Effects of Cellulase and Brewers' Grains Addition on the Fermentation Quality and Nutritive Value of Barley Straw Silage

M. Ridla and S. Uchida[1]
Faculty of Agriculture, Okayama University, Tsushima Naka 1-1-1, Okayama 700, Japan

ABSTRACT : Two experiments were carried out to evaluate the effects of cellulase and brewers' grains addition on improvement of the fermentation quality and the nutritive value of barley straw silages made from dried or fresh straw. In Exp. I : 1 kg dried barley straw + 2 kg wet brewers' grains + 0 (I-0), 2 (I-2), 4 (I-4), 6 (I-6), and 8 (I-8) g of cellulase. In Exp. II : 2 kg fresh barley straw + 2 kg wet brewers' grains + 0 (II-0), 2 (II-2), 4 (II-4), 6 (II-6), and 8 (II-8) g of cellulase. Each prepared material was ensiled into vinyl bag silos (5 L capacity) and stored for 10 (Exp. I) or 7 (Exp. II) months at 21℃. The fermentation quality and nutritive value of barley straw silages produced were markedly improved by mixing them with wet brewers' grains, on the other hand the effect of cellulase addition on the fermentation and reduction of the cell wall components in the silos at ensiling more effectively occurred at low dry matter silages rather than at the high ones. All silages in both Exp. I and II were found well preserved as indicated by their low pH and high lactic acid concentration. Cellulase treated silages had a lower pH ($p<0.05$) and a higher lactic acid concentration ($p<0.05$) than those of without cellulase addition. NDF, ADF, and (Hemi)cellulose contents of cellulase treated silages reduced ($p<0.05$) compare to those of the corresponding silage without cellulase. Increasing levels of cellulase addition caused an increase in fermentation quality and reduction of cell wall components. *In vitro* dry mater digestibility was found similar in all silages. Fermentation quality and nutritive value of barley straw silages were improved by both wet brewers' grains and cellulase addition. Cellulase addition reduced the cell wall components silages, but did not improve the digestibility.

(**Key Words:** Barley Straw, Brewers' Grains, Cellulase, Preserved, Digestibility)

INTRODUCTION

Previous work has shown that by mixing and ensiling barley straw with wet brewers' grains the fermentation quality of the mixed silages was improved, the chemical composition and the nutritive value were also enhanced as indicated by increasing crude protein content and silage digestibility (Ridla and Uchida, 1994). Further more Ridla and Uchida (1993) found that cellulase added was able to break down cell wall components of barley straw during ensilage. Similar results on the reduction of cell wall components due to cellulase addition on grass silages were also reported by many researchers (Henderson and McDonald, 1977; van Vuuren et al., 1989; Jaakkola, 1990; Jacobs and McAllan, 1991; Jacobs et al., 1991). The reduction cell wall components due to cellulase addition, however, was not followed by improving the digestibility of either barley straw or grass silages. van Vuuren et al. (1989) reported that enzyme additives produced the carbohydrate fraction more readily available for the rumen fermentation. Furthermore it could give a potential to improve the digestion in the rumen by providing protein readily available for achieving energy and nitrogen balance. Reducing fibre fraction due to enzymic action on cell wall components of barley straw silage, in this experiment, is expected to provide more readily available carbohydrate fraction, while brewers' grains as protein source would provide nitrogen readily available, which in turn could give advantage for microbial growth and digestion in the rumen. The slightly higher OM digestibility of enzyme treated silage diet with fish meal supplementation was reported by Jaakkola et al. (1990). Jacobs et al. (1992) also noticed that DM and OM digestibilities of the enzyme treated silages were increased by either rapeseed meal or fish meal protein supplementation.

[1] Address reprint requests to S. Uchida.

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The experiment reported herein was designed to evaluate the effects of cellulase and wet brewers' grains addition on improving fermentation quality and nutritive value of barley straw silage.

MATERIALS AND METHODS

Silage Production

The chemical composition of materials barley straws and wet brewers' grains is shown in table 1, the preparation of the materials of silages for both experiment I and II were described elsewhere (Ridla and Uchida, 1994). Samples of about 1 kg (dried, Exp. I) or 2 kg (fresh, Exp. II) of the barley straw were mixed with 2 kg wet brewers' grains before applying the cellulase (Ridla and Uchida, 1993). In experiment I, cellulase was applied at levels of 0 g (I-0) as control, 2 g (I-2), 4 g (I-4), 6 g (I-6) and 8 g (I-8). In experiment II, the cellulase was added at levels of 0 (II-0) as control, 2 g (II-2), 4 g (II-4), 6 g (II-6) and 8 g (II-8). Each prepared material was ensiled into vinyl bag silos (5 L capacity) from which air was excluded by a vacuum pump (Ridla and Uchida, 1993). The dry matter ratio of the mixed barley straw and wet brewers' grains were about 1:0.87 and 1:0.95 for Exp. I and II, respectively.

All silos were placed in a constant chamber temperature of 21℃ for 10 (Exp. I) and 7 (Exp. II) months of storage. At the end of the storage period, the silos were opened and the mouldy part (upper 1/4 and bottom 1/4) of each silages was discarded before sampling (Ridla and Uchida, 1994). The samples were collected and frozen in −32℃ until ready for analysis.

Table 1. Chemical composition and *in vitro* dry matter digestibility of barley straw and wet brewers' grains materials

	Experiment I		Experiment II	
	Dried barley straw	Wet Brewers' grains	Fresh barley straw	Wet Brewers' grains
Dry matter (g/kg)	824.9	273.3	262.4	248.2
Crude protein (g/kg DM)	26.4	262.8	27.4	266.5
Crude ash (g/kg DM)	44.9	30.7	67.2	33.7
Crude fibre (g/kg DM)	479.6	119.1	418.9	109.6
NDF (g/kg DM)	806.9	512.9	751.6	559.1
ADF (g/kg DM)	516.2	165.3	488.6	192.7
Hemicellulose (g/kg DM)	290.7	347.6	263.0	366.4
Cellulose (g/kg DM)	452.1	127.9	402.9	148.2
ADL (g/kg DM)	64.1	37.4	85.7	44.5
Silica (g/kg DM)	14.2	9.5	13.9	9.5
WSC (g/kg DM)	45.4	196.6	36.8	138.4
IVDMD[1)] (%)	35.0	65.9	42.6	62.1
IVDMD[2)] (%)	51.6	68.1	49.9	63.0

Abbreviation: 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.
[1)] Abe and Horii method (1974).
[2)] Tilley and Terry method (1963).

Sampling procedure and chemical analysis

All sampling procedures and methods of chemical analysis for materials and silages were the same as those described by Ridla and Uchida (1994). In summary, dry matter content of silages was determined by a vacuum freeze-dry method (Uchida, 1986), raw materials were dried by oven at 60℃ for 24 h, crude protein was determined by the Kjeldahl method, NDF, ADF, ADL and silica were measured by the method of Goering and Van Soest (1970), organic acid and ethanol were analyzed by gas chromatography (GC - 14A, Shimadzu), lactic acid was measured by the method of Barker and Summerson (1941), WSC was evaluated by using the method of Deriaz (1961), and *in vitro* dry matter digestibility

(IVDMD) was determined by both the method of Tilley and Terry (1963) and Abe and Horii (1974).

RESULTS AND DISCUSSION

The fermentation quality of all experimental silages in both Exp. I and II were well preserved as measured in terms of their low pH (below 4, except for the control in Exp. I was 4.08) and high lactic acid concentration (table 2). Wet brewers' grains as carbohydrate rich material had supplied an adequate substrate of water soluble carbohydrate (WSC) for stimulating fermentation by lactic acid bacteria to rapidly achieve acid condition. The addition wet brewers' grains in these experiments also enriched the chemical composition of silages, especially the crude protein content (table 3). This is similar with our previous results that by mixing and ensiling barley straw and wet brewers' grains, the fermentation quality and nutritive value of silages were improved compared to the corresponding barley straw without wet brewers' grains silages (Ridla and Uchida, 1994).

The pH value of mixed barley straw and brewers' grains cellulase treated silages was found lower ($p < 0.05$) than those without cellulase treatment, and continued to decrease as cellulase addition increase in both Exp. I and II (table 2). This is in agreement with the results observed by the other researchers that cellulase addition had improved silage quality by decreasing pH and increasing lactic acid concentration as a result of the hydrolysis of polysaccharides components in the silo at ensiling and provided more WSC to stimulate a good fermentation by lactic acid bacteria, either in grass (Henderson and McDonald, 1977; van Vuuren et al., 1989; Jacobs et al., 1991; Selmer-Olsen et al., 1993) or in barley straw (Ridla and Uchida, 1993) silages. The effect of cellulase addition on the improvement of the fermentation quality of the silages were more markedly occurred at low dry matter silages (Exp. II) rather than at the high ones (Exp. I) since the appropriate water was needed as a transport medium for distribution of enzyme (van Vuuren et al., 1989; Jacobs et al., 1991), and it was similar to our previous results (Ridla and Uchida, 1993; Ridla and Uchida, 1994)

The cell wall components (NDF, ADF, hemicellulose, cellulose) of cellulase treated silages were found lower ($p < 0.05$) than those without cellulase addition silages both

Table 2. Fermentation quality of mixed barley straw-wet brewers' grains-cellulase treated silages (Exp. I and II)

Treatments	pH	Lactic	Eth	C_2	C_3	$\underline{i}$-C_4	$\underline{n}$-C_4
		....	....	(g/kg DM)	....	....	....
				Experiment I			
I-0	4.1	20.5	4.8	14.9	1.3	0.6	0.2
I-2	3.9	24.6	4.8	14.2	1.3	0.5	0.2
I-4	3.9	31.9	4.1	11.1	1.8	0.6	0.2
I-6	3.9	31.2	4.0	9.1	1.2	0.6	0.1
I-8	3.8	31.8	6.4	11.5	1.4	0.6	0.1
Sig.[a)]	*	*	NS	NS	NS	NS	NS
				Experiment II			
II-0	3.91	60.6	9.6	13.4	0.8	0.2	0.1
II-2	3.74	61.5	10.4	9.1	0.6	0.7	0.1
II-4	3.73	62.6	15.3	12.4	0.8	1.0	0.1
II-6	3.61	63.4	11.0	11.0	0.4	0.4	0.1
II-8	3.58	66.6	15.7	11.2	0.4	0.4	0.1
Sig.[a)]	**	NS	*	NS	NS	NS	NS

Abbreviated: Lactic = Lactic acid, Eth = Ethanol, C_2 = Acetic acid, C_3 = Propionic acid, $\underline{i}$-C_4 = iso Butyric acid, $\underline{n}$-C_4 = normal Butyric acid.

[a)] Significant different: NS $p > 0.05$, *$p < 0.05$, **$p < 0.01$.

Table 3. Chemical composition and *in vitro* dry matter digestibility of mixed barley straw-wet brewers' grains-cellulase treated silages (Exp. I and II)

Treatments	DM	CP	Ash	CF	WSC	IVDMD [1]	IVDMD [2]
	g/kg	....	....	(g/kg DM)	....	.... (%)	....
				Experiment I			
I-0	532.6	110.6	45.3	375.4	18.1	44.7	51.4
I-2	532.6	116.1	45.0	340.4	16.2	45.7	51.6
I-4	525.5	121.6	46.8	341.3	19.2	45.5	50.8
I-6	508.5	123.6	48.1	341.3	19.9	45.5	47.8
I-8	537.0	120.0	49.8	329.9	20.4	45.7	50.4
Sig. [a]	NS	NS	NS	**	NS	NS	NS
				Experiment II			
II-0	255.5	151.2	53.8	295.0	19.9	48.1	53.1
II-2	253.2	158.7	55.9	294.7	19.1	51.5	54.1
II-4	248.7	152.7	58.0	287.0	22.6	51.1	54.1
II-6	252.2	158.6	55.8	289.7	23.5	50.3	53.0
II-8	250.6	156.3	57.2	269.8	24.1	49.2	53.1
Sig. [a]	NS	NS	NS	*	NS	NS	NS

Abbreviated: DM = Dry matter, CP = Crude protein, CF = Crude fibre, WSC = Water soluble carbohydrate, IVDMD = *In vitro* dry matter digestibility.
[1] Abe and Horii method (1974).
[2] Tilley and Terry method (1963).
[a] Significant different: NS $p > 0.05$, * $p < 0.05$, ** $p < 0.01$.

in Exp. I and II (table 4). These result indicated that the activity of cellulase addition on reducing the cell wall components of silages was greatly extended. The decreasing of cell wall components of silages as a result of cellulase addition was reported by many researchers (Henderson and McDonald, 1977; van Vuuren et al., 1989 ; Jaakkola, 1990; Jaakkola et al., 1991; Jacobs and McAllan, 1991; Jacobs et al., 1991; Jacobs and McAllan, 1992; Selmer-Olsen et al., 1993; Ridla and Uchida, 1993). Increasing levels of cellulase addition caused on further increase in reduction of cell wall components both in Exp I. and II, and it was in agreement with our previous finding (Ridla and Uchida, 1993).

There were no differences *in vitro* dry matter digestibilities (IVDMD) in all silages due to cellulase addition, either by Abe and Horii (1974) or Tilley and Terry (1963) method, in both Exp. I and II (table 2). This is similar with our previous finding that the reduction of cell wall components of barley straw silages due to cellulase addition was not followed by the improving of silage digestibility (Ridla and Uchida, 1993). Many reports also noticed the same results in grass silages as cellulase addition did not influence the silage digestibility (van Vuuren et al., 1989; Jaakkola, 1990; Jaakkola et al., 1991; Jacobs and McAllan, 1991; Jacobs et al., 1991). According to Jaakkola (1990) and Jacobs and McAllan (1991) it might be caused by the degradation of the most digestible fraction of the structure of polysaccharides due to enzyme, and left a less digestible material. In relating to the wet brewers' grains addition, the IVDMD had improved and increased as the level of wet brewers' grains addition increased (see Ridla and Uchida, 1994).

In conclusion, the present experiment showed that the fermentation quality and nutritive value of barley straw silages were considerably improved by mixing and ensiling with wet brewers' grains. Cellulase added was able to reduce the cell wall components of barley straw silage and improved the potential nutritive value, however, did not enhance the digestibility.

Table 4. Fibre composition of mixed barley straw-wet brewers' grains-cellulase treated silages (Exp. I and II)

Treatments	NDF	ADF	ADL	Si	Hcel	Cell
	(g/kg DM)					
			Experiment I			
I-0	711.8	442.3	97.3	23.0	262.5	345.0
I-2	674.3	422.2	72.5	22.9	252.1	349.7
I-4	654.7	405.7	87.1	18.3	249.0	318.6
I-6	654.7	412.5	94.0	19.5	242.2	318.5
I-8	633.4	404.1	89.5	19.4	229.3	318.5
Sig.[a]	**	*	*	NS	*	*
			Experiment II			
II-0	650.0	383.4	80.2	18.5	266.6	303.2
II-2	609.7	368.3	85.2	18.3	241.4	283.1
II-4	606.7	360.9	86.7	20.1	245.8	274.2
II-6	594.5	354.9	83.5	17.2	239.6	271.4
II-8	582.9	356.9	82.9	19.8	226.0	274.0
Sig.[a]	**	**	*	NS	**	**

Abbreviated: NDF = Neutral detergent fibre, ADF = Acid detergent fibre, ADL = Acid detergent lignin, Si = Silica, Hcel = Hemicellulose, Cell = Cellulose.
Cellulose = ADF - ADL Hemicellulose = NDF - ADF.
[a] Significant different: NS $p > 0.05$, * $p < 0.05$, ** $p < 0.01$.

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