THE EFFECT OF CELLULASE ADDITION ON NUTRITIONAL AND FERMENTATION QUALITY OF BARLEY STRAW SILAGE

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Summary

Three experiments were conducted to investigate the effect of cellulase addition on high (Exp. Ⅰ) and low (Exp. Ⅱ and Ⅲ) dry matter barley straw silages. In Exp. Ⅰ: 1 kg barley straw + 16 g glucose + 600 g water + 0 g as control (E0G), + 2 g (E2G), + 4 g (E4G), + 6 g (E6G), and + 8 g (E8G) of cellulase as treatments were ensiled. In Exp. Ⅱ and Ⅲ, 10 g glucose was and was not added, respectively, into 2 kg barley straw + 0 g (E0W, E0T) as control, + 2 g (E2W, E2T), + 4 g (E4W, E4T), + 6 g (E6W, E6T), and + 8 g (E8W, E8T) of cellulase as treatments. Samples were stored for 10 (Exp. Ⅰ) and 7 (Exp. Ⅱ and Ⅲ) months at 21℃. The effect of cellulase addition on the fermentation and breakdown of the polysaccharides component in the silos at ensiling occured more markedly at low dry matter silages rather than at the high ones. All cellulase treated silages were well preserved (pH below 5 in Exp. Ⅰ and below 4 in Exp. Ⅱ and Ⅲ), while lactic acid and ethanol concentration increased. The fibrous fraction (ADF, NDF, crude fiber, hemicellulose, and cellulose) significantly ($p < 0.01$) decreased (except hemicellulose content in Exp. Ⅰ) compared with corresponding untreated silages. *In vitro* dry matter digestibility values (IVDMD) were similar for all silages. The present study showed that cellulase addition improved the potential nutritional and fermentation quality of barley straw silage.

(Key Words: Barley Straw Silage, Cellulase Addition, Fermentation, Fiber Composition)

Introduction

The nutritional quality of fibrous material can be improved by biological or chemical treatment. Researchers have used cell wall degrading enzymes i.e. cellulase, hemicellulase, and lignin-modifying enzymes (Jaakkola et al., 1991), lignolytic fungi (Amdjed et al., 1990) or ruminal bacteria and fungi (Akin and Benner, 1988) to improve the nutritional quality of fibrous materials by biological degradation.

Cell wall-degrading enzymes as silage additives improved the potential feed quality of fibrous material by decreasing cell wall content, increasing apparent digestibility of cellulose and enhanced the preservation of grass silage by increasing lactic acid concentration (van Vuuren et al., 1989; Jaakkola et al., 1991).

Breakdown in the structure of polysaccharides in the silo at ensiling due to enzyme treatment might have supplied an adequate substrate (water soluble carbohydrate/WSC) for rapid establishment and growth of favourable microorganism, leading to a good fermentation and preservation (Jacobs and McAllan, 1991).

Jacobs et al. (1991) reported that if such enzymes alter the integral structure of the cell wall polysaccharides, then they might also enhance the intake and digestibility of the resultant silages. However, Jaakkola (1990) and Jaakkola et al (1991) found that there were no differences in the voluntary silage DM intake, and no differences were noticed in DM, OM, NDF, and crude protein digestibility between untreated, formic acid, and enzymes-treated silages. But Jaakkola et al. (1991) found that the apparent digestibility of cellulose was higher with enzymes treated silages than formic acid silage.

The present experiment was designed to study the effect of cellulase addition on nutritional value (fibrous content changes) and fermentation quality of barley straw silages.

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Materials and Methods

Silages

Barley straw materials were taken from a farm at Kozima Gun Nadasaki Cho Okayama Prefecture, on June 6, 1990 for experiment Ⅰ, and on June 2, 1991 for experiment Ⅱ and Ⅲ. The barley straw material for Exp. Ⅰ was dried until 81.38% DM content, on the other hand, the material for Exp. Ⅱ and Ⅲ was used freshly (26.24% DM). After being chopped to 1.3 cm length, they were lacerated by chopper-cracker (Taninaka, Co. Ltd.) until nearly smashed. Sample of about 1 kg was ensiled into vinyl bag silos (5 litre capacity), and then the air was excluded by a vacuum pump. The additives used were anhydrous glucose (Ishizu Pharmaceutical Co., Ltd.) and Cellulase Onozuka FA derived from *Tricoderma viridae* (Yakult Honsya Co. Ltd. Tokyo, Japan). In experiment Ⅰ, 1 kg barley straw, 0.6 kg water, and 16 g glucose were mixed thoroughly. Five treatments were applied by adding cellulase at levels of 0 g (E0G) as control, 2 g (E2G), 4 g (E4G), 6 g (E6G), and 8 g (E8G). In Exp. Ⅱ and Ⅲ, 10 g glucose was and was not added, respectively, into 2 kg barley straw. Cellulase was added at levels 0 g (E0W, E0T), 2 g (E2W, E2T), 4 g (E4W, E4T), 6 g (E6W, E6T), and 8 g (E8W, E8T) to the barley straw mix. The silos were placed in a constant chamber temperature of 21℃ for 10 (Exp. Ⅰ) and 7 (Exp. Ⅱ and Ⅲ) months of storage.

At the end of the storage period, the silos were opened and the mouldy parts (1/4 the upper part and 1/4 the bottom part) of each silage were discarded before sampling. The samples were collected and frozen in −32℃ until they were required for further analysis.

Chemical Analysis

Dry matter values (DM) of silages were determined by freeze and vacuum drying method (Uchida, 1986). Dry samples were then ground and kept for the other analysis. Ash, crude protein, and crude fiber were determined according to AOAC (1992). NDF, ADF, lignin, and silica were examined according to Goering and Van Soest (1970) method. *In vitro* dry matter digestibility values were determined by the cellulase (Abe and Horii, 1974) and Tilley and Terry (1963) methods, and water soluble carbohydrate (WSC) values were determined by the technique of Deriaz (1961).

Water soluble solutions were extracted from 40 g of fresh silage in 400 ml distilled water and were prepared for measuring pH, lactic acid (Barker and Summerson, 1941), volatile fatty acids and ethanol (Uchida and Hayashi, 1985).

Results and Discussion

The chemical composition and *in vitro* dry matter digestibility value of barley straw materials is shown in table 1. Fermentation quality, chemical composition, *in vitro* dry matter digestibility, and fiber composition of resulting silages are shown in table 2, 3, and 4, respectively. Fermentation during ensilage was found to be more extensive in low dry matter silages (Exp. Ⅱ and Ⅲ) than in high dry matter silages (Exp. Ⅰ). It was indicated by the decrease of hemicellulose content (table 4), and the increase of lactic acid and ethanol concentrations (table 2) which greatly occured in low DM silages. These results showed that the capability of enzyme to hydrolyse the

TABLE 1. CHEMICAL COMPOSITION AND *IN VITRO* DRY MATTER DIGESTIBILITY VALUE OF BARLEY STRAW MATERIALS

Composition	Experiment Ⅰ	Experiment Ⅱ & Ⅲ
Dry matter (g/kg)	813.8	262.4
Ash (g/kg DM)	44.9	67.2
Crude protein (g/kg DM)	26.4	27.4
Crude fiber (g/kg DM)	479.6	418.9
NDF (g/kg DM)	806.9	751.6
ADF (g/kg DM)	516.2	488.6
Lignin (g/kg DM)	64.1	85.7
Silica (g/kg DM)	14.2	13.9
Cellulose (g/kg DM)	452.1	402.9
Hemicellulose (g/kg DM)	290.7	263.0
WSC (g/kg DM)	45.4	36.8
IVDMD[1] (%)	35.0	42.6
IVDMD[2] (%)	50.5	51.4

Abbreviated: NDF = Neutral detergent fiber, ADF = Acid detergent fiber, WSC = Water soluble carbohydrate, IVDMD = *In vitro* dry matter digestibility.
Cellulose = ADF Lignin
Hemicellulose = NDF ADF.
[1] Cellulase method
[2] Tilley and Terry method.

TABLE 2. FERMENTATION QUALITY OF BARLEY STRAW SILAGES

Treatment	pH	Lactic	Eth	C2	C3	*i*-C4	*n*-C4	*i*-C5	*n*-C5
					(g/kg DM)				
					Experiment Ⅰ				
E0G	5.0	14.1	1.4	1.4	2.5	2.4	3.1	0.0	0.0
E2G	4.6	17.8	1.1	1.1	2.1	3.4	2.4	0.0	0.0
E4G	4.8	13.3	2.5	0.3	2.9	4.9	3.0	0.0	0.0
E6G	4.8	20.4	3.2	0.9	2.7	4.2	3.1	0.0	0.0
E8G	4.7	20.6	3.1	0.9	1.4	2.4	0.7	0.0	0.0
Sig.[a]	**	*	NS	*	NS	NS	NS	–	–
					Experiment Ⅱ				
E0W	4.0	36.0	3.9	2.4	0.7	0.2	0.0	0.0	0.0
E2W	3.8	52.6	4.6	8.2	1.3	0.9	0.0	0.0	0.0
E4W	3.8	54.1	5.9	4.2	0.7	0.6	0.0	0.0	0.0
E6W	3.7	57.1	8.8	5.1	0.8	0.7	0.0	0.0	0.0
E8W	3.7	64.3	8.7	4.3	0.7	0.5	0.0	0.0	0.0
Sig.[a]	**	**	*	*	NS	*	–	-	
					Experiment Ⅲ				
E0T	4.4	34.5	1.7	6.2	0.8	0.2	0.4	0.8	0.3
E2T	3.9	52.1	2.4	4.7	0.7	0.3	0.1	0.1	0.1
E4T	3.8	57.6	6.9	6.9	1.0	0.4	0.1	0.1	0.1
E6T	3.7	62.3	8.7	5.9	0.9	0.6	0.1	0.1	0.1
E8T	3.7	65.6	6.6	4.9	0.8	0.5	0.1	0.1	0.1
Sig.[a]	*	**	*	NS	NS	*	*	*	*

Abrevated: Lactic = Lactic acid, Eth = Ethanol, C2 = Acetic acid, C3 = Propionic acid, *i*-C4 = iso Butyric acid, *n*-C4 = normal Butyric acid, *i*-C5 = iso Valeric acid, *n*-C5 = normal Valeric acid.

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

structure of polysaccharides in high dry matter silages decreased, as the water, which is used as transport medium for distribution of enzyme decreased (van Vuuren et al., 1989; Jacobs et al., 1991). Less efficient fermentation in high dry matter silages has previously been reported by Henderson and McDonald (1977), van Vuuren et al. (1989), Jacobs et al. (1991), and Tangerdy et al. (1991).

The addition of glucose (10 g glucose/2 kg fresh materials) did not affect the fermentation characteristic of the low DM silages (Exp. Ⅱ and Exp. Ⅲ). But the control without glucose-silage (E0T) contained a higher concentration of butyric acid and valeric acid compared with the control using glucose silage (E0W). It seemed that without glucose addition a good fermentation could be achieved by celltulase.

Cellulase addition significantly ($p < 0.01$) decreased component of fibrous fraction (ADF, NDF, crude fiber, cellulose, hemicellulose) compared with corresponding control untreated silages, in both high and low DM silages. This is in agreement with the result of Henderson and McDonald (1977), van Vuuren et al., (1989), Jaakkola et al., (1991), Jacobs and McAllan (1991), and Jacobs et al. (1991). Hemicellulose content in the low DM silages decreased by 12.6 -38.6 (Exp. Ⅱ) and 26.2-40.4 (Exp. Ⅲ) g/kg DM. This is in contrast to the results of Jacobs and McAllan (1991). In high DM silages the hemicellulose contents were found to be similar.

All cellulase-treated silages appeared to be well preserved as pH was below 5 and 4 in high and low DM silages, respectively. Lactic acid and ethanol concentration increased with the addition of cellulase, however, butyric acid concentration was greater in high DM silages. These results imply that the addition of cellulase more extensively brokendown the structure polysaccharides

and hydrolysed the hemicellulose and cellulose to be glucose in the silos at ensiling, then provided an adequate substrate (water soluble carbohydrate/WSC) for enhancing the rapid establishment of a good fermentation and preservation (van Vuuren et al., 1989; Jacobs et al., 1991).

The increasing levels of added cellulase was followed by the decrease of fibrous component. Henderson and McDonald (1977) and Jaakkola (1990) also found that fibrous component decreased with increasing levels of added cellulase. The results showing that the residual WSC increased in all cellulase-treated silages compared with the corresponding control silages, is in agreement with Jaakkola's (1990). Residual WSC increased with the levels of cellulase added from 0.2-4.21, 2.7-8.7, and 8.3-11.2 g/kg DM for experiment Ⅰ, Ⅱ, Ⅲ, respectively. Crude protein content also tended to increase with increasing levels of added cellulase.

In vitro dry matter digestibility values were similar in all silages, irrespective of the method of analysis (cellulase or Tilley and Terry method). This result is in agreement with that reported by van Vuuren et al. (1989), Jaakkola (1990), Jaakkola et al. (1991), Jacobs and McAllan (1991), and Jacobs et al. (1991) who found that a reduction of fibrous components did not produce a corresponding increase in *in vitro* silage digestibility. Added cellulase may have degraded the most digestible fraction of the structural polysaccharides and left less digestible materials (Jaakkola, 1990; Jacobs and McAllan, 1991). This was also indicated by the increase of silica ($p < 0.05$) and the trend in ash content.

TABLE 3. CHEMICAL COMPOSITION AND *IN VITRO* DRY MATTER DIGESTIBILITY VALUE OF BARLEY STRAW SILAGES

Treatment	DM	CP	Ash	CFi	WSC	IVDMD[1]	IVDMD[2]
	(g/kg)	..... (g/kg DM)				..... (%)	
				Experiment Ⅰ			
E0G	588.7	17.7	48.9	481.6	13.9	34.2	44.9
E2G	624.2	18.4	49.5	465.1	17.6	33.1	45.0
E4G	630.6	17.7	53.5	456.2	19.1	33.9	43.2
E6G	589.5	18.8	55.2	452.5	18.0	32.9	42.4
E8G	588.4	19.1	58.3	448.8	13.7	32.4	42.4
Sig.[a]	NS	NS	*	**	*	NS	NS
				Experiment Ⅱ			
E0W	277.9	28.4	71.5	444.2	21.4	38.5	48.2
E2W	272.1	28.9	72.5	411.1	24.1	39.0	48.5
E4W	273.5	28.7	75.1	401.2	24.1	37.8	50.4
E6W	276.9	29.2	73.5	374.6	29.9	38.8	50.2
E8W	276.9	37.9	73.5	377.7	30.1	38.4	49.3
Sig.[a]	NS	NS	NS	**	*	NS	NS
				Experiment Ⅲ			
E0T	266.7	24.6	72.8	461.7	17.6	38.6	48.4
E2T	268.6	27.7	73.7	421.2	26.5	38.3	48.1
E4T	270.6	29.3	77.1	421.9	25.9	38.2	46.4
E6T	274.6	29.5	74.3	408.4	28.8	39.1	47.4
E8T	275.7	30.0	74.6	390.3	28.8	40.1	50.5
Sig.[a]	NS	NS	NS	*	**	NS	NS

Abbreviated: DM = Dry matter, CP = Crude protein, CFi = Crude fiber, WSC = Water soluble carbohydrate, IVDMD = *In vitro* dry matter digestibility.

[1] Cellulase method.

[2] Tilley and Terry method.

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

TABLE 4. FIBER COMPOSITION OF BARLEY STRAW SILAGES

Treatment	NDF	ADF	Lignin	Si	Cell	HCell
	(g/kg DM)					
			Experiment I			
E0G	818.7	551.5	88.4	17.9	463.1	262.7
E2G	788.7	520.4	94.2	28.6	426.2	268.3
E4G	774.2	510.2	97.4	27.2	412.8	264.0
E6G	778.4	512.2	98.4	24.5	413.8	266.2
E8G	765.7	525.7	96.9	31.2	425.8	240.0
Sig.[a]	**	**	NS	**	**	NS
			Experiment II			
E0W	770.0	521.4	101.7	17.2	419.7	248.6
E2W	733.4	497.4	103.4	27.5	394.0	236.0
E4W	721.8	488.1	105.9	27.4	382.2	233.7
E6W	708.3	478.2	100.5	23.8	377.7	230.1
E8W	689.4	479.4	108.9	19.6	370.5	210.0
Sig.[a]	**	*	NS	NS	**	*
			Experiment III			
E0T	779.8	521.5	98.5	19.9	423.0	254.8
E2T	728.6	500.0	99.2	22.4	400.8	228.6
E4T	709.7	495.3	101.4	23.9	393.9	214.4
E6T	696.1	478.1	95.7	21.8	382.4	218.0
E8T	698.5	475.0	101.9	23.9	373.1	223.5
Sig.[a]	**	*	NS	NS	**	**

Abbreviated NDF = Neutral detergent fiber, ADF = Acid detergent fiber, Si = Silica, Cell = Cellulose, Hcell = Hemicellulose.
Cellulose = ADF − lignin, Hemicellulose = NDF − ADF
[a] Significant difference: NS $p > 0.05$, * $p < 0.05$, ** $p < 0.01$.

It can be concluded that the addition of cellulase in barley straw silage at ensiling improved the potential nutritional quality by reducing the fibrous component and enhancing followed fermentations by increasing its lactic acid and ethanol concentration. The solubility of the fibrous materials increased with the level of added cellulase. Ensiling high dry matter barley straw restricted the establishment of a good fermentation.

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