	1.56 ^b	1.65 ^b	1.53 ^b	0.05
35	1.01 ^a	2.05 ^b	1.85 ^b	1.80 ^b	1.87 ^b	0.09
7 days exp.	0.91 ^a	2.41 ^b	1.74 ^{ab}	1.29 ^{ab}	2.03 ^{ab}	0.29
<i>rapidly degraded soluble true protein – B1, % of DM</i>						
0	0.13	0.15	0.17	0.14	0.15	0.02
3	0.12	0.10	0.10	0.12	0.10	0.02
35	0.06	0.07	0.04	0.06	0.08	0.02
7 days exp.	0.07	0.08	0.06	0.08	0.04	0.03
<i>intermediate degraded insoluble true protein – B2, % of DM</i>						
0	0.42	0.44	0.44	0.41	0.43	0.03

3	0.32 ^a	0.42 ^b	0.37 ^{ab}	0.38 ^{bc}	0.37 ^{ac}	0.01
35	0.28 ^a	0.36 ^b	0.33 ^{ab}	0.28 ^a	0.29 ^{ab}	0.02
7 days exp.	0.38	0.38	0.38	0.33	0.31	0.04

slowly degraded insoluble true protein – B3, % of DM

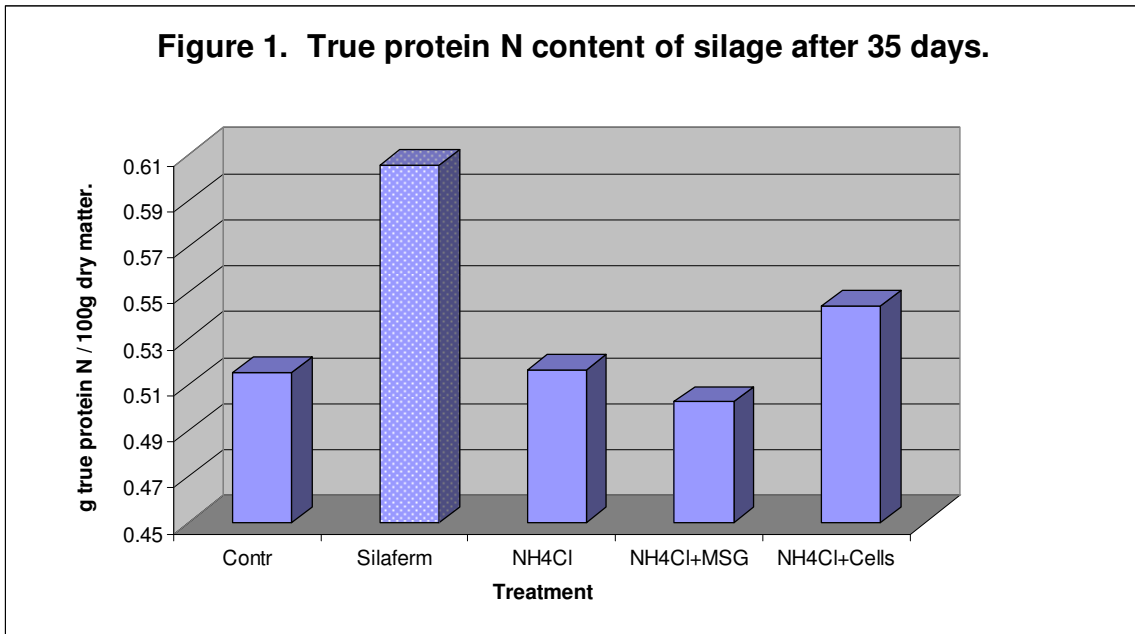
0	0.17	0.16	0.12	0.15	0.13	0.03
3	0.07	0.07	0.05	0.08	0.03	0.01
35	0.07	0.06	0.08	0.06	0.08	0.03
7 days exp.	0.13	0.15	0.16	0.11	0.2	0.05

unavailable insoluble true protein – C, % of DM

0	0.17	0.15	0.14	0.14	0.16	0.01
3	0.11	0.13	0.10	0.11	0.13	0.01
35	0.10	0.12	0.07	0.10	0.09	0.03
7 days exp.	0.12	0.06	0.05	0.13	0.08	0.03

^{abc}Means within rows with unlike superscripts differ. P < 0.05.

Figure 1. True protein N content of silage after 35 days.



Silaferm had lower pH compared with Control on day 0 and tended to have lower pH (P=0.11) on day 35. In a previous trial, Silaferm had no effect on pH of corn silage when added at similar levels. However, corn forage is lower in pH (about 5.5) compared with triticale. During the aerobic exposure period, Silaferm pH changed the least compared with all others.

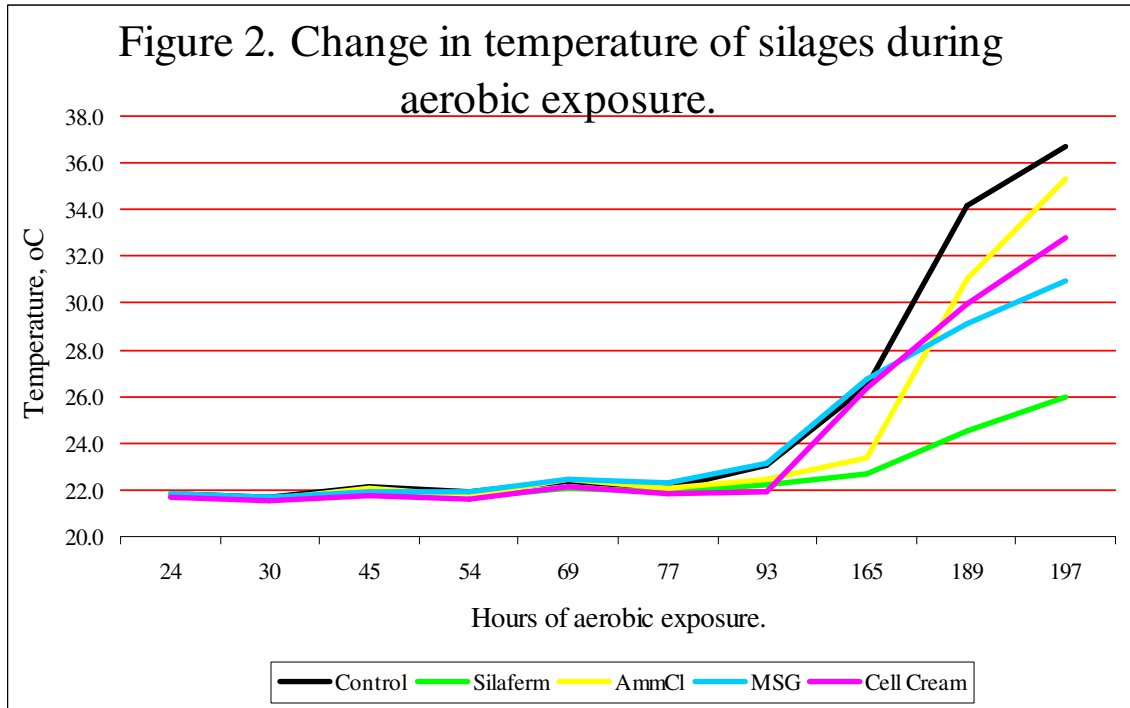
All silages were fairly stable under aerobic conditions (Figure 2). Control silage reached the highest temperature the earliest while Silaferm treated silage was the most stable during exposure to air.

Table 5. Measured pH of silages on different days.

Day	Treatment					SEM
	Control	Silaferm	NH4Cl	NH4Cl + MSG	NH4Cl + Cell Cream	
0	6.37 ^a	6.20 ^b	6.30 ^{ab}	6.39 ^a	6.30 ^a	0.02
3	4.35	4.33	4.33	4.38	4.33	0.03
35	4.17 ^{ab}	4.20 ^b	4.14 ^a	4.15 ^a	4.13 ^a	0.01
7 days exp.	5.51	4.24	4.53	5.41	5.35	0.74

^{abc}Means within rows with unlike superscripts differ. P < 0.05.

Figure 2 shows the core temperature of silos during aerobic exposure. Silaferm had the lowest temperature during the exposure period.



Conclusions

Adding Silaferm to grass silage can decrease true protein degradation during ensiling and also prolong aerobic stability. Ammonium chloride improved aerobic stability compared with Control but not as well as Silaferm. The effectiveness of Silaferm as a silage additive can be partly attributed to its NH_4Cl , but not fully. Monosodium glutamate and bacterial cell cream did not appear to have any affect on the parameters measured in this trial.

References

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