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An ASABE Meeting Presentation

Paper Number: 1008613

Ensiling Characteristics of Alfalfa Leaves and Stems

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Written for presentation at the
2010 ASABE Annual International Meeting
Sponsored by ASABE
David L. Lawrence Convention Center
Pittsburgh, Pennsylvania
June 20 – June 23, 2010

Abstract. *The separate harvesting of alfalfa leaves and stems would provide farmers more flexibility in the harvesting and utilization of alfalfa, but a key issue is storage. In three trials, unwilted alfalfa leaves were ensiled alone or with cell wall degrading enzymes, formic acid or lactic acid bacterial inoculant. Alfalfa stems wilted to 350 g DM/kg were ensiled with the same four treatments. In the two trials where the leaves were above 230 g DM/kg, the leaves ensiled successfully without any additives. Leaves ensiled at 168 g DM/kg eventually underwent a clostridial fermentation with elevated levels of butyric acid and ammonia, regardless of the treatments used. Formic acid could be used to guarantee a good fermentation, but more research is needed to ascertain the addition level as a function of the DM concentration of the leaves. The stems when wilted to approximately 350 g DM/kg ensiled well without additives. The enzyme and inoculant treatments affected fermentation as expected but were not necessary for good preservation by ensiling. Their value to the producer would depend on other factors not measured: improving DM recovery from storage and/or enhancing utilization by livestock or as a biomass feedstock.*

Keywords. Silage, alfalfa, leaf, stem, enzyme, formic acid, inoculant.

Introduction

Alfalfa is the primary hay crop forage grown in the U.S. However in Wisconsin, alfalfa acreage and total production have declined over the past 10 years whereas opposite trends have occurred in corn silage production (NASS, 2010). The vast majority of both crops are raised to feed dairy cattle in Wisconsin.

Various explanations have been offered as to why this trend is occurring. Corn silage is harvested once per year versus 3 to 4 cuttings of alfalfa. Yields of corn silage are higher per hectare. Alfalfa has a relatively short harvest window if you are making high quality forage for lactating dairy cows.

One potential solution to boost alfalfa production might be to harvest alfalfa leaves and stems separately. Alfalfa leaves are high in protein, and the nutritive value of the leaves declines slowly with maturity (Buxton and O'Kiely, 2003). Most of the decline in quality of alfalfa with maturity is due to the reduced fraction of leaves (or increased fraction of stem) as the plant matures. If leaves and stems were harvested and stored separately, they could be blended into the ration at the level needed by the animals being fed. High-producing, lactating dairy cows would receive proportionately higher levels of leaves; dry cows and heifers would receive more stems. Such a scheme may permit a reduction in the number of cuttings per year and/or increase the harvest window for alfalfa.

Another advantage of harvesting alfalfa leaves and stems separately is the potential for alternate uses of the alfalfa. Stems with their lower protein and higher structural carbohydrate content could be a potential source of ligno-cellulose for biofuel production. Alfalfa could possibly be genetically altered to produce valuable proteins or other compounds in the leaves.

For these possibilities to come to fruition, two problem areas need to be addressed. One is the development of equipment for harvesting the leaves and stems. The second is the development of storage systems for leaves and stems. Shinners et al. (2007) developed a prototype leaf harvester that removed 94% of the alfalfa leaves, resulting in a product that was 90% leaf tissue. The leaf tissue was ensiled in mini-silos, untreated or with one of three additives. The addition of corn or formic acid produced stable silage fermentations of low pH.

In the current study, we investigated various alternatives (inoculant, cell-wall degrading enzymes and formic acid) for ensiling both alfalfa leaves and stems using an updated harvester prototype that not only stripped the leaves but also cut the stems, laying them back on the stubble in a swath.

Materials and Methods

Three identical trials (June 11, August 6 and August 27, 2008) were carried out on mature alfalfa (early to late flower) grown at the University of Wisconsin Arlington Agricultural Research Station. The alfalfa was harvested in late morning with a modified prototype harvester, similar in part to that described by Shinners et al. (2007). Like the earlier prototype, leaves were removed with a stripping rotor head that had 16 rows of tines radially protruding 187 mm from the drum. The leaves were blown into a trailing wagon. In a modification of the earlier prototype, a disk cutterbar was added beneath and behind the stripping rotor head to cut the stems, which were laid in a swath back onto the stubble.

Leaves and stems were brought back to the laboratory in Madison for ensiling. Leaves were ensiled directly. Stems were laid out on screens outside to wilt for 2 h to reach approximately

35% dry matter (DM) and then chopped with a stationary chopper (14 mm theoretical length of cut).

Both leaves and stems were ensiled with the same four treatments: untreated control, lactic acid bacterial inoculant (Ecosyl 100, *Lactobacillus plantarum* MTD/1, approximately 10^5 colony-forming units/g alfalfa, Ecosyl Products Ltd., Stokesley, UK), formic acid (4 ml/kg alfalfa) and cell-wall degrading enzyme (Multifect A40 at approximately 5 IU/g alfalfa DM, Genencor Int'l, Rochester, NY). All treatments were diluted so that there would be identical application rates of 1 g/100 g alfalfa. The untreated control received 1 g distilled water/100 g alfalfa. In each trial, 4 mini-silos were made of each treatment. For each silo, 250 g alfalfa were weighed out, sprayed with 2.5 g treatment, mixed by hand and packed by hand in 500 ml Weck canning jars. During the course of filling silos, three initial samples of both leaves and stems were taken for analysis.

The silos were stored at room temperature (~22°C) for 120 d. Then the silos were frozen (-20°C) until the silages were analyzed.

The initial samples were analyzed for DM (freeze drying), pH, water-soluble carbohydrates (WSC) (Dubois et al., 1956), crude protein (CP) (Leco FP-2000A nitrogen analyzer, Leco Corp.,St. Joseph, MI), fiber fractions [neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and lignin (Ankom Technology Corp., Fairport, NY)] and lactic acid bacteria (Rogosa SL Agar, Becton Dickinson and Co., Sparks, MD). The inoculant was analyzed for lactic acid bacteria. The ensiled leaves and stems were analyzed for all of the same constituents as the initial samples except for lactic acid bacteria. In addition, the silages were analyzed for fermentation products (Muck and Dickerson, 1988), ammonia and free amino acids (Broderick et al., 2004).

Statistical analysis was performed across all three trials using the MIXED procedure of SAS (SAS, 2001). The treatment, trial and their interactions were considered fixed effects except as noted. Means were separated using LSMEANS with the PDIFF option ($P < 0.05$).

Results

Initial Alfalfa Characteristics

The alfalfa was harvested on sunny days between 10:00 and 11:00 am, and blossoms were present on the standing crop in all cases. The prototype harvester created two fractions that were considerably different in characteristics (Table 1). The stems were largely free of leaf tissue whereas the leaf fraction did contain petioles and some stem tissue, primarily from the tops of plants. The CP and fiber concentrations indicate that there was a substantial difference between the two fractions. In all three trials, the leaf fraction had a CP concentration more than double that of the stems, and the NDF, ADF and ADL concentrations in the leaf fraction were approximately half those measured in the stems. The WSC concentrations were higher in leaf than stem in all three trials.

The leaf fractions had low DM contents, but in the range that we had expected from earlier research on whole-plant alfalfa (e.g., Muck, 1987). After two hours of wilting, the stems in all trials had DM concentrations near the target of 350 g/kg, ranging from 319 and 367 g/kg.

The application of the lactic acid bacterial inoculant was similar across the three trials, averaging 6.26×10^5 cfu/g alfalfa (5.80 $\log_{10}$ cfu/g). This provided an application rate that was more than 10 times higher than the epiphytic population of lactic acid bacteria in all but one case (Table 1). The leaf fraction in the third trial had an epiphytic population similar to the population of lactic acid bacterial applied in the inoculant.

Table 1. Initial characteristics of alfalfa leaves and stems in the three trials.

Alfalfa Part	DM* Content, g/kg	pH	Lactic acid bacteria, log_{10}cfu/g	CP, g/kg DM	NDF, g/kg DM	ADF, g/kg DM	ADL, g/kg DM	WSC, g/kg DM
11 June 2008								
Leaf	168	5.98	2.79	298	287	196	43	122
Stem	319	6.00	2.84	129	579	441	95	90
6 August 2008								
Leaf	232	6.11	4.22	227	352	262	68	86
Stem	348	6.47	3.06	109	663	513	134	67
27 August 2008								
Leaf	234	6.10	5.61	263	253	181	41	114
Stem	367	6.28	2.95	126	565	424	100	86

*DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; WSC, water-soluble carbohydrates.

Ensiled Alfalfa Leaf Characteristics

The fermentation characteristics of the ensiled leaf fractions are shown in Table 2. There was considerable variation among replicates in the first trial that was due to the degree to which a clostridial fermentation (i.e., butyric acid accumulation) had developed in each silage. The smallest range of butyric acid concentrations occurred in the inoculant treatment (18.2 to 57.0 g butyric acid/kg alfalfa DM) whereas the largest range was in the enzyme treatment (3.0 to 138.3 g/kg DM). Because the variation in fermentation characteristics across replicates was much greater than the other two trials, each trial was statistically analyzed separately. In the first trial,

Table 2. Fermentation characteristics of ensiled alfalfa leaves from the three trials.

Treatment	DM Content, g/kg	pH	Lactic Acid, g/kg DM	Acetic Acid, g/kg DM	Butyric Acid, g/kg DM	Ethanol, g/kg DM	Total Products, g/kg DM
11 June 2008							
Control	158	5.94^{A}*	15.8	49.4	50.5	9.2	168.1
Enzyme	151	5.33^{AB}	24.4	46.9	65.2	6.7	169.5
Formic	163	4.86^{B}	36.7	51.6	29.0	13.7	145.8
Inoculant	156	5.85^{A}	13.8	56.6	36.7	11.2	144.5
6 August 2008							
Control	226^{BC}	4.35^{A}	70.0^{A}	23.6^{A}	0.0^{B}	4.3^{A}	101.8^{A}
Enzyme	224^{C}	4.16^{C}	88.3^{A}	22.2^{AB}	0.4^{A}	2.8^{B}	118.9^{A}
Formic	237^{A}	4.19^{C}	36.9^{B}	5.6^{C}	0.1^{AB}	0.4^{C}	43.8^{B}
Inoculant	228^{B}	4.32^{B}	62.4^{AB}	18.8^{B}	0.0^{B}	1.4^{C}	86.4^{A}
27 August 2008							
Control	234^{AB}	4.48^{A}	85.4	30.7^{A}	0.0	5.9^{A}	132.5^{A}
Enzyme	224^{C}	4.38^{B}	81.5	27.7^{A}	0.1	6.0^{A}	122.8^{A}
Formic	239^{A}	4.20^{C}	58.4	7.8^{B}	0.0	1.1^{B}	68.6^{B}
Inoculant	229^{BC}	4.50^{A}	78.9	26.1^{A}	0.0	4.7^{A}	119.5^{A}

*Numbers in a column within a trial having the same superscript are not statistically different ($P > 0.05$).

the only fermentation parameter that was significantly ($P < 0.05$) affected by treatment was pH. Formic acid produced the lowest pH whereas the control and inoculant treatments had pH values approximately one unit higher.

In the second trial, all treatments produced good fermentations with little or no butyric acid and low pH values (pH < 4.40) (Table 2). The enzyme and formic acid treatments had the lowest pH values. In the enzyme treatment, this was due to the high production of lactic and acetic acids whereas in the formic acid treatment, the formic acid reduced the amounts of fermentation products needed to achieve a low stable pH. The inoculant-treated leaves had a lower pH than that of the control. This appeared due to the reduced acetic acid in the inoculant treatment. Acetic acid has a pK_a of approximately 4.75 so that as acetic acid concentrations increase greater concentrations of other stronger acids like lactic are needed to achieve a given pH below 4.75.

The leaf silages in the third trial all had good fermentations and low pH values (Table 2). Formic acid treatment produced the lowest pH and the lowest concentrations of fermentation products. The enzyme treatment produced a lower pH than the untreated control even though there were no significant differences in products between the two treatments. The inoculant treatment produced similar ($P > 0.05$) results to the control.

Table 3. Nutritive characteristics of the ensiled alfalfa leaves from the three trials.

Treatment	CP, g/kg DM	NH_3-N, g/kg N	Free Amino Acid N, g/kg N	NDF, g/kg DM	ADF, g/kg DM	ADL, g/kg DM	WSC, g/kg DM
11 June 2008							
Control	309	191.3	566^{AB+}	330^{A}	249	64.6	20.4
Enzyme	337	153.8	599^{A}	278^{B}	199	53.3	36.6
Formic	300	139.3	500^{C}	357^{A}	262	51.8	48.7
Inoculant	302	218.5	520^{BC}	352^{A}	264	62.7	23.6
6 August 2008							
Control	249	70.5	512^{A}	388^{A}	311	88.0	16.5
Enzyme	268	63.5	456^{AB}	328^{C}	249	80.5	27.0
Formic	246	35.5	418^{B}	344^{BC}	272	71.2	40.8
Inoculant	257	68.5	482^{A}	365^{AB}	292	78.4	9.8
27 August 2008							
Control	287	47.5	454^{A}	275^{A}	203	48.5	8.9
Enzyme	295	45.0	438^{A}	245^{B}	166	50.8	19.3
Formic	278	35.5	278^{B}	276^{A}	198	50.7	35.9
Inoculant	285	42.3	401^{A}	286^{A}	210	52.9	13.6
Average							
Control	282^{b}*	103.1^{a}	511^{a}	331^{a}	254^{a}	67.1	15.3^{c}
Enzyme	300^{a}	87.4^{ab}	497^{ab}	283^{b}	205^{b}	61.5	27.6^{b}
Formic	274^{b}	63.3^{b}	398^{c}	326^{a}	244^{a}	57.9	41.8^{a}
Inoculant	281^{b}	109.8^{a}	468^{b}	334^{a}	255^{a}	64.7	15.7^{c}

*Numbers in an Average column followed by the same superscript are not statistically different ($P > 0.05$).

$^{+}$Numbers in a Trial column followed by the same superscript are not statistically different ($P > 0.05$). These comparisons are shown when the treatment by trial interaction was significant.

In contrast to the fermentation products, nutritive characteristics (N and fiber fractions) generally had similar variation across trials, and in most constituents there were no significant treatment by trial interactions. A summary of the nutritive characteristics of the leaf silages is shown in Table 3. The enzyme treatment had a higher crude protein concentration than the other treatments (300 vs. 279 g CP/kg DM). Ammonia N was highest in the inoculant and control treatments and lowest in formic acid treatment. The control and enzyme treatments had the highest free amino acid content whereas the lowest occurred in the formic acid treatment. There was a significant treatment by trial interaction for free amino acids. This appears to be due to differences in the ranking of the enzyme treatment across the three trials: highest free amino acid concentration in the first trial, third in the second trial, and second in the third trial.

Across the three trials, the control, formic acid and inoculant treatments had similar NDF concentrations. The enzyme reduced NDF 47 g/kg DM on average. The significant treatment by trial interaction was caused by the NDF of the formic acid treatment being lower than the control in the second trial and not in the other trials. The ADF concentration of the enzyme treatment was 46 g/kg DM lower than the average of the other three treatments. ADL concentrations were not affected by treatment. The levels of WSC at the end of ensiling were highest in the formic acid treatment and lowest in the control and inoculant treatments.

Ensiled Alfalfa Stem Characteristics

The wilted alfalfa stems ensiled well in most cases (Table 4). There were trace levels of butyric acid in some treatments. Given the DM contents and pH values, it is likely that the butyric acid was not formed in a secondary fermentation late in storage but rather early in fermentation before an inhibitory pH was reached (Muck et al., 2003). Overall, there was no significant effect of treatment or treatment by trial interaction on butyric acid content.

For the other fermentation characteristics, treatment was significant, and the treatment by trial interaction was significant for all but ethanol. The inoculant treatment produced the lowest pH and formic acid the highest pH consistently across all three trials. The treatment by trial interaction was caused by the enzyme treatment having a similar pH ($P > 0.05$) to that of the inoculant in the first two trials, but a higher pH than that of the inoculant in the third trial.

Across the three trials, the inoculant and enzyme treatments produced the greatest amount of lactic acid, and the formic acid treatment produced the lowest level of lactic acid. The significant treatment by trial interaction was due to two factors: the variable ranking of the enzyme treatment from trial to trial (highest concentration in the first trial, similar to control and inoculant in the second, and similar to the control in the third) and the lack of significant differences between any treatments in the second trial although there was a trend ($P < 0.10$) for the formic acid treatment to be lower than the enzyme and inoculant treatments.

For both acetic acid and ethanol, the highest concentrations on average were observed in the enzyme treatment and the lowest in the formic acid and inoculant treatments. There were no significant differences between treatments in the second trial for acetic acid, causing the treatment by trial interaction to be significant. Total fermentation products were highest in the enzyme and inoculant treatments and lowest with the formic acid treatment. The principal cause of the significant treatment by trial interaction for total fermentation products was the similarity in values across treatments in the second trial.

The nutritive characteristics of the ensiled alfalfa stems are shown in Table 5. Crude protein followed a similar pattern with treatment as the ensiled leaves. On average, the CP was highest in the enzyme treatment, and the other three treatments were similar to each other. The significant treatment by trial interaction was due to the absence of significant differences between treatments in the second trial.

Table 4. Fermentation characteristics of ensiled alfalfa stems from the three trials.

Treatment	DM Content, g/kg	pH	Lactic Acid, g/kg DM	Acetic Acid, g/kg DM	Butyric Acid, g/kg DM	Ethanol, g/kg DM	Total Products, g/kg DM
11 June 2008							
Control	312^{AB}*	4.16^{A}	36.0^{C}	9.2^{B}	0.0	3.4	52.2^{B}
Enzyme	294^{C}	4.00^{B}	76.5^{A}	16.8^{A}	0.0	6.6	109.2^{A}
Formic	317^{A}	4.21^{A}	19.4^{D}	2.4^{C}	0.1	1.0	24.5^{C}
Inoculant	301^{BC}	3.95^{B}	58.7^{B}	3.8^{C}	0.0	2.3	69.2^{B}
6 August 2008							
Control	340	4.46^{B}	36.0	8.2	1.1	2.9	52.0
Enzyme	345	4.29^{C}	36.7	7.1	0.0	2.8	49.5
Formic	341	4.87^{A}	19.8	7.3	1.9	1.0	48.6
Inoculant	345	4.23^{C}	39.3	9.6	0.0	1.5	53.6
27 August 2008							
Control	369^{A}	4.48^{B}	36.4^{B}	7.5^{B}	0.0	3.6	50.8^{BC}
Enzyme	356^{B}	4.51^{B}	44.1^{B}	10.9^{A}	0.5	5.3	65.8^{AB}
Formic	360^{AB}	4.91^{A}	17.0^{C}	4.0^{C}	0.6	2.0	33.0^{C}
Inoculant	366^{AB}	4.16^{C}	63.8^{A}	5.6^{BC}	0.0	3.7	75.2^{A}
Average							
Control	340	4.37^{b+}	36.1^{b}	8.3^{b}	0.4	3.3^{b}	51.6^{b}
Enzyme	332	4.27^{c}	52.4^{a}	11.6^{a}	0.2	4.9^{a}	74.8^{a}
Formic	339	4.66^{a}	18.7^{c}	4.6^{c}	0.8	1.3^{c}	35.3^{c}
Inoculant	337	4.11^{d}	53.9^{a}	6.3^{c}	0.0	2.5^{bc}	66.0^{a}

*Numbers in a Trial column followed by the same superscript are not statistically different (P > 0.05). These comparisons are shown when the treatment by trial interaction was significant.

$^{+}$Numbers in an Average column followed by the same superscript are not statistically different (P > 0.05).

Ammonia and free amino acid concentrations were affected by treatment (Table 5). Ammonia N as a fraction of total N was highest in the control and lowest in the inoculant treatment across the three trials. There was a significant treatment by trial interaction largely because of the lack of significant differences between treatments in the second trial. Free amino acid N was similar for all treatments except for the formic acid treatment, which was significantly lower than the others. That pattern held across all three trials.

The enzyme treatment reduced NDF and ADF (55 and 53 g/kg DM on average, respectively) compared to the other treatments (Table 5). There was no significant treatment by trial interaction for NDF, but there was for ADF. This significant interaction was not due to the consistency of the enzyme treatment but due to the control ADF being significantly higher than the formic acid and inoculant treatment ADFs in the first trial.

Water-soluble carbohydrates remaining after ensiling followed a similar pattern in the ensiled stems as was observed in the ensiled leaves. On average, the highest WSC concentration occurred in the formic acid treatment, followed in order by the enzyme, control and inoculant treatments. However, the ranking of treatments varied with each trial, resulting in significant treatment by trial interactions.

Table 5. Nutritive characteristics of the ensiled alfalfa stems from the three trials.

Treatment	CP, g/kg DM	NH_3-N, g/kg N	Free Amino Acid N, g/kg N	NDF, g/kg DM	ADF, g/kg DM	ADL, g/kg DM	WSC, g/kg DM
11 June 2008							
Control	138^{B}*	59.5^{A}	524	609	482^{A}	113^{A}	64.7^{B}
Enzyme	145^{A}	51.5^{A}	470	539	404^{C}	103^{B}	52.8^{C}
Formic	139^{AB}	30.5^{B}	409	582	450^{B}	103^{B}	135.3^{A}
Inoculant	139^{AB}	33.5^{B}	430	589	456^{B}	105^{B}	12.0^{D}
6 August 2008							
Control	122	86.3	391	664	525^{A}	139	5.5^{C}
Enzyme	118	72.3	419	624	482^{B}	141	51.1^{A}
Formic	120	80.5	330	668	524^{A}	142	22.5^{B}
Inoculant	121	72.3	428	658	519^{A}	138	4.0^{C}
27 August 2008							
Control	132^{B}	53.5^{A}	391	588	470^{A}	114^{A}	16.9^{B}
Enzyme	150^{A}	61.0^{A}	423	522	418^{B}	105^{B}	54.8^{A}
Formic	129^{B}	50.5^{A}	338	607	487^{A}	116^{A}	56.0^{A}
Inoculant	134^{B}	28.5^{B}	356	591	472^{A}	117^{A}	17.9^{B}
Average							
Control	130^{b+}	66.4^{a}	435^{a}	620^{a}	492^{a}	122^{a}	29.0^{c}
Enzyme	138^{a}	61.6^{ab}	437^{a}	562^{b}	434^{b}	116^{b}	52.9^{b}
Formic	129^{b}	53.8^{bc}	359^{b}	619^{a}	487^{a}	120^{ab}	71.2^{a}
Inoculant	131^{b}	44.8^{c}	405^{a}	613^{a}	482^{a}	120^{ab}	11.3^{d}

*Numbers in a Trial column followed by the same superscript are not statistically different ($P > 0.05$). These comparisons are shown when the treatment by trial interaction was significant.

$^{+}$Numbers in an Average column followed by the same superscript are not statistically different ($P > 0.05$).

Discussion

Ensiled Alfalfa Leaves

Alfalfa silage is known to be susceptible to clostridial fermentation because alfalfa normally has a relatively high buffering capacity and low sugar concentration. As a result, typical recommendations for making alfalfa silage involve wilting the crop to a minimum of 300 g DM/kg (Albrecht and Beauchemin, 2003). Wilting to 300 g DM/kg also minimizes effluent production in most silo types (Muck et al., 2003).

With the current prototype harvester for separating leaves and stems, it is not possible to wilt the leaves prior to ensiling. So an aim of this study was to determine if it would be possible to ensile alfalfa leaves reliably without wilting using only typical additives that are used in making silage.

The Multifect A40 enzyme product was expected to improve fermentation by breaking down cell wall carbohydrates, providing extra sugar for fermentation by the lactic acid bacteria and hopefully lowering pH sufficiently to avoid a clostridial fermentation. The formic acid treatment immediately lowers crop pH but permits lactic acid bacteria to ferment sugars so that a lower final pH should be achieved than is possible in an untreated crop. The inoculant in this study

applied a homofermentative lactic acid bacteria to the crop. If the inoculant overwhelms the natural lactic acid bacteria, this product should produce a lower pH compared to that in an untreated silage by shifting fermentation away from acetic acid and ethanol to lactic acid, a stronger acid than acetic.

The leaves were successfully ensiled in two of the three cases. The first trial was conducted with the wettest leaves (168 g DM/kg), and all treatments underwent a secondary, clostridial fermentation with substantial concentrations of butyric acid and ammonia. The formic acid treatment had the lowest pH and ammonia concentration and numerically lowest butyric acid concentration. However, 4 ml formic acid/kg alfalfa was not sufficient prevent clostridial activity.

The leaves in the second and third trials were drier at ensiling (232 and 234 g DM/kg). In both of these trials, all four treatments ensiled well. The enzyme and formic acid treatments had lower pH values than the control, which would have made them more reliably stable over even longer storage periods than the 120 d in this study. The inoculant treatment did not produce different results from the respective control treatment in either the second or third trial with the exception of a lower pH (0.03) in the second trial.

In an earlier study, Shinners et al. (2007) ensiled alfalfa leaves at 216 g DM/kg. After 123 d ensiling, the ensiled untreated leaves had undergone a clostridial fermentation and were of poor quality. The addition of 2.5 ml formic acid/kg alfalfa was sufficient to avoid a clostridial fermentation with a final pH of 4.3 and no butyric acid detected.

The earlier study and the current one together suggest that the quality of the leaf silages is highly dependent on the DM content at ensiling. At 230 g DM/kg, the leaves ensiled well without additives. This is considerably wetter than what is deemed safe (300 to 350 g DM/kg) in normal wilted alfalfa (Albrecht and Beauchemin, 2003). However, when alfalfa is wilted in the field, there will be respiration losses, reducing the amount of sugar available to the lactic acid bacteria to ferment (Muck et al., 2003). In the current study, the leaves averaged more than 100 g WSC/kg DM at ensiling, which may have accounted for the successful ensiling in the two drier trials.

A wetter crop needs a lower pH from the lactic acid bacterial fermentation to avoid clostridial fermentation (Muck et al., 2003). In the Shinners et al. (2007) study at 216 g DM/kg, the unaided fermentation was not sufficient for a stable silage whereas the low level of formic acid (2.5 ml/kg alfalfa) was. In the first trial of the current study at 168 g DM/kg alfalfa, a higher level of formic acid (4.0 ml/kg) was not sufficient to prevent clostridial activity. This suggests the need for further research to develop a suitable application rate of formic acid as a function of DM content to guarantee a stable alfalfa leaf silage. This additive could potentially guarantee a successful fermentation every time provided that the application is sufficiently high for the DM content of the leaves being ensiled.

The enzyme also has the potential to guarantee a good fermentation. The enzyme consistently reduced ADF and NDF in the three trials by a minimum of 30 g/kg DM so that extra sugar was provided to the lactic acid bacteria. However, this extra sugar was not sufficient with the wettest leaves to prevent clostridial growth. The pH values achieved with the enzyme treatments in the second and third trials together with the modeling work on clostridial growth in silage by Leibensperger and Pitt (1987) suggest that the enzyme could permit successful ensiling at DM concentrations as low as 200 to 225 g/kg. It is not possible without further research to speculate on whether higher application rates would liberate more sugars, permitting successful preservation at lower DM contents.

The inoculant provided the least modification of fermentation of the three additives. It is not possible from these three trials to determine the minimum DM content for the successful use of

an inoculant. However, it is clear that the range of successful DM contents will be smaller than for formic acid or enzyme treatments.

While these results indicate that there are a range of DM contents at which alfalfa leaf silage can be made successfully with or without treatment, silage effluent will be an issue in all cases. Putting leaves into a bag or shallow pile will minimize the amount of effluent, but ensiling at 250 g DM/kg or less will require that the producer has a means to collect and dispose of the effluent. Well-fermented effluent does not have to be a waste and has been fed to beef cattle (O'Kiely, 1989).

Ensiled Alfalfa Stems

The stems in this study wilted to approximately 350 g DM/kg in 2 h. This is similar to or faster than observations in earlier trials (Shinners et al., 2007) where the stems were cut in a separate operation from leaf stripping. This rapid drying is beneficial from the perspective of minimizing the potential for rain damage during harvest. The disadvantage with ensiling stems if they dry this rapidly is that both leaves and stems would need to be ensiled on the same day, requiring that two structures or piles be filled simultaneously.

The untreated stems ensiled well in all three trials to a low pH (< 4.50; Table 4). There was a low level of butyric acid in the second trial, but this was likely caused by clostridia early in fermentation rather than by a secondary fermentation after a long storage period. This is suggested by two factors. First, the low level of lactic acid bacteria on the stems at ensiling (~1000 cfu/g; Table 1) could have increased the time available for clostridia to develop early in storage. Note: the inoculant treatment that should accelerate pH decline did not have detectable amounts of butyric acid. Second, the ensiled pH at 120 d was well below that needed to inhibit clostridia in 340 g DM/kg alfalfa (Leibensperger and Pitt, 1987).

The enzyme treatment was effective and hydrolyzed mostly cellulose in the stem cell walls as noted by similar reductions in both NDF and ADF. In the first and second trials, this activity led to lower pH values than observed in the respective untreated stem silages. There was no effect in the third trial with the driest stems. Added sugars, directly or indirectly, have had similar mixed effects on silage fermentation, depending on the amount of sugar already available in the crop prior to treatment (e.g., Jones et al., 1992). In the current study, the additional sugar was not needed for preservation of the crop during ensiling. However, it is possible that this pretreatment may be beneficial at improving the digestibility of the stems by livestock or increasing the availability of cell wall carbohydrates for production of ligno-cellulosic biofuels.

The formic acid treatment increased pH relative to the control treatment in the second and third trials. This was unexpected and is not easily explained. The numbers of lactic acid bacteria on the stems at ensiling were low in all three trials, suggesting a slow fermentation in the untreated controls. The immediate reduction in pH from formic acid treatment would be expected to further slow the development of lactic acid bacteria. This may have permitted other microbial groups to develop, influencing fermentation products and pH. In the second and third trials, low concentrations of butyric acid were found (Table 4), suggesting clostridial development early in fermentation as observed in the second trial in the control treatment. Not shown in Table 4 are other fermentation products that were measured. The formic acid treatment was the only treatment that had detectable amounts of propionic acid in all silos across all three trials (0.8, 6.1 and 5.8 g/kg DM, respectively, for the first, second and third trials). In contrast, the control had propionic acid concentrations of 0.0, 1.7 and 0.9 g/kg DM, respectively. Propionic acid could come from several sources: propionic acid bacteria and clostridia, being the most likely (Pahlow et al., 2003). With a pK_a of 4.87, the significant levels of propionic acid in the formic

acid treatment, especially in the second and third trials, may have provided the extra buffering to prevent pH from declining as low as in the respective control treatments.

In contrast to the leaf silages, the inoculant worked consistently in the stem silages to reduce pH compared to the control treatments, on average 0.25 units. The consistency of the inoculant effect may be due to the lower competition from the epiphytic lactic acid bacteria on the stem material (Table 1). The inoculant application rate provided more than 100 times more lactic acid bacteria than were naturally present on the stems. In the first and third trials, the reduced pH in the inoculant treatment was caused by increased lactic acid production. Overall the shifts in pH and fermentation products are what would be expected from a homofermentative inoculant (Kung et al., 2003).

Conclusions

Successful preservation of unwilted alfalfa leaves by ensiling in this study was dependent on the DM concentration of the leaves at ensiling. In the two trials where the leaves were above 230 g DM/kg, the leaves ensiled successfully without any additives. The addition of either formic acid or a cell wall degrading enzyme reduced pH compared the untreated control in those trials, further decreasing the likelihood of a clostridial fermentation if longer periods of storage had been investigated.

Leaves ensiled at 168 g DM/kg eventually underwent a clostridial fermentation with elevated levels of butyric acid and ammonia, regardless of the treatments used in this study. In order to successfully preserve leaves this wet, formic acid would be the only treatment of the ones investigated that would be capable of preventing clostridial fermentation, but higher levels of formic acid than used in this study (4 ml/kg) would be needed. Overall, it would appear that formic acid could be used to guarantee a good fermentation when ensiling alfalfa leaves. However, more research is needed to ascertain the addition level as a function of the DM concentration of the leaves.

Of the other two additives studied, the cell wall degrading enzyme appeared to be capable of ensuring a good fermentation of leaves if the leaves were above 200 to 225 g DM/kg. The inoculant appeared to have the narrowest range of improvement compared with untreated leaves.

The stems when wilted to approximately 350 g DM/kg ensiled well without additives. Formic acid did not improve the preservation of the stems. The enzyme and inoculant treatments affected fermentation as expected from ensiling studies in other crops but were not necessary for good preservation of the stems by ensiling. Their value to the producer would depend on other factors not measured: improving DM recovery from storage and/or enhancing utilization by livestock or as a biomass feedstock.

Acknowledgements

The authors wish to express our thanks for technical assistance in carrying out this study by Ursula Hymes-Fecht and assistance in sample analysis from Sharanya Dayal.

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