Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs[1,2,3]

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ABSTRACT: The objectives of this study were to determine the effect of a cellulase (from *Trichoderma longibrachiatum*) alone or combined with a bacterial inoculant (*Lactobacillus plantarum* and *Pediococcus cerevisiae*) or formic acid on composition, intake, and digestibility of orchardgrass (*Dactylis glomerata* L.) and alfalfa (*Medicago sativa* L.) silages. Orchardgrass and alfalfa were harvested at the early heading stage and at the early bloom stage of maturity and wilted to approximately 22 and 32% DM, respectively. Forages were then ensiled in 100-L sealed barrels for at least 60 d before they were fed to lambs. Silage treated with cellulase had lower ($P < .001$) pH and lower ($P < .001$) acetic acid and NH_3 N concentrations than untreated silage of both plant species and a higher ($P = .004$) lactic acid concentration than the control treatment of alfalfa silage. Fermentation characteristics of cellulase-treated silages, especially of alfalfa, were further enhanced by use of inoculant. Formic acid addition increased ($P < .001$), reducing sugar concentration of cellulase-treated orchardgrass and alfalfa silage by 90 and 154%, respectively, and decreased ($P < .001$) NH_3 N concentration of cellulase-treated alfalfa silage by 19%. Averaged across plant species, cellulase, combined with inoculant or formic acid, resulted in 8 and 13% greater ($P = .03$) DMI, respectively, than the control silage. Extensive enzymatic cell-wall degradation during ensiling decreased ($P = .003$) NDF intake of cellulase-treated orchardgrass silage by 25% and decreased ($P = .001$) cellulose intake by 23%, when averaged across plant species. Addition of formic acid increased ($P = .003$) NDF intake of cellulase-treated orchardgrass silage by 19%. Averaged across species, cellulase application decreased ($P < .05$) silage NDF digestibility by 18%. Greater sugar and lower acetic acid, NH_3 N, and NDF concentrations resulted in greater DMI of cellulase-treated silage than of control silage, when cellulase was combined with formic acid or inoculant.

Key Words: Cellulase, Composition, Digestibility, Intake, Silage

Introduction

Forage intake and digestibility are influenced both by forage nutritive value and animal characteristics.

When feeding high-quality forage, the energy demand of the animal is usually most limiting to DMI, whereas rumen fill normally limits intake of low-energy forage (Mertens, 1994). It is, therefore, important to use animals with potential for high-energy intake, such as young, growing lambs and cattle, or dairy cows in early lactation, when feeding high-quality forage to ensure that fill, and not energy demand, limits DMI. The often stated relationship that "increased digestibility results in increased intake" is influenced by the residence time of forage in the rumen (Thornton and Minson, 1973). Thornton and Minson (1973) suggested that the greater intake of legumes than of grasses at equal digestibilities is related to the shorter ruminal retention times for legumes than for grasses. The short ruminal retention time at high intake can decrease the apparent digestibility of DM and NDF (Staples et al., 1984). The DMI by animals has a greater effect on animal performance than digestibility. Buxton and Mertens (1995) concluded that 65 to 75% of the variation in energy intake can be related to DMI and only 20 to 30% to differences in digestibility.

Numerous publications have presented the effects of cell-wall degrading enzymes, bacterial inoculants, or formic acid on intake and apparent digestibility of grass silage (Jaakkola, 1990; Jaakkola et al., 1991; Jacobs et al., 1991, 1992; Sharp et al., 1994), whereas only limited information is available on the effect of enzyme treatment on the feeding value of alfalfa silage (Jaster and Moore, 1988). This experiment was conducted to study the effect of cellulase alone, or combined with a bacterial inoculant or formic acid, on composition, intake, and digestibility of orchardgrass and alfalfa silages. We expect additivity of the treatments based on our previous results (Nadeau et al., 2000).

Materials and Methods

Plant Material and Ensiling

Orchardgrass and alfalfa were grown in adjoining plots at the Agronomy and Agricultural Engineering Research Center of Iowa State University near Ames. The soil was a Webster (Typic Haplaquolls) fertilized with 73 kg K and 38 kg P/ha during the spring of 1993. Orchardgrass was fertilized with 114 kg N/ha on April 23, 1993, and was harvested for silage on June 6, 1993, at the early heading stage of maturity. Alfalfa was harvested for silage on June 9, 1993, at the early bloom stage of maturity. Orchardgrass and alfalfa were mowed, wilted in windrows to approximately 22 and 32% DM, respectively, and chopped to a theoretical length of cut of 19 mm with a forage chopper. Although it was intended that both plant species be dried to an equal moisture level, the difference in DM concentrations between plant species was caused by much better drying conditions with 11°C warmer temperature during wilting of alfalfa.

For treatment application, wilted forages were spread evenly on plastic sheets, aqueous solutions of the treatments were applied with sprinkler water cans, and forages were mixed well. Treatments included 1) wilted herbage before ensiling, 2) wilted control silage with no treatment, and silage treated with 3) cellulase alone or with 4) cellulase combined with a bacterial inoculant, or 5) cellulase combined with formic acid. The liquid cellulase (Multifect CL, Genencor International, Rochester, NY), which also had some hemicellulolytic activity, was derived from *Trichoderma longibrachiatum* and had a minimum carboxymethylcellulase activity of 2,500 IU/mL (pH 4.8, 50°C) as stated by the manufacturer. Application rates of cellulase and formic acid (88%) were 10 and 4 mL/kg of wilted herbage, respectively. Bacterial inoculant (Biomate SI Forage Inoculant, Chr. Hansen's Laboratory, Milwaukee, WI) contained *Lactobacillus plantarum* and *Pediococcus cerevisiae*. A water solution of the inoculant was applied at 10^5 cfu of lactic acid bacteria/g wilted herbage. Water was added to the control treatment as well as to the silage additive treatments so that a total of 4% liquid was added to all wilted herbage weights. Forages were

packed with a manual steelpacker and ensiled in polyethylene bags within 100-L sealed barrels for 70 to 160 d at about 20°C. Eight barrels per treatment of each plant species, each containing 39 kg of forage, were prepared to ensure adequate silage for the feeding trial. After a barrel had been opened, silage quality was maintained by adding Dry Ice to the silage and resealing the barrel after each meal. Additionally, eight samples of untreated wilted herbage within each plant species were taken prior to ensiling and later composited to four samples of wilted herbage per species.

In Vivo Digestibility Trial

Eight male Dorset × Polypay lambs, with an average weight of 23 ± 1.8 kg and 16 wk of age at the beginning of the trial, were placed in individual cages equipped with water nipples and meshed rubber floor to let feces fall through onto the net underneath the base. Four lambs were assigned to orchardgrass silage and four were assigned to alfalfa silage in two 4 × 4 Latin square designs with 3-wk periods. Each period consisted of one 1-wk preliminary phase, when feed, orts, and waste were weighed, and two 1-wk collection phases, when feed, orts, waste, and feces were weighed and sampled. Lambs were fed silage twice daily (0830 and 2030), and orts, waste, and feces were collected before each feeding. During the collection phases, 150-g subsamples of feed and feces and all of the orts and waste were collected twice daily and composited for each collection week and lamb. In vivo digestibility was determined at ad libitum intake during the first collection phase and at a restricted intake of 18 g of DM/(kg BW·d) during the second collection phase to study the effect of rate of passage on digestibility. In vivo digestibility measurements at a restricted intake of 18 g of DM/(kg BW·d) minimize any effects of ruminal retention time and rate of passage on digestibility. Intake was measured during the first 2 wk of each of the four periods, when the lambs were fed at 10% above their ad libitum intake. Digestible intakes were calculated using the digestibility coefficients at ad libitum intakes. To avoid carryover effects between treatments, the lambs were placed in a common pen and fed excess amounts of untreated silage for 1 wk before the start of each 3-wk period.

During feedout, a composited subsample of silage was taken from two of the eight barrels within each treatment and plant species to obtain four replicates per treatment for analysis of the chemical composition of the silages. All samples from the digestion trial were kept frozen at −20°C until they were prepared for chemical analyses.

Chemical Analyses

One 100-g subsample from each sample of wilted herbage and silage was freeze-dried and ground in a UDY cyclone mill (UDY Corp., Fort Collins, CO) to pass a 1-mm screen. Analyses of DM, IVDMD with the NC-

64 direct acidification procedure (Marten and Barnes, 1980), NDF, ADF, and ADL (Goering and Van Soest, 1970) were conducted on the freeze-dried samples. The DM concentration was determined by weighing the samples before and after freeze-drying. Concentrations of NDF, ADF, and ADL were determined sequentially with an α-amylase (Sigma Chemical Co, St. Louis, MO, No. A-6814) addition to the NDF procedure (Van Soest and Robertson, 1980). Hemicellulose concentration was calculated as the difference between NDF and ADF concentrations, and cellulose concentration was calculated as the difference between ADF and the sum of ADL-plus-ash concentrations.

Crude protein concentration was determined on wet samples by using the macro-Kjeldahl technique with a Tecator 1015 digestion block (Tecator AB, Höganäs, Sweden). Digested samples were analyzed for total N concentration according to the QuikChem Method No. 15-107-06-2-B with a salicylate-nitroprusside color reagent by using an automated ion analyzer (QuikChem AE, Lachat Instruments, Milwaukee, WI).

A second 100-g subsample from each sample of wilted herbage and silage was diluted with 100 mL of deionized water, mixed in a Waring blendor (Model 1113, Waring Products Div., Winsted, CT) for 30 s, and squeezed through one layer of cheesecloth. Herbage and silage pH were determined with a glass electrode on fresh plant extracts before the extracts were frozen for later analyses of NH_3 N, reducing sugar, and organic acid concentrations. Plant extracts were centrifuged at $11,200 \times g$ at 5°C for 10 min before analysis. Concentration of NH_3 N was determined according to the QuikChem Method No. 26-107-06-2-B with a salicylate-nitroprusside color reagent by using the same automated ion analyzer as for the total N determination. Concentration of reducing sugars was determined by absorbance measured spectrophotometrically (Ultrospec 4050, LKB Biochrom Ltd., Cambridge, U.K.) at 660 nm and related to a glucose standard curve for calculating concentrations of reducing sugar (Nelson, 1944; Somogyi, 1945). Concentrations of organic acids were determined by gas chromatography (model 5890 GC, HP3396 Series II integrater, HP 7673A auto sampler, Hewlett-Packard Co., Wilmington, DE) of butyl esters, which were prepared as described by Salanitro and Muirhead (1975). Heptanoate was used as an internal standard, and the butyl esters were separated on a HP5 10-m × 530-μm glass column coated with 5% phenylmethyl silica (Hewlett-Packard Co.), using a flame ionization detector and nitrogen as a carrier gas with a flow rate of 6.3 mL/min. The injection port temperature was 180°C, and the detector temperature was 270°C. The oven temperatures were regulated as follows: 50°C for 30 s, followed by an 8°C/min increase to 100°C, and a 30°C/min increase to a final temperature of 180°C.

To determine DMI and in vivo DM digestibility of the silages, 300-g subsamples of feed, orts, waste, and feces were dried in air-forced driers (Siemens-Allis, New Orleans, LA) at 65°C for 48 h. Dried samples were ground in a UDY cyclone mill to pass a 1-mm screen before sequential fiber analysis was conducted on the samples as previously described. Concentrations of NDF, cellulose, and hemicellulose of feed, orts, waste, and feces were used to calculate intakes and in vivo digestibilities of these fiber components in the silages.

Statistical Designs

Silage quality data were analyzed via analysis of variance for a split-plot design by using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Plant species were treated as the whole plot and forage treatment, including wilted herbage and the four silage treatments, was treated as the subplot. The four replicates of each treatment, each composited from two barrels, were nested within plant species. The effect of plant species was tested using replicate nested within species as an error term. When a significant F-value was detected at $P < .05$ or at $.05 < P < .10$, LSD at $P < .05$ or $P < .10$, respectively, was used to determine significant variation among means in the main effect of treatment across plant species and in the plant species × treatment interaction (Cochran and Cox, 1957).

Intake and in vivo digestibility data for orchardgrass and alfalfa were analyzed via analysis of variance for two 4 × 4 Latin square designs by using the GLM procedure of SAS (SAS Inst. Inc.). Lamb, period, and treatment were used as main effects within plant species (squares). Because there was no significant plant species (= square) × treatment interaction, except for the total NDF intake ($P = .087$), in the combined analysis of the two 4 × 4 Latin square designs, treatment effects across plant species were analyzed. When a significant F-value was detected at $P < .05$ or at $.05 < P < .10$, LSD at $P < .05$ or $P < .10$, respectively, was used to determine significant variation among means in the main effect of treatment for the combined analysis across plant species (Cochran and Cox, 1957). For total NDF intake, LSD was used to separate treatment means within species. Differences between means for digestibilities measured at ad libitum and at restricted intakes and differences between means for digestibilities measured with in vivo and in vitro methods were analyzed by a paired t-test (Cochran and Cox, 1957).

Results and Discussion

Silage Composition

There were significant treatment × species interactions for all silage variables, except for CP, hemicellulose, and lignin (Tables 1 and 2). Because of warmer weather conditions during field drying, wilted alfalfa had a greater DM concentration than wilted orchardgrass at ensiling (Table 1). Ensiling resulted in a significant decrease in the DM concentration of alfalfa but not of orchardgrass. Control silages had 5 and 3% lower IVDMD than wilted orchardgrass and alfalfa,

Table 1. Dry matter, IVDMD, CP, NDF, cellulose, hemicellulose, ADL, and reducing sugar concentrations in wilted herbage and silage of orchardgrass and alfalfa

Chemical component and species	Wilted herbage	Control	Cellulase	Cellulase plus inoculant	Cellulase plus formic acid	Species x̄	Treatment P	Treatment SEM	Interaction P	Interaction SEM	LSD[a]
DM, g/kg											
Orchardgrass	272	264	283	283	285	277			<.001	4	12
Alfalfa	401	362	371	371	380	377***					
x̄	337^w	313^y	327^x	327^x	332^{wx}		<.001	3			
IVDMD, g/kg											
Orchardgrass	685	651	649	650	657	658			.09	4	10
Alfalfa	699	680	679	687	696	688***					
x̄	692^w	665^y	664^y	668^y	677^x		<.001	3			
CP, g/kg DM											
Orchardgrass	166	170	168	159	163	165			NS[b]	4	
Alfalfa	239	257	242	235	239	242***					
x̄	203^{xy}	214^w	205^x	197^y	201^{xy}		.002	3			
NDF, g/kg DM											
Orchardgrass	569	539	444	419	427	479***			.004	14	40
Alfalfa	411	432	369	376	374	392					
x̄	490^w	485^w	407^x	397^x	400^x		<.001	10			
Cellulose, g/kg DM											
Orchardgrass	313	306	211	201	220	250			.01	12	35
Alfalfa	255	276	224	231	232	244					
x̄	284^w	291^w	218^x	216^x	226^x		<.001	8			
Hemicellulose, g/kg DM											
Orchardgrass	209	202	184	184	167	189***			NS	12	
Alfalfa	86	81	60	58	64	70					
x̄	148^w	141^{wx}	122^{xy}	121^y	115^y		.05	8			
ADL, g/kg DM											
Orchardgrass	41	30	25	34	33	33			NS	7	
Alfalfa	68	73	84	82	73	76***					
x̄	55	51	54	58	53		NS	5			
Reducing sugar, g/kg DM											
Orchardgrass	69	8	69	74	131	70***			<.001	3	10
Alfalfa	62	0	28	33	71	39					
x̄	66^x	4^z	48^y	53^y	101^w		<.001	2			

[a]LSD at $P < .05$ or $P < .10$ when P for the F-test of species × treatment is $< .05$ or $.05 < P < .10$, respectively.

[b]NS = not significant ($P > .10$).

^{w,x,y,z}Means within a row lacking a common superscript letter differ ($P < .05$ when P for the F-test of treatment is $< .05$; $P < .10$ when P for the F-test of treatment is $.05 < P < .10$) according to LSD test.

***$P < .001$ for the main effect of species.

Table 2. The pH, lactic acid:acetic acid ratio, organic acid, and NH$_3$ N concentrations in wilted herbage and silage of orchardgrass and alfalfa

Chemical component and species	Wilted herbage	Control	Cellulase	Cellulase plus inoculant	Cellulase plus formic acid	Species $\overline{x}$	Treatment P	Treatment SEM	Interaction P	Interaction SEM	LSD[a]
pH											
Orchardgrass	6.43	4.42	4.12	4.11	4.13	4.64			<.001	.01	.04
Alfalfa	5.51	4.83	4.34	4.29	4.28	4.65					
$\overline{x}$	5.97^v	4.63^w	4.23^x	4.20^y	4.21^{xy}		<.001	.01			
Lactic acid, g/kg DM											
Orchardgrass	.0	84.9	87.9	86.7	53.7	62.5***			.004	2.0	5.8
Alfalfa	.0	65.5	77.4	76.2	39.9	51.6					
$\overline{x}$	.0^y	75.2^w	82.6^v	81.5^v	46.8^x		<.001	1.4			
Acetic acid, g/kg DM											
Orchardgrass	1.5	22.1	17.8	16.5	11.4	13.9			<.001	.5	1.5
Alfalfa	.4	32.0	23.6	19.8	12.4	17.7***					
$\overline{x}$	1.0^z	27.1^v	20.7^w	18.2^x	11.9^y		<.001	.4			
Lactic acid:acetic acid											
Orchardgrass	.0	3.9	4.9	5.3	4.7	3.7***			<.001	.1	.4
Alfalfa	.3	2.0	3.3	3.8	3.2	2.5					
$\overline{x}$	.1^y	3.0^x	4.1^w	4.6^v	4.0^w		<.001	.1			
Succinic acid, g/kg DM											
Orchardgrass	4.7	15.8	12.6	11.7	5.8	10.1***			<.001	.4	1.0
Alfalfa	3.3	8.7	7.5	7.0	3.2	5.9					
$\overline{x}$	4.0^y	12.2^v	10.0^w	9.3^x	4.5^y		<.001	.3			
Total acids, g/kg DM											
Orchardgrass	17.1	126.1	123.0	120.1	88.0	94.9**			<.001	2.2	6.3
Alfalfa	26.3	110.4	113.3	110.6	73.3	86.8					
$\overline{x}$	21.7^y	118.2^v	118.1^v	115.3^v	80.7^x		<.001	1.5			
NH$_3$ N, g/kg total N											
Orchardgrass	50	146	120	126	126	114			<.001	4	12
Alfalfa	40	188	160	155	129	135***					
$\overline{x}$	45^y	167^v	140^w	141^w	127^x		<.001	3			

[a]LSD at $P < .05$ for species × treatment.
^{v,w,x,y,z}Means within a row lacking a common superscript letter differ ($P < .05$) according to LSD test.
**$P < .01$ for the main effect of species.
***$P < .001$ for the main effect of species.

respectively. Alfalfa silage treated with cellulase plus formic acid had 2% greater IVDMD than control alfalfa silage; the increase was mostly caused by formic acid. However, the cellulase plus formic acid treatment did not increase IVDMD of control orchardgrass silage. Control silage had a greater CP concentration (total N × 6.25) than wilted herbage or treated silages, when averaged across plant species (Table 1). However, a greater portion of total N in control silage than in wilted herbage or treated silages was in the form of NH_3 N (Table 2).

Compared with the controls, cellulase treatment decreased NDF concentration by 18% in orchardgrass and by 15% in alfalfa, with no additional effects by inoculant or formic acid (Table 1). Similarly, other research by Nadeau (1995) reported no effects of inoculant or formic acid on cell-wall concentrations of cellulase-treated orchardgrass and alfalfa silages. Averaged across plant species, cellulase degraded 25% of the cellulose and 13% of the hemicellulose. Enzymatic hydrolysis of cellulose was 65% greater in orchardgrass than in alfalfa silage (31 vs 19% hydrolysis). Less cell-wall degradation in alfalfa is likely related to the greater lignin concentration and lower initial NDF concentration in alfalfa than in orchardgrass.

Silage fermentation depleted reducing sugars in control alfalfa silage and left only trace amounts in control orchardgrass silage (Table 1). There were sufficient fermentable sugars in wilted orchardgrass for a successful fermentation to occur, as indicated by similar lactic acid concentrations between cellulase-treated and control silage (Table 2). Alfalfa, however, is not as easily ensiled as orchardgrass because of its greater buffering capacity, as indicated in this study by higher NH_3 N and acetic acid concentrations, and, consequently, higher pH (McDonald and Henderson, 1962). Addition of cellulase to alfalfa silage was necessary to stimulate lactic acid production and to decrease pH. Because of greater NDF degradation by cellulase during ensiling and sufficient sugars available in wilted herbage to stimulate fermentation in control orchardgrass silage, cellulase-treated orchardgrass silage had more than twice as much sugar as cellulase-treated alfalfa silage (Table 1). Enzymatic hydrolysis of NDF to soluble sugars supplied as much sugar as was fermented during ensiling of orchardgrass.

Because formic acid restricts silage fermentation, and, consequently, preserves sugars, sugar concentration of orchardgrass silage treated with cellulase plus formic acid was nearly twice as great as for wilted herbage and the other cellulase treatments. Likewise, addition of formic acid to cellulase-treated alfalfa silage more than doubled the concentration of reducing sugars. These results agree with data by Russell (1985) and confirm that the increased sugar concentration in silage treated with cellulase plus formic acid had no inhibitory effect on cell-wall degradation, as shown by similar fiber degradations between silage treated with cellulase and silage treated with cellulase plus formic

acid. The increased sugar concentration, especially in orchardgrass silage treated with cellulase plus formic acid, may reduce the aerobic stability of the silage. However, if the silage is packed well to minimize airflow in the silo during ensiling, the risk for aerobic deterioration during feedout is decreased (Muck and Pitt, 1993).

Silage Fermentation Products

Because of a greater lactic acid concentration and a lower NH_3 N concentration, pH of orchardgrass silage was lower than that of alfalfa silage (Table 2). Orchardgrass silage had a lower acetic acid concentration and, consequently, a higher lactic:acetic acid ratio than alfalfa silage within treatments. The lactic:acetic acid ratio indicates the extent of homolactic fermentation in relation to heterolactic fermentation of sugars to lactic acid during ensiling, where also acetic acid is produced (Jones et al., 1992). A more heterolactic fermentation of alfalfa than of orchardgrass is related to a greater buffering capacity in alfalfa than in orchardgrass (McDonald and Henderson, 1962).

Cellulase treatment decreased pH of control silage in both plant species (Table 2). Addition of inoculant or formic acid caused a further small but significant pH decline of cellulase-treated alfalfa silage, whereas inoculant or formic acid had no effect on pH in orchardgrass silage. Cellulase alone increased lactic acid concentration of control alfalfa silage by 18% with no additional increase by the inoculant. The already high lactic acid concentration in control orchardgrass silage was not increased by use of cellulase or inoculant. Silage treated with cellulase plus formic acid had a significantly lower lactic acid concentration than other silages of both plant species. Addition of formic acid decreased lactic acid concentration of cellulase-treated orchardgrass and alfalfa silage by 39 and 48%, respectively.

Cellulase treatment decreased acetic acid concentration in control orchardgrass and alfalfa silage by 19 and 26%, respectively (Table 2). Addition of inoculant decreased acetic acid concentration of cellulase-treated alfalfa silage by 16%, but inoculant had no effect on acetic acid concentration in orchardgrass silage. Cellulase plus formic acid treatment, which had the largest decrease in acetic acid concentration among the silage treatments, resulted in a 36 and 47% lower acetic acid concentration than cellulase-treatment of orchardgrass and alfalfa silage, respectively.

Untreated alfalfa silage had a low lactic:acetic acid ratio but, as in orchardgrass, the ratio was increased by addition of cellulase (Table 2). Cellulase-treated silages had 26 and 65% higher lactic:acetic acid ratios than control orchardgrass and alfalfa silages, respectively. Addition of inoculant increased the lactic:acetic acid ratio of cellulase-treated orchardgrass and alfalfa silage by 8 and 15%, respectively. Results by others (Jaakkola et al., 1991; Jacobs and McAllan, 1991; Kung et al.,

1991) have shown variable effects of cellulase enzyme mixtures on fermentation of grass and alfalfa silages.

There were usually only traces of organic acids in wilted herbage, except for acetic and succinic acid (Table 2). Trace amounts of propionic acid were present in control silage of both plant species, with nearly four times greater amounts in alfalfa than in orchardgrass (orchardgrass: .17 g/kg DM; alfalfa: .78 g/kg DM). Treated silages had no propionic acid. Succinic acid concentration was three times as high in control silage as in wilted herbage of both plant species. The increase in succinic acid concentration might be related to the relatively extensive proteolysis in the silages partly caused by enterobacteria. In addition to their proteolytic activity, enterobacteria can ferment glucose to succinic acid (McDonald et al., 1991). Cellulase treatment decreased succinic acid concentration by 20 and 14% compared with control orchardgrass and alfalfa silages, respectively. Formic acid treatment decreased succinic acid concentration of cellulase-treated orchardgrass and alfalfa silages by 54 and 57%, respectively, to a level close to that of wilted herbage. Orchardgrass had a significantly greater succinic acid concentration than alfalfa both within and across treatments.

Formic acid present in wilted herbage of orchardgrass and alfalfa was nearly depleted during ensiling, but addition of formic acid to cellulase-treated silage increased formic acid concentration (orchardgrass: 1.3 vs 14.1 g/kg DM; alfalfa: .6 vs 10.6 g/kg DM for cellulase and cellulase plus formic acid, respectively). There was no detectable butyric acid in the silages.

Total acid concentration, which indicates the extent of fermentation during ensiling, was greater in orchardgrass than in alfalfa. The greater fermentation of orchardgrass than of alfalfa silage was probably related to the lower DM concentration in orchardgrass. Control silage had six and three times greater total acid concentration than wilted orchardgrass and alfalfa, respectively. Treatment with cellulase alone and with cellulase combined with inoculant resulted in total acid concentrations similar to those in control silage of both plant species. Addition of formic acid decreased total acid concentrations in cellulase-treated orchardgrass and alfalfa silage by 28 and 35%, respectively.

Extensive proteolysis occurred during ensiling, which was shown by the nearly two and four times greater NH_3 N concentrations in control silages than in wilted orchardgrass and alfalfa, respectively (Table 2). As a result of a decreased pH, cellulase-treated orchardgrass and alfalfa silages had 18 and 15% lower NH_3 N concentrations than control orchardgrass and alfalfa silages. Addition of formic acid to cellulase-treated alfalfa silage decreased NH_3 N concentration by 19%, but addition of formic acid had no effect on NH_3 N concentration of cellulase-treated orchardgrass silage.

Ad Libitum Dry Matter and NDF Intake

Cellulase combined with inoculant or formic acid increased total DMI by 8 and 13%, respectively, compared with control silage, when averaged across plant species (Table 3). The same treatments had similar effects on the digestible DMI. Because NDF concentration of forage is a good predictor of the voluntary DMI by sheep (Van Soest, 1965), the higher DMI of the silages treated with cellulase and inoculant or formic acid may have been associated with their lower NDF concentrations compared with the control silages (Table 1). Additionally, silage fermentation characterisics have been associated with voluntary intake of silage (Rook and Gill, 1990). Thus, the improved homolactic fermentation of silages treated with cellulase and inoculant or formic acid compared with the control silages may be another reason for the increased DMI of these silages (Table 2).

Although we cannot separate the effect of plant species from the effect of square in the statistical design, numerical data from Table 3 indicate that the ad libitum intake by lambs fed alfalfa silage was nearly twice the DMI by lambs fed orchardgrass silage. The difference in DMI between plant species may be related to the lower DM concentration of orchardgrass than of alfalfa silage. However, orchardgrass silage had a more homolactic fermentation than alfalfa silage, which would counteract the negative effect of a lower DM concentration on DMI (Rook and Gill, 1990).

Because of extensive degradation of the more digestible portion of NDF by cellulase during ensiling, lambs fed cellulase-treated orchardgrass silage had 25% lower total NDF intake than lambs fed control silage (Table 3). When formic acid was added to cellulase-treated orchardgrass silage, total NDF intake was increased by 19% to a level close to that of control silage. Similar trends were found in alfalfa, but the differences were not significant. Across species, lambs fed cellulase-treated silage had 35% lower digestible NDF intake than lambs fed control silage. Similar to total NDF intake, digestible NDF intake was increased to a level similar to that of control silage when formic acid was added to cellulase-treated silage. This increased NDF intake may be related to the increased DMI of silage treated with cellulase plus formic acid. Average calculated daily intakes of undigested NDF were 3.7 and 9.3 g/kg BW in lambs fed orchardgrass and alfalfa silages, respectively. Lambs fed orchardgrass silage treated with cellulase and inoculant consumed on average 25% less undigested NDF than lambs fed the other silages, whereas there were no differences in intakes among the treatments of alfalfa silage (data not shown). In agreement with our results, Jacobs et al. (1992) observed a reduced NDF intake by steers fed grass silage treated with a cellulase/hemicellulase/glucose oxidase mixture, but they found no effect of formic acid on NDF intake. Data from an earlier study (Jacobs et al., 1991) suggested, however, improved NDF intake by formic acid-treated silage fed to sheep.

Lambs fed cellulase-treated silage had 23 and 41% lower total and digestible cellulose intakes than lambs fed control silage, when averaged across species. However, addition of formic acid to cellulase-treated silage

Table 3. Daily ad libitum intakes of total and digestible DM, NDF, cellulose, and hemicellulose in orchardgrass and alfalfa silages[a]

Intake and species	Treatment				Treatment	
	Control	Cellulase	Cellulase plus inoculant	Cellulase plus formic acid	P	SEM
	g/kg of BW					
Total DM						
Orchardgrass	20.1	20.6	21.4	23.7	NS[b]	1.06
Alfalfa	37.4	40.3	40.9	41.5	NS	1.15
$\bar{x}$	28.8[y]	30.5[xy]	31.2[x]	32.6[x]	.03	.78
Digestible DM						
Orchardgrass	13.0	13.4	14.4	15.4	NS	.90
Alfalfa	23.1	24.8	25.4	26.0	.06	.61
$\bar{x}$	18.0[y]	19.1[xy]	19.9[x]	20.7[x]	.03	.54
Total NDF						
Orchardgrass	11.7[x]	8.8[y]	8.3[y]	10.5[x]	.003	.40
Alfalfa	15.1	13.8	15.1	15.4	NS	.74
$\bar{x}$	13.4	11.3	11.7	12.9	.01	.42
Digestible NDF						
Orchardgrass	7.9	4.9	5.3	6.1	.004	.35
Alfalfa	6.2	4.3	5.5	5.9	NS	.66
$\bar{x}$	7.1[x]	4.6[z]	5.4[yz]	6.0[xy]	.005	.38
Total cellulose						
Orchardgrass	6.7	4.4	4.2	5.6	.03	.46
Alfalfa	9.7	8.2	8.9	8.9	.02	.22
$\bar{x}$	8.2[x]	6.3[z]	6.5[z]	7.3[y]	.001	.26
Digestible cellulose						
Orchardgrass	4.9	2.7	2.9	3.5	.03	.39
Alfalfa	4.8	3.0	4.1	4.0	.03	.30
$\bar{x}$	4.9[x]	2.9[z]	3.5[yz]	3.8[y]	.001	.25
Total hemicellulose						
Orchardgrass	4.2	3.3	3.1	3.9	NS	.33
Alfalfa	2.6	2.3	2.6	3.1	NS	.24
$\bar{x}$	3.4[x]	2.8[y]	2.9[y]	3.5[x]	.07	.21
Digestible hemicellulose						
Orchardgrass	2.8	1.8	2.0	2.3	NS	.39
Alfalfa	1.0	.5	.7	1.4	NS	.31
$\bar{x}$	1.9	1.1	1.3	1.8	NS	.25

[a]Interaction of species (= square) and treatment was detected for total NDF intake only ($P = .087$).
[b]NS = not significant ($P > .10$).
[x,y,z]Means within a row lacking a common superscript letter differ ($P < .05$ when P for the F-test of treatment is $< .05$; $P < .10$ when P for the F-test of treatment is $.05 < P < .10$) according to LSD test.

increased total and digestible cellulose intakes by 16 and 31%, respectively (Table 3). The cellulase treatment also decreased the intake of total hemicellulose by 18%. Addition of formic acid to cellulase-treated silage increased total hemicellulose intake to a level similar to that of the control silage.

Dry Matter and NDF Digestibility

Ad libitum intake of orchardgrass was only 19% greater than restricted intake (18 g of DM/kg BW·d), whereas alfalfa silage had twice as great ad libitum as restricted intake (Table 3). Despite this large difference in DM intakes of alfalfa silage, DM and NDF digestibilities at the two intake levels were usually similar (Table 4).

Orchardgrass silage had similar in vitro and in vivo DM digestibilities, but alfalfa silage had, on average,

10% greater ($P < .05$) in vitro than in vivo DM digestibilities within treatments (Tables 1 and 4). The lower in vivo than in vitro DM digestibilities in alfalfa but not in orchardgrass can be related to a lower fermentable NDF concentration and, therefore, a shorter rumen retention time of the fiber particles in alfalfa than in orchardgrass (Allen, 1996; Nadeau et al., 1996). Furthermore, alfalfa had greater in vitro but numerically lower in vivo DM digestibility at ad libitum intake than orchardgrass (Tables 1 and 4). Thus, when comparing DM digestibilities of legumes with those of grasses, it is important to consider the digestibility technique that was used.

Averaged across species, cellulase-treated silage had 18% lower NDF digestibility at ad libitum and restricted DMI than the control (Table 4). However, addition of inoculant or formic acid to cellulase-treated silage tended to increase the NDF digestibility. The de-

Table 4. Digestibility coefficients of DM, NDF, cellulose, and hemicellulose at ad libitum and restricted[a] intake of orchardgrass and alfalfa silages[b]

Digestibility and species	Control	Cellulase	Cellulase plus inoculant	Cellulase plus formic acid	P	SEM
			Treatment		Treatment	
			g/kg consumed			
DM, ad libitum						
Orchardgrass	648	659	672	655	NS[c]	13
Alfalfa	619	615	622	626	NS	5
x̄	633	637	647	641	NS	7
DM, restricted						
Orchardgrass	634	642	652	647	NS	14
Alfalfa	615	618	621	639	.07	5
x̄	624	630	637	643	NS	7
NDF, ad libitum						
Orchardgrass	685	580	641	586	.08	26
Alfalfa	409	314	365	388	NS	31
x̄	547[x]	447[y]	503[xy]	487[xy]	.03	20
NDF, restricted						
Orchardgrass	661	572	601	582	NS	28
Alfalfa	450	336	338	408	.007	16
x̄	556[x]	454[y]	470[y]	495[y]	.005	16
Cellulose, ad libitum						
Orchardgrass	742	636	700	639	NS	29
Alfalfa	501	364	464	453	NS	32
x̄	622[x]	500[z]	582[xy]	546[yz]	.01	22
Cellulose, restricted						
Orchardgrass	731	673	667	593	NS	40
Alfalfa	526	476	408	502	.03	20
x̄	629[x]	574[xy]	538[y]	548[y]	.06	22
Hemicellulose, ad libitum						
Orchardgrass	667	519	627	577	NS	61
Alfalfa	369	179	230	441	NS	122
x̄	518	349	429	509	NS	68
Hemicellulose, restricted						
Orchardgrass	615	511	610	609	NS	30
Alfalfa	454	303	367	347	NS	120
x̄	534	407	489	478	NS	62

[a]Intake restricted to 18 g of DM/(kg BW·d).
[b]Interactions of species (= square) and treatment were not detected.
[c]NS = not significant ($P > .10$).
[x,y,z]Means within a row lacking a common superscript letter differ ($P < .05$ when P for the F-test of treatment is $< .05$; $P < .10$ when P for the F-test of treatment is $.05 < P < .10$) according to LSD test.

creased NDF digestibility of cellulase-treated silages is probably related to degradation of the easily digestible portion of NDF by cellulase during ensiling, leaving the less-digestible portion of NDF for microbial degradation in the rumen and in the lower tract (Nadeau et al., 1996). In agreement with our results, Jaakkola (1990) reported a decreased NDF digestibility of cellulase/glucose oxidase-treated silage, containing a mixture of timothy (*Phleum pratense*) and red clover (*Trifolium pratense*), compared with control silage when fed to sheep.

Cellulase treatment decreased cellulose digestibility at ad libitum DMI by 20% compared with control silage, when averaged across plant species (Table 4). Addition of inoculant increased cellulose digestibility of cellulase-treated silage by 16% to a level similar to that of control silage. Although nearly twice as much cellulose as hemicellulose degradation occurred during ensiling,

cellulose was more digestible than hemicellulose in both plant species (Tables 1 and 4). Similar results were obtained in our earlier rumen in situ digestibility study (Nadeau et al., 1996), using the same plant species. However, because a larger portion of hemicellulose might be digested in the lower tract, whereas more of the cellulose might be digested in the rumen (Van Soest, 1994), results from our rumen in situ experiment must be interpreted carefully.

Results from our experiment indicate the importance of adding cellulase to a sugar-limiting crop, such as alfalfa, at ensiling to ensure silage of good quality. To potentially improve the performance of ruminants, cellulase should be combined with formic acid or inoculant, both of which increased silage DMI in our study. Results from this study are limited to one maturity and, as we reported previously (Nadeau et al., 2000), there are

interactions between these silage treatments and maturity on silage quality.

Implications

Cell-wall degrading enzymes, such as cellulase, are most useful in crops such as alfalfa that have a limited supply of sugars for a succesful fermentation to occur. The improved fermentation and increased sugar concentration in cellulase-treated silage has the potential to increase silage DMI by sheep. According to our study, addition of formic acid to cellulase-treated silage is necessary to improve silage DMI, and, consequently, the performance of ruminants. Adding a bacterial inoculant to cellulase-treated silage can also improve silage DMI, but to a lesser extent. There are, however, great differences in the bacterial contents of silage inoculants that may affect silage quality and, thereby, silage DMI by ruminants.

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