

William H. Miner Agricultural Research Institute

THE EFFECTIVENESS OF A KILLED LACTOBACILLUS ACIDOPHILUS SILAGE INOCULANT APPLIED TO ALFALFA/GRASS SILAGE STORED IN CONTRETE MIDI-SILOS

FINAL REPORT

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Research Report



Introduction:

Some farmers rely on silage additives to maintain consistent forage quality. A producer can benefit most from silage additives when good silo management is being practiced. Previous work has shown that “good” silages can be made “better” through the proper use of silage additives however, they will not make “poor” silage “good”. Silage additives can be classified into four broad categories: 1) bacterial inoculants, 2) enzymes, 3) acids and 4) nutritive additives. This study will evaluate the use of a killed bacterial inoculant and its effect on ensiling characteristics of alfalfa/grass silage stored in concrete midi silos.

Objectives

1. Determine if the use of silage inoculant results in a reduction in dry matter losses in alfalfa/grass silage.
2. Evaluate silage quality of treated and control silages to determine if silage quality is improved and/or fermentation losses are reduced.
3. Measure silage temperature to determine if use of the silage inoculant results in faster fermentation and/or reduced temperatures during fermentation.
4. Measure silage temperature after “feed-out” to determine aerobic stability of silage.

Materials and Methods

On June 8, first cut alfalfa/grass was harvested at 7:30 am, from a single field, raked at 1:00 pm and then chopped for ensiling at 1:45 pm at a DM ranging from 27.8 to 34.8%. Forage was treated at the chopper with one of two treatments: 1) Control – no additive; and 2) killed bacterial silage inoculant (Dyna Sile). The two treatments were replicated (4x) in midi silos for a total of 8 silos. Midi silos were made from 3' X 4' concrete culverts with 4" thick walls and a poured concrete bottom. These silos were buried 2-3 feet in the ground on a slope to simulate conditions found in standard bunker silos (see Figure 2). Effluent was collected through a center drain and measured via a collection system (see Figure 3).



Figure 1. Alfalfa/grass field prior to harvest.

The silage inoculant was applied at the rate recommended by the manufacturer as the silage was chopped (6 ounces per ton). Dyna Sile was applied as a liquid spray using an AFP applicator at the chopper head. In order to achieve the appropriate rate of application, 120 ounces of Dyna Sile was diluted to a total of 5 gallons of inoculant plus water. Silage treatments were randomly assigned within 4 plots while the field was being chopped into a dump truck bed. Between treatments, one load of forage was chopped as a “clean-out” to remove any residual products from the spray system. Between treatments, the dump truck bed was flushed with water to remove any inoculant residue.

At the midi silo, forage was dumped in a pile and ~1000 lbs. (455 kg) of forage was added to each silo, three inches at a time and manually packed with an attempt to obtain a theoretical packing density of 40 lbs./cubic ft (~14 lbs./cubic ft dry density). As each midi-silo was filled, grab samples of silage were taken for every 80 lbs. added, composited and divided into two samples. One sample from each midi-silo (approximately 1 lb.) was submitted for nutrient analysis immediately. The second sample (approximately 2 lbs.) was frozen at -20°C. A Tiny Talk[®] temperature data logger was placed midway in the center of the silo encased in a PVC tube (Figure 2). After packing, the silo was sealed with black plastic, and cut tire for weight and covered with plywood to prevent water from pooling and seeping down silo walls.

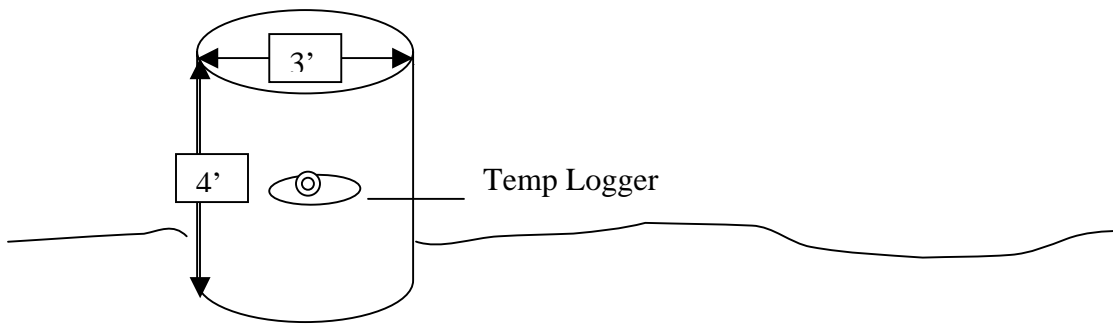


Figure 2. Buried concrete Midi-silo showing placement of temperature data logger.

Silage temperatures were measured every 2 hours for 35 days of the trial using a Tiny Talk[®] temperature data logger unit to record at the desired interval. Silage effluent was collected and measured after 28 days by external collection (see Figure 3). On July 13th, after 35 days of ensiling, the silos were opened and approximately 14 inches of silo face was removed daily, weighed and sampled until the silos were empty. Total dry matter loss was assessed. Amount of spoiled silage was estimated. Samples of good quality silage were frozen and later composited and



Figure 3. View of midi silos positioned on sloping hill to allow for external collection of effluent.

analyzed as the top third, middle third and bottom third for each silo. Silage quality was assessed for the following parameters: pH, DM, crude protein, soluble protein, ADF, NDF, NSC, ash, lignin, starch, sugar, titratable acidity, lactic acid, acetic acid, propionic acid, iso-butyric acid, butyric acid, total VFAs, lactic acid-VFA, ammonia, mold and yeast. Samples were analyzed in the Miner Institute Forage Laboratory for *in vitro* analysis, which included: organic matter, DM disappearance-true, and NDF disappearance. The temperature information recorded by the Tiny Talk[®] system was uploaded to a computer and change of temperature during fermentation was determined. A subsample of each third of the silo removed daily was placed into a Honig Box with a Tiny Talk[®] and placed into a control temperature room (80°F) where temperature of silage was recorded over 24 hours to evaluate the stability of the silage.

All response variables were analyzed using analysis of variance procedures of SAS to determine if differences exist between treatments.

Results and Discussion

Actual fresh forage DM, chemical composition and packing density of fresh forage are presented in Table 1. No significant treatment differences were found for any of the initial parameters evaluated, indicating similar forages were ensiled for all treatments tested. Although no significant differences were found, the forage treated with Dyna Sile tended to have a slightly lower CP and sugar content prior to ensiling. The forage DM at the time of ensiling and packing density was similar for both control and treated silages.

No effluent was collected from any of the silos indicating that the forage was sufficiently dry to limit effluent flow.

Table 1. DM and nutrient parameters of fresh chopped forages prior to ensiling and packing density of silos.

Item	Control	DynaSile	SE	<i>P</i> -value
DM	32.38	32.20	0.32	0.725
CP (%DM)	21.73	20.9	0.31	0.155
Lignin (%DM)	7.03	7.95	0.57	0.333
NDF (%DM)	45.62	46.50	0.85	0.518
Sugar (%DM)	5.98	5.70	0.10	0.151
Starch (%DM)	1.93	2.10	0.22	0.608
DM Density*	12.60	12.60	0.16	0.993

*lbs/cubic foot



Figure 4. Spoilage on surface of silo after opening.

The effect of Dyna Sile inoculant on amount of DM loss during ensiling and spoiled haycrop silage is presented in Table 2. There was no significant difference between treatments for DM loss however numerically there was slightly less loss for haycrop silage treated with Dyna Sile. Spoilage was measured by subjectively removing haylage that appeared to be moldy (Fig. 4). For all silos, moldy silage was only removed from the top of the silo after uncovering the plastic sheeting. The Dyna Sile treatment did not appear to minimize

any surface spoilage with both treatments having 8 to 8.5% spoilage on the surface of the silo.

Table 2. The %DM loss and % silo spoilage by forage treatment.

Item	Control	DynaSile	SE	<i>P</i> -value
% DM loss in silo	10.56	8.99	1.01	0.387
% spoilage in silo	8.53	7.92	0.86	0.668

The chemical composition and forage quality parameters of the forages following ensiling were evaluated to determine treatment effects (Tables 3 and 4). There were no significant treatment differences in chemical composition of ensiled forages. However, forage treated with Dyna Sile tended to have higher sugar values when compared to untreated forage.

Table 3. Chemical composition of treated forages after ensiling. (Ismean +/-se)

Item	Control	DynaSile	SE	<i>P</i> -value
DM (%)	30.03	30.29	0.18	0.696
OM (%DM)	88.86	89.13	0.06	0.203
CP (%DM)	22.04	21.95	0.09	0.334
SP (%CP)	60.25	61.00	0.37	0.267
ADICP (%DM)	1.54	1.58	0.08	0.698
Lignin (%DM)	8.83	8.97	0.12	0.384
NDF (%DM)	44.07	44.14	0.32	0.896
NSC (%DM)	3.55	3.48	0.19	0.858
Starch (%DM)	1.41	1.04	0.16	0.470
Sugar (%DM)	2.14	2.43	0.12	0.117

The fermentation profile and digestibility parameters are presented in Table 4. The pH and isobutyric acid for the untreated forage tended to be slightly higher than for the DynaSile forage. Both true DM and NDF disappearance was similar for both the control and Dyna Sile treatments.

Table 4. Fermentation profile and digestibility parameters of treated forages after ensiling.

Item	Control	DynaSile	SE	<i>P</i> -value
pH	4.36	4.28	0.03	0.052
Ammonia (% DM)	2.30	2.20	0.05	0.273
Lactic Acid (% DM)	4.97	4.95	0.45	0.938
Acetic Acid (% DM)	2.57	2.44	0.11	0.645
Lactic Acid/Acetic Acid	1.92	2.21	0.22	0.522
Propionic Acid (% DM)	0.041	0.044	0.006	0.721
Isobutyric Acid (% DM)	0.038	0.025	0.008	0.145
Butyric Acid (% DM)	0.025	0.030	0.005	0.664
Total Silage Acids (% DM)	7.64	7.49	0.50	0.434
True DM disapp. (%DM)	73.11	73.03	0.40	0.914
NDF disapp. (%NDF)	39.50	40.26	0.88	0.544

For each silo, mold and yeast counts were evaluated on the top, middle and bottom section of the silo. The silo treatments were replicated 4 times, resulting in 12 total samples. Mold and yeast counts were fairly low for both treatments regardless of silo section, however the Dyna Sile treated forages had a strong tendency to have lower mold and yeast counts when compared to the untreated forage (Table 5). Over 90% of all Dyna Sile treated samples had mold counts less than or equal to 100 colonies/g compared to 58% of the untreated control samples.

Table 5. Frequency table of mold and yeast counts for treated forages after ensiling (colonies/g).

Item	Control	DynaSile	<i>P</i> -value
Mold Counts			0.143
≤ 100	7	11	
100-1000	3	1	
>1000	2	0	
Yeast Counts			0.140
≤ 100	10	12	
100-1000	2	0	
>1000	0	0	

The average temperatures recorded by the data loggers during the ensiling process are presented in Figure 5. Temperature patterns indicate similar fermentations for both the control and Dyna Sile treated forages. The first stage of the fermentation process appeared to occur within the first few days after forage was ensiled. This fermentation was characterized by a rapid heating from 87°F to 97°F followed by a rapid decline in forage temperature to approximately 78°F. There was also no treatment difference between the start temperature and the highest temperature recorded during the first stage of the fermentation process (Control =9.20°F and Dyna Sile = 9.98°F; SE = 0.81°; $P=0.548$).

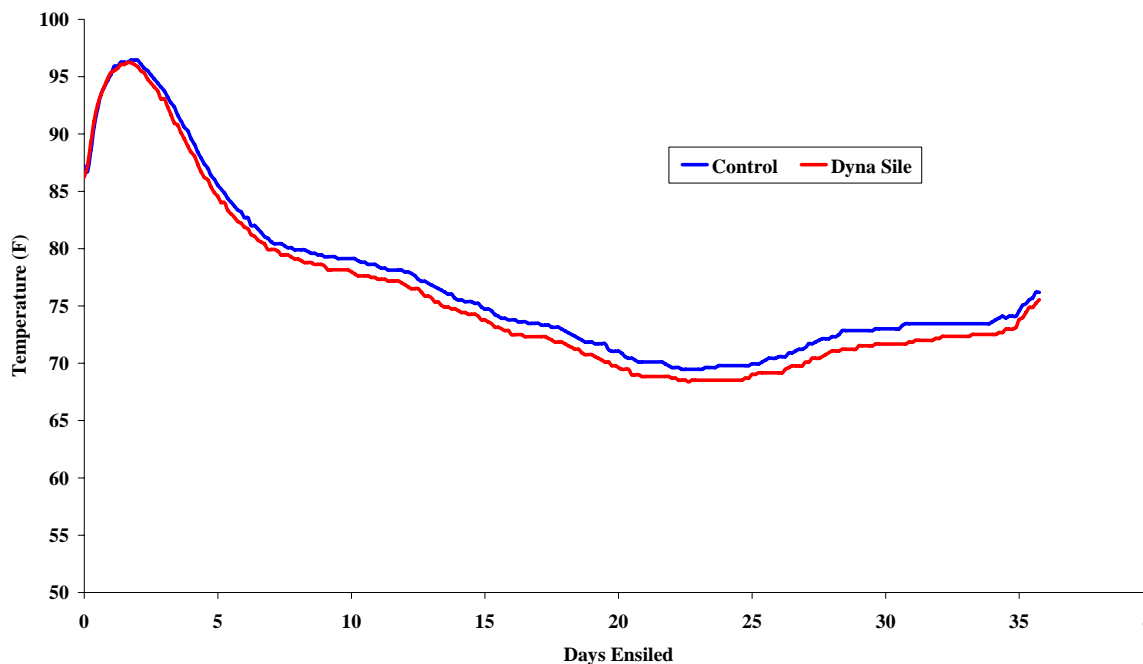


Figure 5. Midi silo temperature during ensiling process.

After 35 days of ensiling, the midi silos were opened and 14 inches of face material was removed daily until the silos were emptied. On a daily basis, a subsample of silage removed from the silos was placed into a Honig box and stored at 80°F to evaluate the stability of the forage. Temperature differences after 12 and 24 hours of storage were evaluated by section of midi silo where silage was removed: top, middle or bottom third (Table 6). The top section of the silo showed less heating at 12 and 24 hours after removal for silage treated with Dyna Sile ($P=0.067$ and $P=0.028$, respectively). There was no significant difference between treatments for the silage removed from either the middle or bottom section of the silo. These findings indicate that the top section of the silo was most susceptible to aerobic instability, however the inoculant treatment minimized the heating over the 24-hour period measured. These results are supported by

the higher mold and yeast counts found in the untreated forage, especially in the top third of each silo.

Table 6. Difference in temperature at 12 and 24 hrs. after removal of silage from top, middle and bottom third of midi silo and stored in Honig box at 80°F for 24 hrs. (°F)

Section	Hour	Control	Dyna Sile	SE	P-value
Top	12	2.65	0.65	0.50	0.067
	24	9.40	3.48	1.05	0.028
Middle	12	3.10	2.58	0.40	0.417
	24	3.43	3.28	0.30	0.743
Bottom	12	3.40	4.43	0.52	0.259
	24	3.75	4.43	0.61	0.488

The 24 hour heating pattern of the top, middle and bottom third of each silo after silage removal and storage in the Honig box system at 80°F is characterized in Figures 6-8. The untreated forage heated at a more rapid rate resulting in a 6°F difference in temperature after 24 hrs (Figure 6). Similar heating was observed for both untreated and Dyna Sile treated forage when silage was removed from the middle and bottom third of the silo, indicating aerobic stability of the forages since no heating occurred once the samples reached room temperature (Figures 7 and 8).

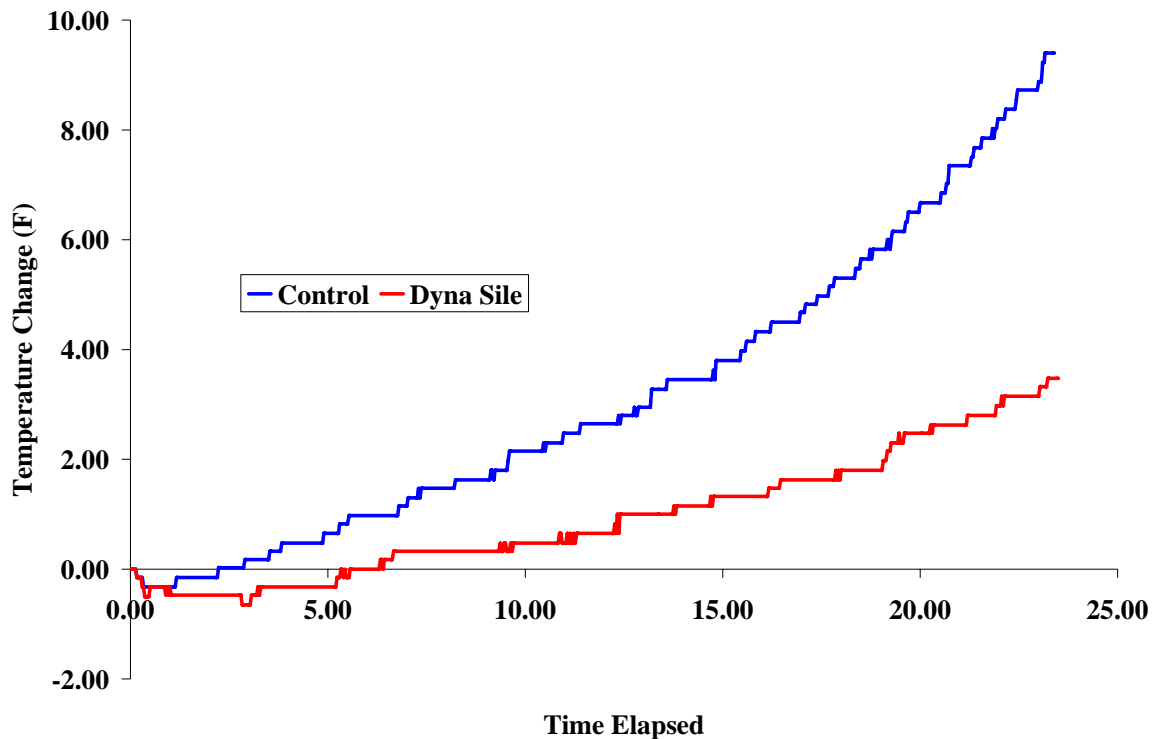


Figure 6. Temperature change of silage from top third of silo stored in Honig box for 24 hrs.

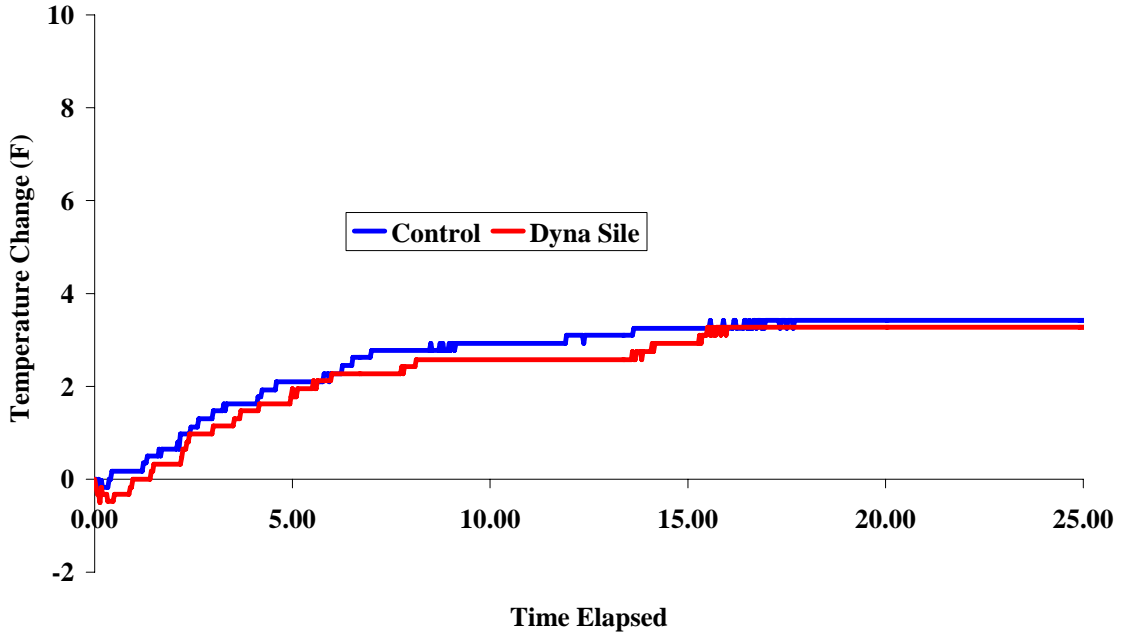


Figure 7. Temperature change of silage from middle third of silo stored in Honig box for 24 hrs.

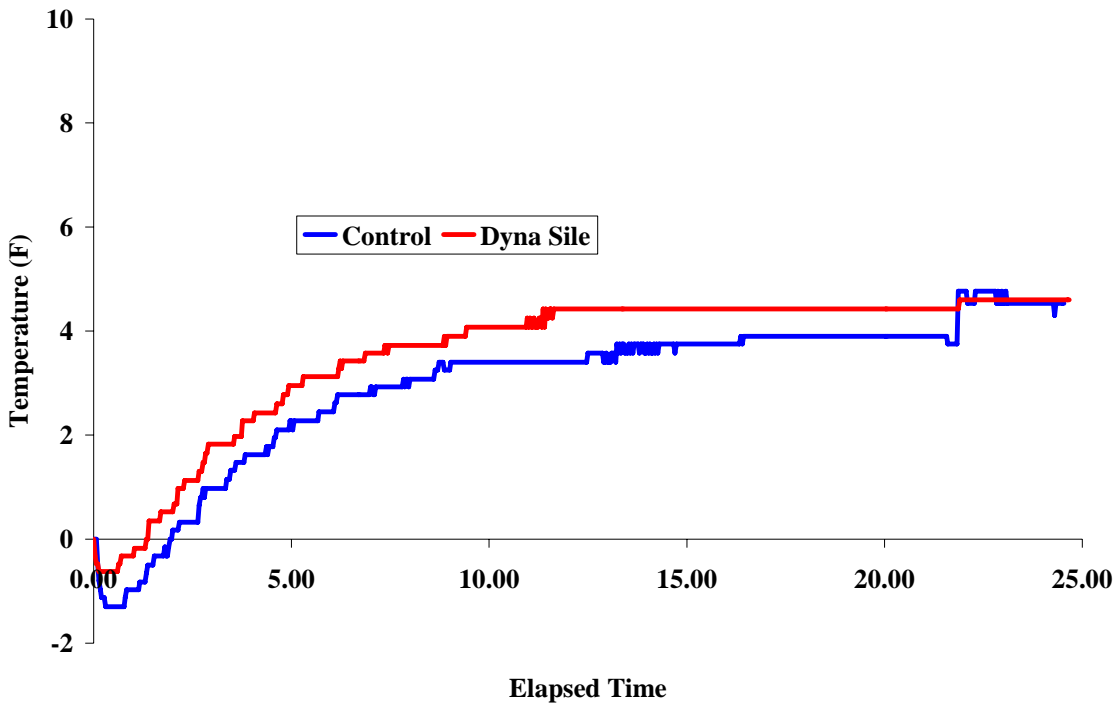


Figure 8. Temperature change of silage from bottom third of silo stored in Honig box for 24 hrs.

Summary

The use of Dyna Sile inoculant on alfalfa/grass silage did not appear to affect the fermentation of the forage and resulting chemical composition. Digestibility of the silage was also not influenced by the use of the Dyna Sile inoculant at the time of ensiling. Dyna Sile inoculant tended to reduce the amount of mold and yeast present in the top third of the silo and subsequently enhanced the aerobic stability of the silage removed from this section of the silo.

Appendix I. Chemical composition of silage treatments by section of removal from midi silo. (Ismean +/-se)

Item	Section	Control	Dyna Sile	SE	<i>P</i> -value
DM (%)	Top	29.98	30.23	0.41	0.697
	Middle	29.98	30.58	0.58	0.521
	Bottom	30.15	30.08	0.51	0.923
CP (%DM)	Top	22.28	22.10	0.13	0.402
	Middle	21.83	22.03	0.15	0.415
	Bottom	22.03	21.73	0.12	0.173
SP (%CP)	Top	58.75	61.00	0.53	0.058
	Middle	61.75	62.00	0.73	0.959
	Bottom	60.25	60.00	0.78	0.836
ADICP (%DM)	Top	1.65	1.40	0.13	0.269
	Middle	1.43	1.73	0.16	0.279
	Bottom	1.55	1.63	0.07	0.486
Lignin (%DM)	Top	8.98	8.88	0.09	0.495
	Middle	8.95	8.93	0.32	0.959
	Bottom	8.58	9.10	0.08	0.018
NDF (%DM)	Top	44.17	45.08	0.58	0.353
	Middle	44.35	43.65	0.47	0.363
	Bottom	43.67	43.70	0.80	0.982
NSC (%DM)	Top	3.45	3.08	0.39	0.549
	Middle	3.73	3.25	0.41	0.477
	Bottom	3.48	4.13	0.02	0.002
Starch (%DM)	Top	1.20	0.75	0.46	0.542
	Middle	1.50	0.83	0.46	0.372
	Bottom	1.53	1.55	0.07	0.809
Sugar (%DM)	Top	2.25	2.30	0.23	0.887
	Middle	2.20	2.43	0.13	0.319
	Bottom	1.98	2.58	0.05	0.003
OM (%DM)	Top	88.82	89.02	0.19	0.511
	Middle	88.88	89.17	0.05	0.029
	Bottom	88.88	89.21	0.16	0.250

Appendix II. Mean fermentation profile of treated silages by section of removal from midi silo. (lsmean +/-se)

Item	Section	Control	Dyna Sile	SE	P-value
pH	Top	4.47	4.28	0.06	0.104
	Middle	4.30	4.24	0.02	0.171
	Bottom	4.29	4.32	0.03	0.656
Ammonia (% DM)	Top	2.25	2.24	0.10	0.936
	Middle	2.47	2.29	0.10	0.279
	Bottom	2.18	2.07	0.08	0.304
Lactic Acid (% DM)	Top	4.52	4.99	0.73	0.683
	Middle	5.42	4.36	0.53	0.253
	Bottom	4.97	5.49	0.55	0.548
Acetic Acid (% DM)	Top	2.70	2.93	0.31	0.632
	Middle	2.49	2.24	0.06	0.057
	Bottom	2.52	2.15	0.25	0.368
Lactic Acid/Acetic Acid	Top	1.61	1.80	0.34	0.726
	Middle	2.15	1.91	0.24	0.533
	Bottom	1.99	2.92	0.53	0.301
Propionic Acid (% DM)	Top	0.05	0.07	0.02	0.459
	Middle	0.04	0.03	0.003	0.092
	Bottom	0.03	0.03	0.003	0.638
Isobutyric Acid (% DM)	Top	0.038	0.028	0.008	0.423
	Middle	0.028	0.020	0.008	0.547
	Bottom	0.050	0.028	0.009	0.186
Butyric Acid (% DM)	Top	0.033	0.033	0.006	1.00
	Middle	0.023	0.023	0.010	1.00
	Bottom	0.020	0.035	0.014	0.495
Total Silage Acids (% DM)	Top	7.34	8.05	0.77	0.560
	Middle	8.00	6.67	0.52	0.171
	Bottom	7.59	7.73	0.62	0.882

Appendix III. Mean forage digestibilities of treated silages by section of removal from midi silo. (lsmean +/-se)

Item	Section	Control	Bacterial	SE	P-value
True DM disapp	Top	73.12	71.70	0.42	0.097
	Middle	73.63	74.10	0.56	0.593
	Bottom	72.58	73.28	1.11	0.684
NDF disapp.	Top	40.09	37.86	0.69	0.105
	Middle	40.49	41.69	1.80	0.668
	Bottom	37.93	41.24	1.80	0.284