Comparative Study on the Effects of Combined Treatments of Lactic Acid Bacteria and Cellulases on the Cell Wall Compositions and the Digestibility of Rhodesgrass (*Chloris gayana* Kunth.) and Italian Ryegrass (*Lolium multiflorum* Lam.) Silages

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ABSTRACT : This study was conducted to compare the effects of lactic acid bacteria (LAB) or LAB+cellulases on the cell wall compositions and the *in vitro* dry matter digestibility (IVDMD) of Rhodesgrass (RG) and Italian ryegrass (IRG) silages. LAB (*Lactobacillus cassei*) at a concentration of 1.0×10^5 cfu.g^{-1} fresh forage was added to all ensiling samples (except the untreated control) of RG and IRG. The cellulases used were Acremoniumcellulase (A), Meicelase (M) or a mixture of both (AM). Each cellulase was applied at levels of 0.005, 0.01 and 0.02 % fresh sample. The samples were incubated at 20, 30 and 40℃ for about 2 months of storage. LAB inoculation did not affect cell wall components or IVDMD of both the RG and IRG silages, but LAB+cellulase treatments did. Increasing the amount of cellulase addition resulted in further decreases of cell wall concentrations. This reduction more markedly occurred with cellulases A and AM than it did with cellulase M. Cell wall components losses were higher in the IRG silages than in the RG silages. LAB+cellulase treatments decreased IVDMD of the RG silages, but had no effect on the IRG silages. The different effect of LAB+cellulase treatments on cell wall degradation and IVDMD of the RG and IRG silages suggested that RG contains more structural carbohydrates, which were difficult to degrade with cellulase, than did IRG. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 4 : 531-536*)

Key Words : Cellulase, Cell Wall Composition, Italian Ryegrass, IVDMD, Lactic Acid Bacteria, Rhodesgrass

INTRODUCTION

Many studies have been reported that treatment with enzymes (i.e., cellulases, hemicellulases, amylases, and pectinases) are capable of reducing the cell wall components (NDF, ADF, cellulose and hemicellulose) of silages during ensiling (McDonald et al., 1991; Nadeau et al., 1996; Ridla and Uchida, 1993, Ridla and Uchida, 1997; Selmer-Olsen et al., 1993; Sheperd et al., 1995; Sheperd and Kung, Jr., 1996a,b). The extents of the cell wall degradation by enzymes were varied depending on the chemical composition of the fresh crops, environmental conditions, enzyme application rate, enzyme activity, pH optimum, hydrolysis rate, and ensiling time (Kung et al., 1990; Selmer-Olsen et al., 1993; Sheperd and Kung, Jr., 1996b; van Vuuren et al., 1989; Weinberg et al., 1993). The addition of cell wall degrading enzymes to the silage before ensiling is expected to reduce some cell wall components, which in the steps of the ensiling process it would provide more fermentable sugar for silage fermentation by lactic acid bacteria. Furthermore, it may improve the silage dry matter digestibility (Hoffman et al., 1995; Kung, Jr. et al., 1990; McDonald et al., 1991).

The effect of enzyme treatments on silage digestibility has been inconsistent since different results were reported by many researchers. Stokes (1992) and Tengerdy et al. (1991) found the digestibility of resulting silages improved due to enzyme addition. However, no effects of enzyme treatments on silage digestibilities (Huhtanen et al., 1985; van Vuuren et al., 1989; Jacobs et al., 1991; Jaakkola et al., 1991; Jacobs and McAllan, 1992; Ridla and Uchida, 1993; Selmer-Olsen, 1994; Ridla and Uchida, 1997) or lowered silage digestibilities that might be due to cellulase addition (Jaakkola, 1990; Jaakkola and Huhtanen, 1990; Jacobs and McAllan, 1991) have been reported.

The objectives of this study were to compare the effects of lactic acid bacteria (LAB) or LAB+cellulase with different types and levels of application, incubated at different temperatures, on the changes in cell wall compositions and *in vitro* dry matter digestibility of Rhodesgrass (*Chloris gayana* Kunth.) and Italian ryegrass (*Lolium multiflorum* Lam.) silages.

MATERIALS AND METHODS

Silage additives

The cellulase enzymes and lactic acid bacteria (LAB) used in this experiment were provided by Yukijirushi Syubyo Co. Ltd., Hokkaido, Japan. The first cellulase was Acremoniumcellulase (derived from *Acremonium cellulolyticus*, cellulase A), the second one was Meicelase (derived from *Tricoderma viride*, 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 and the inoculant LAB (Snow Lact-L) was prepared 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 bases). The inoculant LAB was used at a theoretical application rate of 1.0×10^5 cfu.g^{-1} fresh sample forage. On the day of the production of silages, a certain amount of each cellulase preparation or inoculant LAB was diluted with distilled water designed

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to achieve the required concentration, and kept for silage making.

Silage making

On 9 August 1995, the first growth of Rhodesgrass (RG) was harvested at the heading stage with a hand cutter. The harvested material was chopped into approximately 1.3 cm lengths and then lacerated with a chopper-cracker (Taninaka Co. Ltd.). One ml of inoculant LAB solution with or without 1 ml cellulase solution was sprayed over 1 kg 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:

Treatment Silage additive

1. Untreated (Control, CTL)
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, and then 3 silages of each treatment were incubated at 20, 30 or 40℃ for approximately 2 months of storage period.

On 9 May 1996, the first growth of Italian ryegrass (IRG) was harvested at the heading stage and used for silage production as described above. The chemical composition of both grasses is shown in table 1.

Table 1. Chemical compositions and IVDMD of Rhodesgrass and Italian ryegrass materials prior to ensiling

Content	Rhodesgrass	Italin ryegrass
Dry matter (%)	21.76	21.76
Crude ash (% DM)	10.28	12.22
Organic matter (% DM)	89.72	87.78
Crude protein (% DM)	10.38	18.03
NDF (% DM)	66.42	59.22
ADF (% DM)	38.18	32.13
Hemicellulose (% DM)	28.24	27.09
Cellulose (% DM)	31.04	28.15
ADL (% DM)	7.14	3.98
WSC (% DM)	5.01	7.17
IVDMD (%)	69.10	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.

After the incubation period, the silos were opened and the upper 1/5 of each silage was discarded before sampling. The samples were collected and kept frozen at -32℃ until they were used for further analysis.

Chemical analysis

Dry matter content of the grasses and silages were determined by a vacuum freeze-drying method (Uchida, 1986). The dried samples were ground and kept for the another analysis. 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 using the method of Deriaz (1961), and *in vitro* dry matter digestibility (IVDMD) was determined by the method of Tilley and Terry (1963).

Statistical analysis

To compare cell wall components disappearances and *in vitro* dry matter digestibility between the RG and IRG silages, a two-sample t test was applied. Data from all incubation temperatures were combined for the comparison of additive treatments, and data from all additive treatments were combined for the comparison of incubation temperatures.

RESULTS AND DISCUSSION

Cell wall composition

Addition of microbial inoculant to the forage had no effect on the degradation of cell wall components, since there were no changes in NDF and ADF contents between the untreated controls and the LAB-treated silages, regardless of incubation temperature, both in the RG and IRG silages (table 2). It was suggested that LAB inoculant had no capability on reducing the cell wall components of both the RG and IRG silages. This finding was consistent with the result of Kung, Jr., et al. (1987) who reported that microbial inoculant possessed a negligible ability to degrade cell wall components. No effects of LAB inoculation on the NDF and/or ADF content in silages have been reported by many researchers (Keady and Steen, 1994; Keady and Murphy, 1996; Kent et al., 1989; Kung, Jr., et al., 1987; Kung, Jr., et al., 1991; Rooke et al., 1988). In contrast, Harrison et al. (1989) reported that applying inoculant to grass-legume silage decreased NDF and ADF concentrations. Conversely, Gordon (1989) reported an increased ADF concentration in the inoculated silage.

Combined treatments of LAB+cellulases significantly reduced ($p<0.05$) NDF and ADF contents of both the RG and IRG silages, regardless of the incubation temperature (table 2). Increasing the amount of cellulase application resulted in a linearly significant decrease ($p<0.05$) in contents of these fibers, with the highest level of cellulase addition (0.02%) resulted in the greatest reduction. Compared with untreated control silages, the LAB+cellulase treatments significantly decreased ($P<0.01$) NDF and ADF contens, regardless of the levels of cellulase application and incubation

Table 2. Differences in dry matter contents and cell wall components disappearances between the RG and IRG silages made with various treatments

Treatments	Dry matter (DM)			NDF[1] (% DM)			ADF[2] (% DM)		
	RG	IRG	Sig[3]	RG	IRG	Sig[3]	RG	IRG	Sig[3]
Control	21.98	22.81	NS	-	-	-	-	-	-
LAB	22.35	22.58	NS	0	0	NS	0	0	NS
LAB+0.005 A	22.22	22.38	NS	3.43	3.26	NS	2.21	2.67	NS
LAB+0.01 A	21.92	22.27	NS	2.47	5.50	**	1.61	4.18	**
LAB+0.02 A	22.05	22.20	NS	5.06	6.79	*	3.43	6.26	**
LAB+0.005 M	21.94	22.74	NS	0.85	1.90	*	0.80	2.02	**
LAB+0.01 M	22.26	22.68	NS	1.12	2.49	**	1.15	2.66	**
LAB+0.02 M	21.88	22.54	NS	1.85	3.48	**	1.92	3.41	*
LAB+0.005 AM	22.06	22.47	NS	2.26	2.80	NS	1.37	2.41	*
LAB+0.01 AM	22.00	22.49	NS	2.38	4.70	**	1.74	3.89	**
LAB+0.02 AM	22.05	22.54	NS	3.62	6.31	**	4.00	5.34	NS

[1,2] See table 1. [3] Significant differences: NS $p>0.05$, * $p<0.05$, ** $p<0.01$.

temperature. The NDF contents of RG silages were reduced by 3.62, 1.27 and 2.75 (unit %) by treatments with LAB+cellulase A, LAB+cellulase M and LAB+cellulase AM, respectively, and those of IRG silages were reduced by 5.18, 2.62 and 4.60 (unit %), respectively (figure 1). Similarly, the ADF contens of RG silages were reduced by 2.42, 1.29 and 2.37 (unit %), and those of IRG silages were reduced by 4.37, 2.70 and 3.88 (unit %) (figure 2). For both the RG and IRG silages, losses of cell wall components were higher after treatment with cellulase A and AM ($P<0.05$) than with cellulase M (figures 1 and 2). This might indicate that the decomposition of cell wall components was more markedly occurred in silages treated with cellulase A and AM than in silages treated with cellulase M (Ridla and Uchida, 1998a,b; Tomoda et al., 1996; Zhang et al., 1997a,b). There were several reports of reductions of cell wall components (NDF and ADF contents) by enzyme treatments, either alone or in combination with microbial inoculant (Chamberlain and Robertson, 1992; Hoffman et al., 1995; Ridla and Uchida, 1993; Ridla and Uchida, 1997; Selmer-Olsen et al., 1993; Sheperd et al., 1995; Sheperd and Kung, Jr., 1996a,b; Stokes and Chen, 1994; Weinberg et al., 1993).

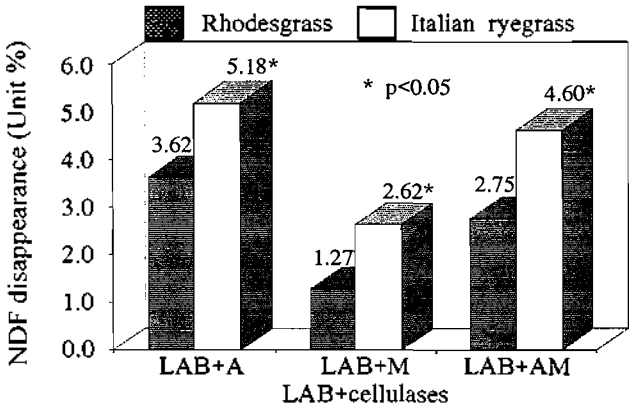

Figure 1. Difference in NDF disappearance between the RG and IRG silages treated with LAB+cellulases. The values were significantly different ($p<0.05$) between the two silages

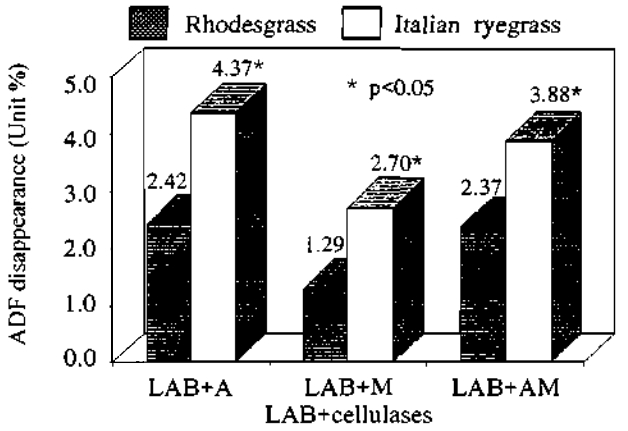

Figure 2. Difference in ADF disappearance between the RG and IRG silages treated with LAB+cellulases. The values were significantly different ($p<0.05$) between the two silages

The effects of the LAB+cellulase treatments on the rate of cell wall reduction was higher in the IRG silages than in the RG silages. This was indicated by the greater losses of NDF and ADF ($p<0.05$) in the IRG silages than in the RG silages (table 2). It was suggested that the cell wall components in RG were more resistant to degradation by the cellulases than those in IRG. This could be due to the higher ADL content in RG than in IRG (table 1). A similar result was reported by Nadeau et al. (1996) who treated alfalfa and orchardgrass with cellulase combined with formic acid. They found that the decreasing rates of NDF, cellulose and hemicellulose concentrations were lower in the alfalfa silage, and attributed them to the higher lignin content in alfalfa than in orchardgrass.

The effect of incubation temperature on the rates of reduction of NDF and ADF is presented in table 3. The data indicate that the NDF and ADF losses were lower ($p<0.05$) in the RG silages than in the IRG silages. One exception was that there was no difference in NDF losses at 20℃ between the RG and IRG silages. The reductions in the NDF and ADF concentrations at 30 and 40℃ were higher ($p<0.05$) than those at 20℃ in

Table 3. Differences in dry matter, crude protein, WSC, IVDMD percentages and cell wall components disappearances between the RG and IRG silages at different incubation temperatures

Incubation temperature	20℃			30℃			40℃		
	RG	IRG	Sig[5]	RG	IRG	Sig[5]	RG	IRG	Sig[5]
Dry matter (%)	22.36	22.76	NS	21.72	22.19	NS	22.62	22.18	NS
Crude protein (% DM)	9.97	18.16	**	10.67	17.47	**	9.80	17.35	**
WSC[1] (% DM)	1.26	1.13	NS	1.08	1.24	*	1.25	1.71	**
IVDMD[2] (%)	66.22	69.1	**	68.39	69.63	NS	65.79	68.95	**
NDF[3] (Unit %)	2.54	2.14	NS	2.26	5.20	**	2.88	5.09	*
ADF[4] (Unit %)	1.63	2.15	*	2.81	4.18	*	1.65	4.62	**

[1,2,3,4] See table 1. [5] See table 2.

the IRG silages. These reductions were inconsistent in the RG silages, but the data showed that NDF reduction at 40℃ was higher ($p<0.05$) than those at 20 and 30℃, and ADF reduction at 30℃ was higher ($p<0.05$) than those at 20 and 40℃. This might be due to the decomposition of cell wall components were more effectively occurred at 30 and 40℃ than at 20℃ (Ridla and Uchida, 1998a,b).

Silage digestibility

Inoculant treatment did not affect the silage digestibility as evidenced by the fact that the *in vitro* dry matter digestibility (IVDMD) was similar between the untreated control and the LAB-treated silages in both the RG and IRG silages, regardless of incubation temperature (table 4). It might indicate that the LAB inoculant used in this experiment did not possess an ability to improve digestibility of both the RG and IRG silages. According to Kung, Jr. et al. (1987) addition of microbial inoculants to forage had different effects on the digestion of the resulting silage. Improvement of silage digestibility by bacterial inoculation was reported by many researchers based on both *in vitro* (Harrison et al., 1989; Stokes, 1992) and *in vivo* (Anderson et al., 1989; Gordon, 1989; Keady and Steen, 1994) results. However, other studies reported no effect of bacterial inoculation on silage digestibility (Keady and Murphy, 1996; Kung, Jr. et al., 1991; Sharp et al., 1994; Stokes, 1992; Tengerdy et al., 1991; Weinberg et al., 1993).

In the present study, the effect of LAB+cellulase treatments on the IVDMD of the resulting silages was inconsistent, since the IVDMD decreased in the RG silages and there was no difference in the IRG silages, in all cellulase types regardless of incubation temperature (table 4). It was suggested that cellulase treatments might have degraded the most digestible fraction of the structural carbohydrates and that the residual fraction might be less digestible. Many researchers reported that the enzyme treatments, either alone or in combination with microbial inoculant, had varied effects on silage digestibility. Improving silage digestibility by addition of enzymes has been reported by Jacobs et al. (1992), Stokes (1992), and Tengerdy et al. (1991). However, most of published studies on enzyme treatments were reported to have no effect on silage digestibilities, in either *in vitro* (Jacobs et al., 1991; Ridla and Uchida, 1993; Ridla and Uchida, 1997; Selmer-Olsen, 1994; van Vuuren et al., 1989) or *in vivo* (Huhtanen et al., 1985; Jaakkola et al., 1991; Jacobs and McAllan, 1992) results. A lowered silage digestibility that might be due to cellulase addition was also reported (Chamberlain and Robertson, 1992; Jaakkola, 1990; Jaakkola and Huhtanen, 1990; Jaakkola et al., 1991). According to Jaakkola (1990), Jacobs and McAllan (1991), McDonald et al. (1991), Sheperd and Kung, Jr. (1996a) and Sheperd et al. (1995) the lower digestion in enzyme-treated silages

Table 4. Differences in crude protein, WSC and IVDMD between the RG and IRG silages made with various treatments

Treatments	Crude protein (% DM)			WSC[1] (% DM)			IVDMD[2] (%)		
	RG	IRG	Sig[3]	RG	IRG	Sig[3]	RG	IRG	Sig[3]
Control	10.06	17.58	**	1.02	1.02	NS	67.12	68.47	NS
LAB	10.04	17.51	**	0.96	1.03	NS	68.13	69.12	NS
LAB+0.005 A	9.79	17.81	**	1.33	1.36	NS	68.81	67.77	NS
LAB+0.01 A	10.25	17.76	**	1.12	1.57	**	66.63	69.37	**
LAB+0.02 A	10.27	17.88	**	1.56	1.74	NS	66.59	68.93	*
LAB+0.005 M	10.18	17.58	**	1.22	1.30	NS	67.73	69.07	NS
LAB+0.01 M	10.12	17.63	**	0.99	1.31	*	65.44	70.02	**
LAB+0.02 M	10.18	17.63	**	1.37	1.18	NS	64.95	69.02	**
LAB+0.005 AM	10.21	17.50	**	1.35	1.33	NS	67.72	68.97	NS
LAB+0.01 AM	10.36	17.57	**	1.10	1.42	*	65.54	68.31	**
LAB+0.02 AM	10.27	17.62	**	1.29	1.49	NS	65.93	69.60	**

[1,2] See table 1. [3] See table 2.

might be due to fact the added enzymes had already hydrolyzed the most readily digestible portion of forage during ensilage and left less digestible materials, which in turn might result in the treated silage having a lower digestibility than that of the untreated silage. On the other hand, Jaakkola (1990) reported that the lower digestion in enzyme-treated silages might be due to fact that the cellulases were not able to degrade the lignin-polysaccharide complexes or plant cell walls, which the rumen microbes were unable to digest.

The IVDMD was higher ($p<0.05$) in the IRG silages than in the RG silages (69.15 versus 66.80%), regardless of the treatment and incubation temperature. The lower digestibility in the RG silages than in the IRG silages might be due to the different origins of these herbage species. RG, which is of tropical origin, and IRG, which is of temperate origin, have different chemical, physical and physiological properties. The cell wall concentrations are especially higher in RG than in IRG. These differences may lead to differences in the silage digestibility as well as in the silage fermentation characteristics (McDonald et al., 1991).

In conclusion, LAB inoculation did not affect the cell wall components or *in vitro* dry matter digestibility of both the RG and IRG silages. LAB+cellulase treatments reduced the cell wall components of silages, with increasing the amount of cellulase resulted in increasing the reduction. However, decreasing the cell wall components by the LAB+cellulase treatments did not improve the silage digestibility. This may indicate that the material that is easily digested by rumen microbes was loss due to cellulase activity, and that the remaining material was less digestible. The lower cell wall reduction in the RG silages than in the IRG silages suggested that RG, as a tropical origin herbage species, contains more structural carbohydrates, which were more difficult to degrade by cellulase, than did the temperate origin IRG.

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