Effects of Combined Treatments of Lactic Acid Bacteria and Cell Wall Degrading Enzymes on Fermentation and Composition of Italian Ryegrass (*Lolium multiflorum* Lam.) Silage

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ABSTRACT : This experiment was carried out to study the effects of lactic acid bacteria (LAB) inoculation and addition of cell wall degrading enzymes on the fermentation characteristics and chemical compositions of Italian ryegrass silage. An inoculant LAB with or without a cell wall degrading enzyme of Acremoniumcellulase (A), or Meicellulase (M) or a mixture of both (AM), was applied to 1 kg of fresh Italian ryegrass sample. The treatments were control untreated, LAB-treated (application rate 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 (a total of 99 silages). Three silages of each treatment were incubated at 20, 30 and 40℃ for an approximately 2-months storage period. All silages were well preserved as evidenced by their low pH values (3.79-4.20) and high lactic acid concentrations (7.71-11.34 % DM). The fermentation quality and chemical composition of the control untreated and the LAB-treated silages were similar, except that for volatile basic nitrogen (VBN) content was lower ($p < 0.05$) in the LAB-treated silages. LAB + cellulase treatments improved the fermentation quality of silages by decreasing ($p < 0.01$) pH values and increasing ($p < 0.01$) lactic acid concentrations, in all of cellulase types and incubation temperatures. Increasing amount of cellulase addition resulted in further decrease ($p < 0.01$) of pH value and increases ($p < 0.01$) of lactic acid and residual water soluble carbohydrate (WSC) concentrations. LAB + cellulase treatments reduced ($p < 0.01$) NDF, ADF, hemicellulose and cellulose contents of silages compared with both the control untreated and LAB-treated silages. LAB + cellulase treatments did not affect the silage digestibility due to fact of *in vitro* dry matter digestibility (IVDMD) was similar in all silages. The silages treated with cellulase A resulted in a better fermentation quality and a higher rate of cell wall reduction losses than those of the silages treated with cellulases M and AM. Incubation temperature of 30℃ seemed to be more suitable for the fermentation of Italian ryegrass silages than those of 20 and 40℃.

(Key Words: Italian Ryegrass, Lactic Acid Bacteria, Cellulase, Fermentation Quality, Digestibility)

INTRODUCTION

The sugar components (glucose, galactose, mannose, xylose and arabinose) of carbohydrates in the crop are not immediately available as fermentable substrates for the lactic acid bacteria (LAB). They may eventually become available as a result of hydrolysis brought about by the action of enzymes present in the plant itself, by enzymes addition to the crop at the time of ensiling or by acid hydrolysis (McDonald et al., 1991). In order to obtain the necessary level of fermentable sugars (water soluble carbohydrate/WSC) for the lactic fermentation in the crops which are low in WSC, the use of cell wall degrading enzymes (cellulases, hemicellulases and pectinases) have been suggested (Weinberg et al., 1993).

The use of cell wall degrading enzymes as silage additives is expected to improve both the fermentation quality and the digestibility of silages (Ridla and Uchida, 1997b). The degradation of plant fibre by enzyme will provide an extra fermentable sugars as a substrate for a rapidly growing LAB to increase fermentation quality and to enhance preservation. In addition, the reduction of fibre components may improve the silage digestibility (Kung, Jr., et al., 1990; McDonald et el., 1991; Chamberlain and Robertson, 1992; Hoffman et al., 1995). The good fermentation and preservation of silage can be achieved when the rate and extent of hydrolysis of the cell wall coincide with early growth of LAB, and the digestibility

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in the animal can be improved when the alteration in the cellulose or hemicellulose-lignin relationship of silage occur (Kung, Jr., et al., 1990).

The increasing residual WSC content in the silage as a result of enzymes addition has been reported in many studies (Jaakkola, 1990, McDonald et al., 1991; Jacobs and McAllan, 1992; Jacobs et al., 1992; Stokes, 1992; Ridla and Uchida, 1993; Ridla and Uchida, 1997a; Ridla and Uchida, 1997b). However, the improvement of silage digestibility due to (enzymes addition) has not been shortcoming, since most of studies reported that the degradation of the components of structural carbohydrates by enzyme treatments did not enhanced the silage digestibility (Van Vuuren et al., 1989; Jaakkola, 1990; Jaakkola et al., 1991; Jacobs and McAllan, 1991; Jacobs et al., 1991; Ridla and Uchida, 1993; Ridla and Uchida, 1997a; Ridla and Uchida, 1997b).

Kung, Jr., et al. (1990) and Weinberg et al. (1993) reported that the efficiency of biological additives (inoculants and enzymes) for silages depends on the chemical and microbiological compositions of the fresh crops, environmental condition, differences in application rate, enzyme activity, pH optimum, hydrolysis rate, and ensiling time, which they can 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 20, 30 and 40℃, on fermentation characteristics and chemical compositions of Italian ryegrass silage.

MATERIALS AND METHODS

Silage additives

The cellulase enzymes and lactic acid bacteria (LAB) used in this experiment were provided by Yukijirushi Syubio Co. Ltd., Hokkaido, Japan. The first cellulase was derived from *Acremonium cellulolyticus* (Acremonium-cellulase, cellulase A), the second one was derived from *Tricoderma viride* (Meicellulase, cellulase M), and the third cellulase was a mixture of A and M at 1:2 ratio (cellulase AM). According to supplier all cellulase enzymes were prepared to contain of 424 $U.g^{-1}$ avicelase activity. The inoculant LAB (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 preparation was applied at levels of 0.005, 0.01, and 0.02% (fresh matter). The inoculant LAB was used at a theoretical application rate of 10^5 $cfu.g^{-1}$ fresh sample forage. Before being added to the grass sample, a certain amount of each cellulase preparation or inoculant LAB was diluted with distilled water designed to achieve the required concentration, and kept for silage production.

Silage production

The material grass used in this experiment was the first growth of Italian ryegrass (*Lolium multiflorum* Lam.) harvested at the heading stage with a hand cutter on May 9, 1996. The grass was first chopped into approximately 1.3 cm leghts and then macerated with a chopper-cracker (Taninaka Co. Ltd.). The chemical composition and *in vitro* dry matter digestibility of the grass is shown in table 1. A treatment of 1 ml inoculant LAB solution with or without 1 ml cellulase solution was sprayed over to 1 kg of grass sample with a 2.5-ml syringe. The sample was mixed thoroughly and then ensiled into a 2-L vinyl bottle silo. The silage additive treatments were as follows:

Table 1. Chemical composition of Italian ryegrass material used for silage

Composition	Content
Dry matter (%)	21.76
Crude ash (% DM)	12.22
Organic matter (% DM)	87.78
Crude protein (% DM)	18.03
NDF (% DM)	59.22
ADF (% DM)	32.13
ADL (% DM)	3.98
Hemicellulose (% DM)	27.09
Cellulose (% DM)	28.15
WSC (% DM)	7.17
IVDMD (%)	71.00

Abbreviated: 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.

Treatment	Silage additive
1	Non additive (control untreated)
2	LAB-treated (application rate 10^5 cfu/g 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 (a total of 99 silos). Three silages of each treatment were incubated at 20, 30 or 40℃ for about 2 months of storage period. After the incubation period, the silages were opened and the upper 1/5 of each silage was discarded before sampling. The samples were collected and stored at −32℃ until they were used for further analysis.

Chemical analysis

Dry matter content of material 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), water soluble carbohydrate (WSC) was evaluated by 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 sample silage in 400 ml distilled water. The pH of the extracts were measured by electric pH-meter (Horiba F-12), organic acid 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 destilation method.

Statistical analysis

Analysis of variance was applied to all data using a general linier model procedure to analyze the effects of additive treatments on silage fermentation characteristics and chemical compositions. Treatment means were compared by LSD and the significance was declared at $p < 0.05$.

RESULTS AND DISCUSSION

The effects of additive treatments on the fermentation characteristics, chemical compositions and *in vitro* dry matter digestibility of Italian ryegrass silages incubated at 20, 30 and 40℃ are shown in tables 2, 3 and 4,

Table 2. Fermentation characteristics and chemical compositions of control untreated, LAB-treated and LAB + cellulases-treated silages at incubation temperature 20℃

Composition	CTL	LAB	LAB + A			LAB + M			LAB + AM			SEM[+)]
			0.005	0.01	0.02	0.005	0.01	0.02	0.005	0.01	0.02	
pH	4.08^{a}	4.04^{a}	3.93^{d}	3.86^{f}	3.81^{g}	3.99^{b}	3.99^{b}	3.97^{bc}	3.99^{b}	3.95^{cd}	3.89^{e}	0.007
Dry matter (%)	22.99	23.16	22.30	22.50	22.09	23.02	23.07	23.04	22.60	22.68	22.88	0.229
Crude protein (% DM)	17.88	17.89	17.52	17.43	17.84	17.77	17.63	17.99	17.83	17.44	17.34	0.148
NDF (% DM)	51.28^{a}	51.92^{a}	49.89^{cd}	48.00^{e}	46.40^{g}	50.79^{bc}	49.91^{cd}	50.25^{bc}	50.07^{cd}	49.24^{d}	47.49^{f}	0.375
ADF (% DM)	31.44^{a}	31.28^{a}	30.13^{bc}	28.75^{d}	27.27^{e}	30.42^{b}	29.91^{bc}	29.39^{cd}	30.47^{b}	29.40^{cd}	27.89^{e}	0.302
ADL (% DM)	3.55	3.47	3.49	3.84	3.83	3.36	3.56	3.79	3.69	3.94	3.62	0.131
Hemicellulose (% DM)	19.84^{ab}	20.65^{a}	19.76^{b}	19.25^{b}	19.13^{b}	20.36^{a}	20.00^{ab}	20.86^{a}	19.60^{b}	19.84^{ab}	19.60^{b}	0.165
Cellulose (% DM)	27.89^{a}	27.81^{a}	26.64^{b}	24.62^{de}	23.44^{f}	27.06^{b}	26.35^{bc}	25.59^{cd}	26.78^{b}	25.46^{d}	24.27^{e}	0.263
WSC (% DM)	0.98^{fg}	0.93^{g}	1.10^{cd}	1.42^{b}	1.55^{a}	1.15^{c}	1.04^{ef}	1.08^{cde}	1.06^{def}	1.10^{cd}	1.00^{efg}	0.030
Ethanol (% DM)	1.33	1.47	1.33	1.19	1.34	1.18	1.43	1.24	1.39	1.22	1.25	0.130
Lactic acid (% DM)	8.26^{d}	8.72^{be}	8.37^{d}	9.08^{bc}	9.67^{ab}	8.13^{d}	8.11^{d}	9.33^{abc}	8.07^{d}	9.05^{abc}	10.04^{a}	0.432
Acetic acid (% DM)	0.86	0.89	0.96	0.91	1.20	1.00	0.95	0.78	1.00	0.92	0.83	0.085
Propionic acid (% DM)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	−
Butyric acid (% DM)	0.05	0.03	0.03	ND	0.05	0.03	0.04	ND	ND	0.02	ND	0.001
VBN (% TN)	1.96^{abc}	1.29^{e}	1.49^{de}	0.81^{abcd}	1.72^{bcde}	2.13^{ab}	1.91^{abcd}	2.05^{abc}	2.16^{a}	1.66^{cde}	1.87^{abcd}	0.147
IVDMD (%)	68.30	68.00	68.23	68.47	69.33	69.63	69.30	68.77	70.13	68.87	70.40	0.445

[a-g)] Means within same row with different superscript letters differ ($p < 0.05$).

[+)] Standard error of means.

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, ND = Not detectable. Hemicellulose = NDF − ADF, Cellulose = ADF − ADL.

Table 3. Fermentation characteristics and chemical compositions of control untreated, LAB-treated and LAB + cellulases-treated silages at incubation temperature 30℃

Composition	CTL	LAB	LAB + A			LAB + M			LAB + AM			SEM[+)]
			0.005	0.01	0.02	0.005	0.01	0.02	0.005	0.01	0.02	
pH	4.12[a]	4.09[a]	3.89[b]	3.82[b]	3.79[c]	3.99[ab]	3.98[ab]	3.97[ab]	3.96[ab]	3.87[b]	3.80[c]	0.064
Dry matter (%)	22.34	21.96	22.22	22.16	22.15	22.34	22.59	22.23	22.02	22.02	22.08	0.236
Crude protein (% DM)	17.53	17.42	17.42	17.22	17.22	17.74	17.64	17.42	17.07	17.44	17.34	0.141
NDF (% DM)	53.44[a]	53.57[a]	49.69[bc]	47.28[cd]	45.79[d]	51.12[b]	50.20[b]	48.65[bc]	49.70[bc]	47.00[c]	44.70[d]	1.666
ADF (% DM)	32.65[a]	32.51[a]	29.89[b]	28.35[bc]	25.89[c]	30.63[b]	29.23[b]	28.52[bc]	29.76[b]	28.12[bc]	25.92[c]	1.279
ADL (% DM)	3.61[bc]	3.88[ab]	3.92[ab]	3.89[ab]	3.28[d]	3.85[ab]	3.54[cd]	3.56[cd]	4.04[a]	3.88[ab]	4.00[a]	0.167
Hemicellulose (% DM)	20.79[a]	21.06[a]	19.81[ab]	18.93[b]	19.90[ab]	20.49[a]	20.98[a]	20.13[ab]	19.94[ab]	18.89[b]	18.78[b]	0.525
Cellulose (% DM)	29.05[a]	29.12[a]	25.96[bc]	24.47[bc]	22.62[cd]	26.79[b]	25.69[bc]	24.96[bc]	25.72[bc]	24.23[bc]	21.92[d]	1.301
WSC (% DM)	1.03[c]	1.02[c]	1.23[ab]	1.50[a]	1.51[a]	1.13[bc]	1.14[bc]	1.31[ab]	1.12[bc]	1.28[ab]	1.39[ab]	0.102
Ethanol (% DM)	0.79[b]	0.88[ab]	0.83[b]	0.90[ab]	0.82[b]	0.85[ab]	0.90[ab]	0.82[b]	0.97[ab]	1.04[a]	0.92[ab]	0.008
Lactic acid (% DM)	9.33[b]	9.52[b]	10.74[a]	10.90[a]	10.12[ab]	9.10[b]	10.07[b]	10.20[ab]	11.34[a]	11.32[a]	11.14[a]	0.764
Acetic acid (% DM)	1.69	1.75	1.66	1.74	1.56	1.63	1.63	1.49	1.44	1.69	1.35	0.149
Propionic acid (% DM)	0.56	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–
Butyric acid (% DM)	0.16[a]	0.07[b]	0.06[b]	0.06[b]	0.05[b]	0.04[b]	0.07[b]	0.06[b]	0.07[b]	0.06[b]	0.06	0.019
VBN (% TN)	2.36[a]	1.92[b]	1.69[b]	1.85[b]	1.99[b]	1.80[b]	1.72[b]	1.79[b]	1.98[b]	2.16[ab]	2.02[ab]	0.180
IVDMD (%)	70.00	70.13	68.00	70.07	69.73	68.40	70.73	68.60	68.20	69.10	70.10	1.318

[a-d)] Means within same row with different superscript letters differ ($p < 0.05$).

[+)] Standard error of means.

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, ND = Not detectable. Hemicellulose = NDF – ADF, Cellulose = ADF – ADL.

respectively. It can be seen that all silages were well preserved as measured by their low pH values (3.79-4.20), high lactic acid concentrations (7.17-11.34% DM) and low acetic acid (0.78-1.75% DM) and butyric acid (0.01-0.09% DM) concentrations, irrespective of incubation temperature. These silages contain low level of VBN concentrations (1.49-3.29% total nitrogen/TN) which was lower than the concentration generally accepted that a well preserved silage should have an ammonia-N content less then 8% TN (Hendeson, 1993).

LAB inoculation

LAB inoculation had almost no effect on the silage fermentation. This was indicated by the fermentation quality and the chemical composition of the control untreated and the LAB-treated silages were similar, in all of incubation temperatures. LAB inoculation resulted only in a significant decreased ($p < 0.05$) in the VBN concentration. The low VBN concentration in the LAB-treated silages may indicate that LAB inoculation resulted in rapidly fall pH that partially inhibit plant proteolysis and possible clostridial deamination of amino acids (Kung, Jr., et al., 1990). The absence of a positive effect of LAB inoculation on improving silage quality might be due to the WSC content (7.17% DM) in original grass was not enough to allow the LAB to produce more lactic acid to further decrease the final pH value. These results are in line with our previous findings (Ridla and Uchida, 1997b) in Rhodesgrass silage and with the data of Tamada et al. (1996) in napier grass silage, in terms of LAB inoculation did not reduce the pH value and increase the lactic acid concentration as may be due to the low WSC contents (5.10% DM for Rhodesgrass and 4.22% DM for Napier grass) in the original grasses. Keady and Murphy (1996) reported similar results in ryegrass silages that inoculant treatment did not alter the silage fermentation relative to untreated silages, as the WSC contents were 1.82% and 1.66% for untreated and inoculant-treated fresh herbage, respectively. The failure of the LAB inoculation to improve the silage quality in this experiment might also be due to the high numbers of epiphytic LABs were present in the original grass. Although the microflora analysis was not carried out in this experiment, it is assumed that the epiphytic LABs could allow to sustain a satisfactory fermentation in the silo without the need of LAB inoculation (Ridla and Uchida, 1997b). According to

Table 4. Fermentation characteristics and chemical compositions of control untreated, LAB-treated and LAB+cellulases-treated silages at incubation temperature 40℃

Composition	CTL	LAB	LAB+A			LAB+M			LAB+AM			SEM[+)]
			0.005	0.01	0.02	0.005	0.01	0.02	0.005	0.01	0.02	
pH	4.20[a]	4.18[a]	3.88[e]	3.89[e]	3.81[f]	4.00[c]	3.99[e]	3.98[c]	3.98[c]	3.92[d]	3.86[e]	0.012
Dry matter (%)	22.77	22.62	22.61	22.14	22.36	22.86	22.39	22.36	22.79	22.77	22.67	0.156
Crude protein (% DM)	17.34	17.20	17.48	17.63	17.60	17.21	17.61	17.48	17.27	17.04	17.19	0.147
NDF (% DM)	53.08[a]	53.92[a]	48.43[c]	46.02[e]	44.84[f]	50.17[b]	50.21[b]	48.45[c]	49.63[b]	47.45[cd]	46.69[de]	0.384
ADF (% DM)	32.61[a]	33.06[a]	28.68[bc]	27.06[d]	24.76[e]	29.60[b]	29.61[bc]	28.58[c]	29.25[b]	27.51[d]	26.88[d]	0.338
ADL (% DM)	4.06[cd]	4.00[cd]	3.90[de]	4.25[b]	3.84[e]	3.89[de]	4.21[b]	4.14[bc]	4.15[bc]	3.86[de]	4.51[a]	0.698
Hemicellulose (% DM)	20.47[bc]	20.86[b]	19.75[c]	18.96[d]	20.07[c]	20.58[b]	21.61[a]	20.82[b]	20.38[bc]	19.94[c]	19.81[c]	0.255
Cellulose (% DM)	28.55[a]	29.06[a]	24.78[c]	22.80[e]	21.03[f]	25.71[b]	24.39[cd]	23.49[e]	25.10[bc]	23.65[d]	22.37[e]	0.307
WSC (% DM)	1.07[f]	1.15[f]	1.76[cd]	1.78[cd]	2.16[a]	1.62[e]	1.73[cd]	1.71[d]	1.81[bc]	1.88[b]	2.10[a]	0.031
Ethanol (% DM)	0.78	0.68	0.73	0.95	0.68	0.74	0.73	0.72	0.83	0.77	0.82	0.130
Lactic acid (% DM)	7.17[e]	7.60[cde]	8.82[abc]	8.54[ace]	9.58[ab]	7.77[cde]	8.40[de]	9.04[abc]	8.57[bcd]	8.86[ab]	10.09[a]	0.455
Acetic acid (% DM)	1.69	1.75	1.66	1.74	1.56	1.63	1.63	1.49	1.44	1.69	1.35	0.095
Propionic acid (% DM)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–
Butyric acid (% DM)	0.07	0.04	0.01	ND	ND	ND	ND	0.03	0.01	ND	ND	0.008
VBN (% TN)	3.02[abc]	2.89[bcd]	2.77[bcd]	2.48[bd]	2.23[d]	2.39[cd]	2.44[cd]	3.29[a]	2.77[bcd]	2.14[cd]	2.89[bcd]	0.190
IVDMD (%)	69.13	68.53	67.87	69.57	67.73	69.17	70.03	69.37	68.57	69.67	69.80	0.520

[a-f)] Means within same row with different superscript letters differ ($p < 0.05$).

[+)] Standard error of means.

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, ND=Not detectable. Hemicellulose=NDF−ADF, Cellulose=ADF−ADL.

McDonald et al. (1991) and Henderson (1993) epiphytic LABs in the standing crops are present in dormant state, and that the harvesting procedures, macerating the crop, and increasing use of inoculant additives in the field might have encouraged the epiphytic LABs to the higher numbers. In addition, Henderson (1993) reported that the numbers of the epiphytic LABs 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+cellulase (all cellulase types) to Italian ryegrass silages improved their fermentation quality as indicated by decreasing ($p < 0.01$) pH value and increasing ($p < 0.05$) lactic acid concentration compared with those of the LAB-treated silages, in all incubation temperatures. The combined treatments of LAB+cellulase had no effect on the ethanol, acetic acid, butyric acid and VBN concentrations (tables 2, 3, 4). Increasing the amount of cellulase resulted in further decrease ($p < 0.01$) of pH value and increase ($p < 0.01$) of lactic acid concentration, in all of cellulase types and incubation temperatures. These findings, i.e., that cellulase addition improved the preservation of silages by decreasing the pH value and increasing lactic acid concentration are consistent with our previous results (Ridla and Uchida, 1993; Ridla and Uchida, 1997a; Ridla and Uchida, 1997b) and those of other studies (Henderson and McDonald, 1977; van Vuuren et al., 1989; Jacobs et al., 1991; Selmer-Olsen et al., 1993; Sheperd et al., 1995). This might be due to a higher amount of fermentable carbohydrates (WSC) provided by the hydrolysis of cell wall components, which, in turn, stimulate fermentation by lactic acid bacteria (Ridla and Uchida, 1997b).

The cell wall components (NDF, ADF, Hemicellulose and Cellulose) of silages reduced ($p < 0.01$) due to combined treatments of LAB+cellulase and continuously decreased with increasing the amount of cellulase addition, in all cellulase types and incubation temperatures. These reduction more markedly occured in the highest level of 0.02% than in both the 0.005 and 0.01% of cellulase added. Compared with the LAB-treated silages, the NDF contents of silages treated with 0.02% cellulase A were reduced ($p < 0.01$) by 5.52, 7.78 and 9.08 (unit %), respectively for silages incubated at 20, 30 and 40℃. Similarly, the NDF contents of silages treated with 0.02%

cellulase M were reduced ($p < 0.01$) by 1.67, 4.94 and 5.47% (unit %), and those of silages treated with 0.02% cellulase AM were reduced ($p < 0.01$) by 4.43, 8.87 and 7.23 (unit %). The reduction of cell wall components of silages due to enzyme treatments are in line with the results of Ridla and Uchida, 1993, Sheperd et al., 1995, Sheperd and Kung, Jr., 1996, Nadeau et al., 1996 and Ridla and Uchida, 1997a).

The residual WSC content of the silages treated with LAB+cellulase (all cellulase types) was higher ($p < 0.01$) than those of the LAB-treated silages, in all incubation temperatures. Increasing the amount of added cellulase resulted in a significant increase ($p < 0.05$) in the residual WSC content of all cellulase types. This suggests that the cellulase action through reduction of cell wall components was able to provide more WSC for fermentation by LAB (Jaakkola, 1990; McDonald et al., 1991; Jacobs and McAllan, 1992; Jacobs et al., 1992; Stokes, 1992; Ridla and Uchida, 1993; Sheperd et al., 1995; Ridla and Uchida, 1997a). According to Sheperd et al. (1995) increases in the glucose content of cellulase treated silages suggested that added enzymes continued to hydrolyze substrate even after the fermentation was complete, but large amounts of residual WSC could lead to aerobic instability of silage.

The combined treatments of LAB+cellulase did not affect the silage digestibility as evidenced by *in vitro* dry matter digestibility (IVDMD) were similar in all silages of all cellulase types and incubation temperatures. This results were consistent with our previous findings (Ridla and Uchida, 1993; Ridla and Uchida, 1997a) in barley straw silages and with the data reported by van Vuuren et al. (1989), Jaakkola (1990), Jaakkola et al. (1991), Jacobs and McAllan (1991) and Jacobs et al. (1991) in grass silages, in terms of lowering cell wall components due to enzyme treatments were not effective in enhancing the digestibility of silages. This may indicate that cellulase enzymes were not able to degrade the lignin-polysaccharide complexes or plant cell walls, which are indigestible by the rumen microbes (Jaakkola, 1990), or cellulase enzymes degraded only the same cell wall structures in the silo as would be digested in the rumen (Jaakkola and Huhtanen, 1990).

Cellulase type

The fermentation quality and the rate of cell wall components losses of silages treated with LAB+cellulase A and LAB+cellulase AM were higher ($p < 0.01$) than with LAB+cellulase M. The data showing that the silages treated with cellulase LAB+cellulase A and LAB+cellulase AM had a lower pH values ($p < 0.01$) and a higher ($p < 0.05$) lactic acid concentrations than those of silages treated with LAB+cellulase M. The silages received LAB+cellulase A and LAB+cellulase AM treatments had also higher ($p < 0.01$) residual WSC and lower ($p < 0.01$) NDF, ADF, hemicellulose and cellulose contents than silages treated with LAB+cellulase M. This findings was similar with our previous results (Ridla and Uchida, 1997b) in Rhodesgrass silage and with the data reported by Tomoda et al. (1996) in alfalfa silage in terms of silages treated with cellulase preparation originated from *Acremonium cellulolyticus* resulted in a lower pH and a higher lactic acid concentration than were obtained with silages treated with cellulase preparation originated from *Tricoderma viride*. Moreover, this results are in agreement with the results of Zhang et al. (1997a,b) who reported that silages received Acremonium cellulase treament resulted in higher fermentation quality than that of silages received Meicellulase treatment, and that silages received a mixture of these cellulases treatment had an intermediate quality.

Incubation temperature

Incubation temperature of 30℃ seemed to be more appropriate for stimulating fermentation than both of 20 and 40℃, and these temperatures might not relate with the additive treatments. The silages at incubation temperature 30℃ had lower ($p < 0.05$) pH values and VBN concentrations, and higher ($p < 0.01$) lactic acid concentrations than those of silages at incubation temperatures 20 and 40℃. Incubation temperatures had no effect on dry matter, crude protein, NDF, ADF, hemicellulose and cellulose contents and *in vitro* dry matter digestibility of silages. The present data were inconsistent with our previous result obtained from Rhodesgrass silage that the incubation temperature 40℃ might have been the most appropriate environment for stimulating fermentation compared with temperatures 20 and 30℃ (Ridla and Uchida, 1997b).

It can be concluded that LAB inoculation did not improve the silage quality, but the treatment of LAB+cellulases improved the fermentation quality as evidenced by reducing pH, increasing lactic acid and residual WSC concentrations. Cellulase addition increased the solubility of cell wall components and continuously with the increasing amount of cellulase addition. Since the silage digestibility was not affected by the cellulase addition, it can be suggested that the lowest level of 0.005% was likely to be enough for sustaining fermentation in the silo.

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