Effects of Combined Treatment of Lactic Acid Bacteria and Cell Wall Degrading Enzymes on Fermentation and Composition of Rhodesgrass (*Chloris gayana* Kunth.) Silage

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ABSTRACT : This experiment was conducted to study the effects of lactic acid bacteria (LAB) inoculation either alone or in combination with cell wall degrading enzymes on the fermentation characteristics and chemical compositions of Rhodesgrass silage. Over to 1 kg of fresh Rhodesgrass sample a treatment of inoculant LAB with or without addition of an enzyme of Acremoniumcellulase (A) or Meicelase (M) or a mixture of both enzymes (AM) was applied. The treatments were control untreated, LAB-treated (application rate 1.0×10^5 cfu/g fresh sample), LAB+A 0.005%, LAB+A 0.01%, LAB+A 0.02%, LAB+M 0.005%, LAB+M 0.01%, LAB+M 0.02 %, LAB+AM 0.005%, LAB+AM 0.01%, and LAB+AM 0.02%. The sample was ensiled into 2-L vinyl bottle silo, with 9 silages of each treatment were made. Three silages of each treatment were incubated at 20, 30 and 40℃ for 2-months of storage period. All silages were well preserved with their fermentation quality has low pH values (3.91-4.26) and high lactic acid concentrations (4.11-9.89 %DM). No differences were found in fermentation quality and chemical composition of the control untreated silage as compared to the LAB-treated silage. Combined treatment of LAB+cellulases improved the fermentation quality of silages measured in terms of lower ($p < 0.01$) pH values and higher ($p < 0.05$) lactic concentrations than those of LAB-treated silages. Increasing amount of cellulase addition resulted in decrease ($p < 0.05$) of pH value and increase ($p < 0.05$) of lactic acid concentration. LAB+cellulase treatments (all cellulase types) reduced ($p < 0.01$) NDF, ADF and *in vitro* dry matter digestibility of silages compared with the control untreated silages. The fermentation quality and the rate of cell wall reduction were higher ($p < 0.01$) in the silages treated with LAB+cellulase A than in the silages treated with either LAB+cellulase M or LAB+cellulase AM. Incubation temperature of 40℃ was likely to be more appropriate environment for stimulating the fermentation of Rhodesgrass silages than those of 20 and 30℃.
(**Key Words**: Rhodesgrass Silage, Lactic Acid Bacteria, Cellulases, Fermentation Quality, Digestibility)

INTRODUCTION

The use of cell wall degrading enzymes (cellulolytic and hemicellulolytic) as silage additives, ideally would have a dual action in the silo. Firstly, the degradation of plant fibre by enzyme will provide the extra fermentable sugars or water soluble carbohydrate (WSC) as substrate for rapidly growing of lactic acid bacteria (LAB) to increase fermentation quality and enhance preservation. Secondly, the breakdown of fibre component as a result of enzyme action may improve the silage digestibility (Kung, Jr., et al., 1990; McDonald et al., 1991; Chamberlain and Robertson, 1992; Hoffman et al., 1995). According to Kung, Jr., et al. (1990), to achieve the first goal, the rate and extent of hydrolysis of the cell wall must coincide with early growth of lactic acid bacteria, and to improve the digestibility in the animal, alteration in the cellulose or hemicellulose-lignin relationship of silage must occur.

It was well documented that the added enzymes to silages were capable of degrading the component of structural carbohydrates during ensiling, and provided more WSC as substrate for the silage fermentation (Jaakkola, 1990; McDonald et al., 1991; Jacobs and McAllan, 1992; Jacobs et al., 1992; Stokes, 1992; Ridla and Uchida, 1993; Ridla and Uchida, 1997). The reduction of cell wall components due to cellulase addition, however, was not followed by the improving of silage digestibility, since many researchers have found that cellulase addition did not affect the silage digestibility (van Vuuren et al., 1989; Jaakkola et al., 1991; Jacobs et al., 1991; Ridla and Uchida, 1993; Ridla and Uchida, 1997). The lowered silage digestibility which

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might be due to cellulase addition was also reported by Jaakkola (1990), Jaakkola and Huhtanen (1990), and Jacobs and McAllan (1991).

Weinberg et al. (1993) reported that the efficiency of biological additives (inoculants and enzymes) for silages depended very much on the chemical and microbiological composition of the fresh crops, and on environmental condition such as ambient temperature and air that penetrated into the silage during storage. In addition, Kung, Jr., et al. (1990) reported that the differences in application rate, enzyme activity, pH, temperature, hydrolysis rate, and ensiling time could have major effects on the usefulness of cellulase enzymes added to silage.

This experiment was conducted to study the effects of combined treatments of lactic acid bacteria inoculation with different types and levels of cellulase addition, incubated at different temperatures, on the fermentation characteristics and chemical compositions of Rhodesgrass (*Chloris gayana* Kunt.) silage.

MATERIALS AND METHODS

Silage additives

All additives used in this experiment were provided by Yukijirushi Syubyo Co. Ltd., Hokkaido, Japan. The cellulase enzymes used were Acremoniumcellulase (cellulase A, derived from *Acremonium cellulolyticus*), Meicelase (cellulase M, derived from *Tricoderma viride*), and the mixture of cellulase A and M at 1:2 ratio (cellulase AM). The LAB inoculant (Snow Lact-L) was guaranteed by supplier to contain a minimum of 2.5×10^{10} cfu.g^{-1} powder of *Lactobacillus casei*. Each cellulase enzyme was applied at rate of 0.005, 0.01, and 0.02% fresh sample. Inoculant LAB was added at a theoretical rate of 1.0×10^5 cfu.g^{-1} fresh forage sample. On the day of the experiment a certain amount of each cellulase preparation or inoculant LAB was diluted with distilled water designed to achieve the required concentration, and keep for silage production.

Silage production

The silages produced in this experiment were made from a primary growth of Rhodesgrass harvested at the heading stage with a hand cutter on August 9, 1995. The grass was firstly chopped into approximately 1.3 cm lengths and then lacerated with a chopper-cracker (Taninaka Co. Ltd.). The chemical composition and *in vitro* dry matter digestibility of the grass is shown in table 1. Over a 1 kg grass sample treatment of 1 ml LAB inoculant solution with or without 1 ml cellulase solution was sprayed with 2.5-ml syringe followed by thorough mixing. The sample then ensiled into 2-L vinyl bottle silo. The silage additives were used as following design:

Treatment	Silage additive
1	Non additive (control untreated)
2	LAB (application rate 1.0×10^5 cfu.g^{-1} fresh sample)
3	LAB + A 0.005%
4	LAB + A 0.01%
5	LAB + A 0.02%
6	LAB + M 0.005%
7	LAB + M 0.01%
8	LAB + M 0.02%
9	LAB + AM 0.005%
10	LAB + AM 0.01%
11	LAB + AM 0.02%

Nine silages were made for each treatment. Three silages of each treatment were incubated at 20, 30 and 40℃ for 2 -months of storage period. After the incubation period the silages were opened and the upper part 1/5 of silages were discarded before sampling. The samples were collected and kept frozen at −32℃ until used for further analysis.

Table 1. The chemical composition and *in vitro* dry matter digestibility of Rhodesgrass material prior to ensiling

Composition	Contents
Dry matter (%)	21.76
Organic matter (% DM)	98.72
Crude protein (% DM)	10.38
Crude fiber (% DM)	28.15
NDF (% DM)	66.42
ADF (% DM)	38.18
ADL (% DM)	7.14
Hemicellulose (% DM)	28.24
Cellulose (% DM)	31.04
WSC (% DM)	5.01
IVDMD (%)	69.10

Note: NDF = Neutral detergent fibre, ADF = Acid detergent fibre, ADL = Acid detergent lignin, WSC = Water soluble carbohydrate, IVDMD = *In vitro* dry matter digestibility.
Hemicellulose = NDF − ADF, Cellulose = ADF − ADL.

Chemical analysis

Procedures for sampling and chemical analysis of all samples were the same as those described by Ridla and Uchida (1993). In summary, dry matter content of grass and silages was determined by a vacuum freeze-drying method (Uchida, 1986). The dried samples were ground and then crude protein was determined by the Kjeldahl

method. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were measured by the method of Goering and Van Soest (1970). WSC was evaluated by using the method of Deriaz (1961), and *in vitro* dry matter digestibility (IVDMD) was determined by the method of Tilley and Terry (1963).

Water soluble extracts were prepared by macerating of 40 g fresh silage sample in 400 ml distilled water. The pH of the extracts were measured by electric pH-meter (Horiba F-12). Organic acids and ethanol were determined by gas chromatography (GC-14A, Shimadzu) as described by Uchida and Hayashi (1985). Lactic acid was analyzed by the method of Barker and Summerson (1941) and volatile basic nitrogen (VBN) was measured by steam distillation method.

Statistical analysis

Analysis of variance was applied to all data by using a general linear model procedure for factorial experiment to analyze the effects of additive treatments on silage fermentation characteristics and chemical compositions (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

All silages were well preserved as indicated by their low pH values (3.91-4.26), high lactic acid concentrations (4.11-9.79% DM), low of butyric acid (0.01-0.77% DM), acetic acid (0.75-2.24) and propionic acid (0.01-0.17% DM) concentrations, regardless of treatment and incubation temperature (tables 2, 3, 4 and 5). The good fermentation in this silages was also shown by low VBN concentrations (1.92-3.05% of total nitrogen). This ammonia-N content was lower than the concentration recommended by Henderson (1993) that a well preserved silage should have less than 8% TN. This findings are not in agreement with the data of Kim and Uchida (1990) who reported that the acetic acid was the main end-product of Rhodesgrass silages that caused on a high pH value. The ideal level of dry matter content (above 20% DM) and sufficient substrate of WSC (5,01% DM) in grass used in this study might lead to a good fermentation of resulting silages.

LAB inoculation

There no mark differences were found in all fermentation characteristics and chemical compositions between the control untreated and LAB-treated silages in all incubation temperatures (table 2). The absence of effect of LAB inoculation on fermentation quality in this silages might be due to the WSC content in original grass (5.01% DM) was not enough to allow the LAB to

Table 2. The chemical composition and *in vitro* dry matter digestibility of control untreated (Ctl) and LAB-treated (LAB) silages at incubation temperature 20, 30 and 40℃

Incubation temperature	20℃		30℃		40℃		t test results		
Treatments	Ctl	LAB	Ctl	LAB	Ctl	LAB	20℃	30℃	40℃
pH	4.22	4.26	4.26	4.18	4.16	4.20	NS	NS	NS
Dry matter (%)	22.23	22.35	21.65	21.88	22.06	22.83	NS	NS	NS
Crude protein (% DM)	9.89	9.73	10.79	10.79	9.49	9.59	NS	NS	NS
NDF (% DM)	64.22	64.21	65.34	65.37	64.56	64.13	NS	NS	NS
ADF (% DM)	39.15	38.81	40.51	40.20	39.44	39.26	NS	NS	NS
ADL (g/kg DM)	3.59	3.64	3.57	3.98	3.92	3.76	NS	NS	NS
Hemicellulose (% DM)	25.08	25.40	24.83	25.17	25.12	24.87	NS	NS	NS
Cellulose (% DM)	35.56	35.18	36.94	36.23	35.52	35.50	NS	NS	NS
WSC (% DM)	0.95	0.93	1.06	1.03	1.06	0.92	NS	NS	NS
Ethanol (% DM)	0.40	0.42	0.24	0.35	0.57	0.51	NS	NS	NS
Lactic acid (% DM)	4.63	4.31	4.39	4.74	8.89	8.22	NS	NS	NS
Acetic acid (% DM)	1.66	1.66	1.52	1.32	0.87	1.00	NS	NS	NS
Propionic acid (% DM)	0.07	0.06	0.01	0.01	0.17	0.09	NS	NS	NS
Butyric acid (% DM)	0.17	0.15	0.02	0.02	0.28	0.23	NS	NS	NS
VBN (% TN)	2.09	2.48	2.59	2.20	2.66	1.93	NS	NS	NS
IVDMD (%)	67.64	68.01	67.27	68.93	68.32	67.45	NS	NS	NS

Note: NDF=Neutral detergent fibre, ADF=Acid detergent fibre, ADL=Acid detergent lignin, WSC=Water soluble carbohydrate, VBN=Volatile basic nitrogen, IVDMD=*In vitro* dry matter digestibility.
Hemicellulose=NDF−ADF, Cellulose=ADF−ADL.
Levels of significance = ** $p < 0.01$, * $p < 0.05$, NS $p > 0.05$.

Table 3. The chemical composition and *in vitro* dry matter digestibility of combined treatment LAB+Cellulase silages at incubation temperature 20℃

Cellulase type	LAB + A				LAB + M				LAB + AM				SEM[+)]
Cellulase level (%)	0	0.005	0.01	0.02	0	0.005	0.01	0.02	0	0.005	0.01	0.02	
pH	4.26	4.13	4.04	4.07	4.26	4.19	4.27	4.02	4.26	4.09	4.38	3.99	0.038
Dry matter (%)	22.35	22.51	22.31	22.23	22.35	22.28	22.61	22.56	22.35	22.35	22.21	22.29	0.123
Crude protein (% DM)	9.73	9.76	9.89	10.15	9.73	9.90	9.87	10.01	9.73	10.12	10.25	10.08	0.110
NDF (% DM)	64.21	60.80	61.20	59.93	64.21	63.08	62.54	61.83	64.21	62.09	61.88	61.78	0.198
ADF (% DM)	38.81	37.75	37.45	36.45	38.81	38.62	37.87	37.47	38.81	38.23	37.42	37.08	0.135
ADL (% DM)	3.64	3.81	3.93	3.82	3.64	3.81	3.85	3.96	3.64	3.35	3.96	4.13	0.084
Hemicellulose (% DM)	25.40	23.05	23.74	23.49	25.40	24.66	24.67	24.36	25.40	23.86	24.46	24.70	0.163
Cellulose (% DM)	35.18	33.94	33.52	32.63	35.18	34.61	34.02	33.51	35.18	34.87	33.46	32.96	0.236
WSC (% DM)	0.93	1.32	1.24	1.68	0.93	1.33	1.04	1.34	0.93	1.41	1.09	1.21	0.067
Ethanol (% DM)	0.42	0.30	0.33	0.38	0.42	0.34	0.39	0.42	0.42	0.32	0.63	0.66	0.053
Lactic acid (% DM)	4.31	5.11	5.82	6.98	4.31	4.65	5.34	5.34	4.31	6.78	3.93	6.59	0.460
Acetic acid (% DM)	1.66	0.94	0.75	1.15	1.66	1.32	1.29	1.32	1.66	1.29	1.72	2.24	0.095
Propionic acid (% DM)	0.06	0.04	0.02	0.01	0.06	0.04	0.04	0.04	0.06	0.01	0.05	0.06	0.006
Butyric acid (% DM)	0.15	0.10	0.06	0.08	0.15	0.12	0.10	0.11	0.15	0.12	0.12	0.13	0.026
VBN (% TN)	2.48	1.90	2.31	2.07	2.48	2.06	2.81	2.21	2.48	2.34	2.65	2.14	0.128
IVDMD (%)	68.01	67.64	66.74	67.44	68.01	66.58	64.01	64.44	68.01	68.11	65.14	65.61	0.669

Note: NDF=Neutral detergent fibre, ADF=Acid detergent fibre, ADL=Acid detergent lignin, WSC=Water soluble carbohydrate, VBN=Volatile basic nitrogen, TN=Total nitrogen, IVDMD=*In vitro* dry matter digestibility.
Hemicellulose=NDF−ADF, Cellulose=ADF−ADL.
[+)] Standard error of means.

Table 4. The chemical composition and *in vitro* dry matter digestibility of combined treatment LAB+Cellulase silages at incubation temperature 30℃

Cellulase type	LAB + A				LAB + M				LAB + AM				SEM[+)]
Cellulase level (%)	0	0.005	0.01	0.02	0	0.005	0.01	0.02	0	0.005	0.01	0.02	
pH	4.18	3.96	4.09	3.91	4.18	4.09	4.18	4.11	4.18	3.98	4.07	4.15	0.031
Dry matter (%)	21.88	21.48	21.77	21.85	21.88	21.18	21.81	21.34	21.88	21.82	21.80	22.32	0.141
Crude protein (% DM)	10.79	10.16	10.84	10.70	10.79	10.68	10.53	10.61	10.79	10.64	10.79	10.87	0.127
NDF (% DM)	65.37	62.66	63.03	60.89	65.37	65.11	64.84	63.32	65.37	62.96	63.40	61.80	0.222
ADF (% DM)	40.20	37.60	38.24	36.53	40.20	39.64	38.88	37.35	40.20	38.94	38.57	33.64	0.125
ADL (% DM)	3.98	3.07	3.71	3.32	3.98	4.15	3.51	3.87	3.98	4.09	4.14	4.24	0.088
Hemicellulose (% DM)	25.17	25.06	24.79	24.37	25.17	25.47	25.96	25.98	25.17	24.02	24.94	28.16	0.156
Cellulose (% DM)	36.23	34.53	34.54	33.21	36.23	35.49	35.37	33.48	36.23	34.84	34.43	29.40	0.174
WSC (% DM)	1.03	1.14	0.94	1.26	1.03	1.10	1.01	1.03	1.03	1.25	0.99	1.11	0.024
Ethanol (% DM)	0.35	0.54	0.36	0.43	0.35	0.30	0.27	0.50	0.35	0.35	0.45	0.54	0.108
Lactic acid (% DM)	4.74	4.66	5.95	7.05	4.74	4.85	4.11	6.09	4.74	4.94	4.12	5.28	0.354
Acetic acid (% DM)	1.32	1.59	1.23	1.53	1.32	1.48	1.40	1.39	1.32	1.29	1.35	1.75	0.109
Propionic acid (% DM)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.008
Butyric acid (% DM)	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.036
VBN (% TN)	2.20	2.16	2.80	2.60	2.20	2.11	2.21	2.84	2.20	1.90	1.85	2.33	0.134
IVDMD (%)	68.93	71.13	68.40	68.00	68.93	69.93	68.53	65.90	68.93	69.10	66.43	65.67	0.818

Abbreviated: NDF=Neutral detergent fibre, ADF=Acid detergent fibre, ADL=Acid detergent lignin, WSC=Water soluble carbohydrate, VBN=Volatile basic nitrogen, TN=Total nitrogen, IVDMD=*In vitro* dry matter digestibility.
Hemicellulose=NDF−ADF, Cellulose=ADF−ADL.
[+)] Standard error of means.

Table 5. The chemical composition and *in vitro* dry matter digestibility of combined treatment LAB+Cellulase silages at incubation temperature 40℃

Cellulase type	LAB + A				LAB + M				LAB + AM				SEM[*)]
Cellulase level (%)	0	0.005	0.01	0.02	0	0.005	0.01	0.02	0	0.005	0.01	0.02	
pH	4.20	3.96	4.24	3.96	4.20	4.08	4.29	4.16	4.20	4.06	4.13	3.94	0.058
Dry matter (%)	22.83	22.68	21.69	22.07	22.83	22.34	22.35	21.74	22.83	22.00	22.00	22.19	0.120
Crude protein (% DM)	9.59	9.45	10.04	9.97	9.59	9.66	9.95	9.92	9.59	9.88	10.03	9.87	0.092
NDF (% DM)	64.13	60.37	62.48	58.12	64.13	63.48	63.69	64.42	64.13	62.30	61.60	59.68	0.378
ADF (% DM)	39.26	37.14	38.63	35.83	39.26	38.65	39.19	38.53	39.26	37.88	37.88	36.37	0.268
ADL (g/kg DM)	3.76	3.99	4.88	4.40	3.76	4.01	4.35	4.50	3.76	38.88	4.27	3.99	0.105
Hemicellulose (% DM)	24.87	23.23	23.85	22.29	24.87	24.83	24.50	25.90	24.87	24.42	23.72	23.31	0.285
Cellulose (% DM)	35.50	33.15	33.75	31.44	35.50	34.65	34.84	34.03	35.50	34.00	33.61	32.38	0.242
WSC (% DM)	0.92	1.54	1.17	1.74	0.92	1.22	0.91	1.16	0.92	1.38	1.23	1.42	0.047
Ethanol (% DM)	0.51	0.64	1.26	0.96	0.51	0.73	0.84	1.16	0.51	0.59	0.84	0.84	0.068
Lactic acid (% DM)	8.22	7.72	6.69	6.20	8.22	9.79	5.32	8.41	8.22	4.13	7.14	5.26	0.837
Acetic acid (% DM)	1.00	1.03	0.95	0.96	1.00	0.97	1.14	1.09	1.00	0.99	1.04	1.00	0.099
Propionic acid (% DM)	0.09	0.04	0.05	0.02	0.09	0.08	0.09	0.05	0.09	0.02	0.01	0.02	0.006
Butyric acid (% DM)	0.20	0.24	0.77	0.28	0.20	0.19	0.32	0.03	0.20	0.21	0.30	0.29	0.019
VBN (% TN)	1.93	2.55	3.35	2.42	1.93	2.50	3.05	3.02	1.93	1.78	2.06	2.56	0.278
IVDMD (%)	67.45	67.65	64.75	64.18	67.45	66.68	63.78	64.52	67.45	65.95	65.03	65.35	0.588

Note: NDF=Neutral detergent fibre, ADF=Acid detergent fibre, ADL=Acid detergent lignin, WSC=Water soluble carbohydrate, VBN=Volatile basic nitrogen, TN=Total nitrogen, IVDMD=*In vitro* dry matter digestibility.
Hemicellulose=NDF−ADF, Cellulose=ADF−ADL.
[*)] Standard error of means.

produce more lactic acid to further reduce the final pH value. This result is in line with the data of Tamada et al. (1996) in napier grass silage in term of the addition of LAB inoculant was not effective on lowering pH value and increasing lactic acid concentration was due to the low WSC content in original grass. The numbers of epiphytic LAB in original grass might have also affected the fermentation quality of this silages. Although the investigation of microflora was not carried out in this trial, it is assumed that the initial numbers of LAB in grass material prior to ensiling was high enough to sustain a satisfactory fermentation in the silo without the need of LAB inoculation. According to Henderson (1993) the LAB in the standing crop are present in dormant state, and the harvesting procedures which lacerate the crop and release cell contents from the ruptured plant tissue resulting in the recovery of LAB. McDonald et al. (1991) reported that increasing use of inoculant additives in the field and improvements in harvesting machinery might have encouraged the higher number of LAB. In addition, Henderson (1993) reported that the numbers of the epiphytic LAB on grass can increase during the summer months and may be as high as 10^7 colony forming unit (cfu).g^{-1} grass.

Cellulase addition

The combined treatments of LAB+cellulases (all cellulase types) produced the silages which contain lower pH values ($p < 0.01$) and higher ($p < 0.05$) lactic acid concentrations than those of the LAB-treated silages, in all incubation temperatures. The increasing amount of cellulase addition resulted in a significant decrease ($p < 0.05$) in pH value and increase in lactic acid concentration in all enzymes types and incubation temperatures (tables 3, 4, 5 and 6). These results are similar with our previous findings (Ridla and Uchida, 1993; Ridla and Uchida, 1997) and in agreement with the results of Henderson and McDonald (1977), van Vuuren et al. (1989), Jacobs et al. (1991), and Selmer-Olsen et al. (1993) that cellulase addition improved the fermentation quality of silages by decreasing pH value and increasing lactic acid concentration. It might be due to more substrate of fermentable carbohydrates (WSC) were provided from the hydrolysis of cell wall components, which stimulate a good fermentation by lactic acid bacteria.

The combine treatments of LAB+cellulases reduced ($p < 0.01$) the cell wall components (NDF and ADF) of silages, in all cellulase types and incubation temperatures, continuously with the increase amount of cellulase

Table 6. Statistical significant of effect of the treatments

	Cellulase Type (CT)	Cellulase Level (CL)	Incubation Temp. (IT)	CT vs. CL	CT vs. IT	CL vs. IT	CT vs. CL vs. IT
pH	*	**	NS	NS	NS	*	NS
Dry matter (%)	NS	NS	NS	NS	NS	NS	NS
Crude protein (% DM)	NS	NS	NS	NS	NS	NS	NS
NDF (% DM)	**	**	NS	**	NS	NS	NS
ADF (% DM)	**	**	NS	**	**	NS	**
ADL (g/kg DM)	NS	**	NS	**	**	**	**
Hemicellulose (% DM)	**	**	NS	**	**	**	**
Cellulose (% DM)	**	**	NS	**	**	**	**
WSC (% DM)	**	**	NS	**	**	*	NS
Ethanol (% DM)	NS	**	**	NS	*	*	*
Lactic acid (% DM)	NS	*	**	NS	*	*	*
Acetic acid (% DM)	**	*	**	**	**	*	NS
Propionic acid (% DM)	**	**	**	**	**	**	**
Butyric acid (% DM)	**	**	**	NS	**	**	**
VBN (% TN)	NS	**	NS	NS	NS	**	NS
IVDMD (%)	NS	**	NS	NS	NS	NS	NS

Note: NDF = Neutral detergent fibre, ADF = Acid detergent fibre, ADL = Acid detergent lignin, WSC = Water soluble carbohydrate, VBN = Volatile basic nitrogen, TN = Total nitrogen, IVDMD = *In vitro* dry matter digestibility.
Hemicellulose = NDF − ADF, Cellulose = ADF − ADL.
Levels of significance = ** $p < 0.01$, * $p < 0.05$, NS $p > 0.05$.

addition. Silages with the highest level of 0.02% added cellulase contain the lowest ($p < 0.05$) of NDF and ADF contents. Compare to the LAB-treated silages, the NDF reduction of these silages were 3.62, 1.27 and 2.75 (unit %) for silages treated with LAB + cellulase A, LAB + cellulase M and LAB + cellulase AM, respectively, regardless of the cellulase level and incubation temperature. Similarly, the ADF contents reduction were 2.42, 1.29 and 2.37 (unit %).

Unlike in LAB-treated silages, the reduction of cell wall components in combined treatments of LAB + cellulases silages was followed by the increasing residual WSC ($p < 0.01$), in all cellulase types and incubation temperatures. It might indicate that enzyme action upon cell wall components reduction was able to provide more WSC, which ultimately could give an addition substrate for sustaining the fermentation by LAB. These results are in agreement with the findings of Jaakkola (1990), McDonald et al. (1991), Jacobs and McAllan (1992), Jacobs et al. (1992), Stokes (1992), Ridla and Uchida (1993), and Ridla and Uchida (1997), who reported that enzymes addition was capable to breakdown the component of structural carbohydrates during ensiling and provide more WSC as substrate for the silage fermentation.

The reduction of cell wall components due to cellulase addition did not enhance the digestibility of silages. It was indicated by the *in vitro* dry matter digestibility (IVDMD) of silages was lower ($p < 0.01$) in combine treatments of LAB + cellulases than that in LAB-treated silages, in all cellulase types and incubation temperatures. The decreased silage digestibility which might be resulted from cellulase addition was reported by Jaakkola (1990), Jaakkola and Huhtanen (1990), and Jacobs and McAllan (1991). It was likely that the cellulase enzymes reduced only the same cell wall structures in the silo as would be degraded in the rumen (Jaakkola and Huhtanen, 1990), or the cellulase enzymes were not able to degrade the lignin-polysaccharide complexes or plant cell walls, which are indigestible by the rumen microbes (Jaakkola, 1990). On the other hand, Sheperd et al. (1995) and Sheperd and Kung, Jr. (1996) reported that the lower *in vitro* digestion in enzymes-treated silages than in untreated silages might be due to the fact the added enzymes had already hydrolyzed the most readily digestible portion of forage in the silo.

Cellulase type

Silages treated with LAB + cellulase A resulted in higher ($p < 0.05$) fermentation quality and greater losses

of cell wall components than with LAB+cellulase M, and silages treated with LAB+cellulase AM had an intermediate effect. The results showed that the silages treated with LAB+cellulase A and LAB+cellulase AM had lower pH values ($p < 0.05$) and higher ($p < 0.05$) lactic acid concentrations than silages treated with LAB+cellulase M. The silages treated with LAB+cellulase A and LAB+cellulase AM had also lower ($p < 0.01$) cell wall components (NDF, ADF) than silages treated with LAB+cellulase (tables 3, 4, 5 and 6). These results are consistent with the data of Tomoda et al. (1996) and Zhang et al. (1997a,b) who reported that the silages treated with a cellulase preparation originating from *Acremonium cellulolyticus* resulted in a lower pH and a higher lactic acid concentration than was obtained with a cellulase preparation originating from *Tricoderma viride*.

Incubation temperature

Organic acids concentrations of silages were different due to incubation temperature and these temperatures might be independent of silage additive. Silages incubated at 40℃ resulted in higher ($p < 0.01$) lactic acid and ethanol concentrations, and lower acetic acid ($p < 0.01$) and propionic acid ($p < 0.05$) concentrations than those of silages incubated at either 20℃ or 30℃ (tables 3, 4, 5 and 6). It was likely that the most appropriate incubation temperature for the best fermentation in Rhodesgrass silages was at 40℃ rather than at 20 or 30℃.

In conclusion, LAB inoculation did not affect the silage quality, but the combined treatments of LAB+cellulases to Rhodesgrass silage improved their fermentation quality by reducing pH value, increasing lactic acid and decreasing acetic acid concentrations. The increased solubility of cell wall components was caused by cellulase addition and continue to increase with the increasing amount of cellulase addition. Since the cellulase addition did not enhance the silage digestibility, the lowest level of 0.005% seen to be enough for supporting the fermentation by lactic acid bacteria in the silo.

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REFERENCES

Barker, S. B. and W. H. Summerson. 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138:535-554.

Chamberlain, D. G. and S. Robertson. 1992. The effects of the addition of various enzyme mixtures on the fermentation of perennial ryegrass silage and on its nutritional value for milk production in dairy cows. Animal Feed Science and Technology. 37:257-264.

Deriaz, R. E. 1961. Routine analysis of carbohydrates and lignin in herbage. J. Sci. Food Agric. 12:152-160.

Goering, H. K. and P. J. Van Soest. 1970. Forage fiber analysis, U. S. Department of Agriculture, Handbook. No. 379:1-20.

Henderson, A. R. and P. McDonald. 1977. The effect of cellulase preparation on the chemical changes during the ensilage of grass in laboratory silos. J. Sci. Food Agric. 28:486-490.

Henderson, N. 1993. Silage additives. Anim. Feed Sci. Technol. 45:35-56.

Hoffman, P. C., D. A. Welch and N. M. Brehm. 1995. Potential of enzyme mixtures to improve silage quality and lactation performance of dairy cattle. J. Prod. Agric. 8:552-557.

Jaakkola, S. 1990. The effect of cell wall degrading enzymes on the preservation of grass and on the silage intake and digestibility in sheep. J. Agric. Sci. Finl. 62:52-62.

Jaakkola, S. and P. Huhtanen. 1990. Response to cellulase treatment of silage and replacement of barley by unmolassed sugar beet pulp in the diet of growing cattle. Acta Agric. Scand. 40:415-426.

Jaakkola, S., P. Huhtanen and K. Hissa. 1991. The effect of cell wall degrading enzymes or formic acid on fermentation quality and on digestion of grass silage by cattle. Grass and Forage Sci. 46:75-87.

Jacobs, J. L. and A. B. McAllan. 1991. Enzymes as silage additive 1. Silage quality, digestion, digestibility and performance in growing cattle. Grass and Forage Sci. 46:63-73.

Jacobs, J. L., J. E. Cook and A. B. McAllan. 1991. Enzymes as silage additive 2. The effect of grass dry matter content on silage quality and performance in sheep. Grass and Forage Sci. 46:191-199.

Jacobs, J. L. and A. B. McAllan. 1992. Protein supplementation of formic acid- and enzyme-treated silages. 1. Digestibilities, organic matter and fibre digestion. Grass and Forage Sci. 47:103-113.

Jacobs, J. L, M. J. Haines and A. B. McAllan. 1992. The effect of different protein supplements on the utilization of untreated, formic acid-treated or enzyme-treated silages by growing steers. Grass and Forage Sci. 47:121-127.

Kim, K. H. and S. Uchida. 1990. Comparative studies of ensiling characteristics between temperate and tropical species. 1. The effect of various ensiling conditions on the silage quality of Italian ryegrass (*Lolium multiflorum* Lam.) and Rhodesgrass (*Chloris gayana* Kunt.). J. Japan. Grassl. Sci. 36(3):292-299.

Kung, L., Jr., B. R. Carmean and R. S. Tung. 1990. Microbial inoculation or cellulase enzyme treatment of barley and vetch silage harvested at three maturities. J. Dairy Sci. 73:1304-1311.

McDonald, P., A. R. Henderson and S. J. E. Heron. 1991. The Biochemistry of Silage. Second edition. Chalcombe Publication. pp. 184-223.

Ridla, M. and S. Uchida. 1993. The effect of cellulase addition on nutritional and fermentation quality of barley straw silage. AJAS. 6(3):383-388.

Ridla, M. and S. Uchida. 1997. Effects of cellulase and brewers' grains addition on fermentation quality and nutritive value of barley straw silage. AJAS. 10(6):575-580.

Selmer-Olsen, I., A. R. Henderson, S. Robertson and R. McGinn. 1993. Cell wall degrading enzyme for silage. 1. The fermentation of enzyme-treated ryegrass in laboratory silos. Grass and Forage Sci. 48:45-54.

Sheperd, A. C., M. Maslanka, D. Quinn and L. Kung, Jr. 1995. Additives containing bacteria and enzymes for alfalfa silage. J. Dairy Sci. 78:565-572.

Sheperd, A. C. and L. Kung, Jr. 1996. An enzyme additive for corn silage: Effects on silage composition and Animal performance. J. Dairy Sci. 79:1760-1766.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics, with special reference to the biological sciences. McGraw-Hill Book Company, Inc. pp. 194-210.

Stokes, M. R. 1992. Effects of enzyme mixture, an inoculant, and their interaction on silage fermentation and dairy production. J. Dairy Sci. 75:764-773.

Tamada, J., H. Yokota, M. Oshima and I. Choi. 1996. Effect of application of cellulase and lactic acid bacteria inoculant on the fermentation quality of napier grass (*Pennisetum purpureum* Schum.) silage. Proceedings of The 8th AAAP Animal Science Congress. Jap. Soc. Zootech. Sci. Tokyo Japan. Vol. 2. pp. 256-257.

Tilley, J. M. A. and R. A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. British Grassl. Soc. 18:104-111.

Tomoda, Y., S. Ohmomo, O. Tanaka, H. Kitamoto, T. Hamaya, T. Kono and Y. Tanno. 1996. Effect of cellulase preparation originated from *Acremonium cellulolyticus* Y-94 on alfalfa silage fermentation(In Japanese with English Summary). Grassl. Sci. 42:155-158.

Uchida, S. and H. Hayashi. 1985. Studies on the evaluation of dry matter content and feeding value in silage. 1. Volatility of volatile matter and loss of dry matter by oven drying (In Japanese with English Summary). J. Japan. Grassl. Sci. 31:248-256.

Uchida, S. 1986. Studies on the evaluation of dry matter content and feeding value in silage. 3. Sample drying method of accurate determination of nutritive value (In Japanese with English Summary). J. Japan. Grassl. Sci. 32:59-65.

van Vuuren, A. M., K. Bergsma, F. Frol-Kramer and J. A. C. van Beers. 1989. Effects of addition of cell wall degrading enzymes on the chemical composition and the *in sacco* degradation of grass silage. Grass and Forage Sci.44:223-230.

Weinberg, Z. G, G. Ashbell, A. Azrieli and I. Brukental. 1993. Ensiling peas, ryegrass and wheat with additives of lactic acid bacteria (LAB) and cell wall degrading enzymes. Grass and Forage Sci. 48:70-78.

Zhang, J., S. Kumai, R. Fukumi, I. Hattori and T. Kono. 1997. Effects of additives of lactic acid bacteria and cellulases on the fermentation quality and chemical composition of naked barley (*Hordeum vulgare* L. emand Lam) straw silage. Grassl. Sci. 43:88-94.

Zhang, J., S. Kumai and R. Fukumi. 1997. Effects of temperature, moisture and cellulases on the fermentation quality and chemical composition of naked barley (*Hordeum vulgare* L. emand Lam) straw silage. Grassl. Sci. 43, 95-102.