環境温度,水分含量およびセルラーゼの添加がハダカムギ麦稈サイレージの発酵品質と化学成分に及ぼす影響

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Effects of Temperature, Moisture and Cellulases on the Fermentation Quality and Chemical Composition of Naked Barley (*Hordeum vulgare* L. emand Lam) Straw Silage

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Synopsis

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Effects of temperature levels, moisture contents and cellulases on the fermentation quality and chemical composition of naked barley straw silage were investigated in two laboratory-scale experiments. In experiment 1, naked barley straw added with Acremonium cellulase (AC) or Meicelase (MC) was ensiled and stored in incubators at 10, 20, 30, 40 and 50℃, respectively. In experiment 2, naked barley straw with moisture contents of approximately 50, 60, 70 and 75% adjusted with distilled water, was added with or without the above cellulases, ensiled and kept in the laboratory at room temperature.

All silages below 40℃ had a more extensive fermentation than that at 50℃, and the highest butyric acid contents occurred at 30℃, irrespective of types of cellulase additives. The silages added with cellulase were well-preserved with pH values of 4.3 or less and high lactic acid content compared with the control. Moreover, AC additive was more effective than that of MC throughout temperature condition.

Moisture contents had marked effect on the fermentation quality of silages. There were lower pH and higher lactic acid content in silages with a moisture content of 60%. The silages with moisture contents of 50% and 75% were of high pH values and the latter produced high butyric acid and VBN contents. Cellulase additives obviously improved the silage fermentation quality, particularly for the silages with the moisture content from 60% to 70%. Contrasting two cellulases, AC was superior to MC under any moisture condition.

Cellulase as an additive partially degraded the structural carbohydrates of silages and decreased the contents of NDF, ADF and cellulose in comparison with the control silage, although it was not significant in both experiments.

Key words : Cellulase, Fermentation quality, Moisture, Naked barley straw silage,Temperature.

Introduction

It is well known that silage is the material produced by the controlled fermentation of a crop[12]. Although various strategies[1-3,5,7,8] have been adopted to manipulate the silage fermentation in order that the silage is well preserved, the temperature, moisture content and the content of water-soluble carbohydrates (WSC), as the environmental conditions and a major nutritive source for microflora survival, are of considerable significance.

During ensilage, differences in temperature and/or moisture contents could affect the development of different species of microorganisms[21]. A number of workers have concluded that the lactate fermentation is stimulated by lower temperatures[3,15,16] and lower moisture contents ; on the contrary, it is depressed by high temperatures and high moisture contents[5,11,14,20]. At the same time several different results had also been reported[11,21].

As for increasing WSC contents, many attempts have been practiced to be efficient. In recent years, cellulases, as a safe additive for silage, have received considerable attention. Cellulase enzymes capable of breaking down structural polysaccharides may enhance preservation during ensilage by increasing levels of lactic acid due to the increase of WSC[1,9,18,23]. It has also been suggested that if such enzymes alter the integral structure of the cell wall polysaccharides then they might also enhance the digestibility of the resulting silage[4]. Therefore, the additive of cellulases will be of great importance to cereal straw

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with low WSC and high structural polysaccharides. However, its benefit will probably be influenced by environmental temperature and moisture content of materials as the silage fermentation is done.

There have been many findings on the factors controlling fermentation quality on grass silages but a few for straw silage have been reported. The aim of the present study was to examine the effects of temperature and moisture content on the silage fermentation of naked barley straw added cellulases.

Materials and Methods

Additives

Additives used in the experiments were lactic acid bacterial (LAB) inoculant containing *Lactobacillus casei*, a commercial silage additive produced by Snow Brand Seed Co., Ltd. (Sapporo, Japan) and two cellulases obtained from Meiji Seika Kaisha Ltd (Tokyo, Japan). One of cellulases was Meicelase (MC) which is derived from *Trichoderma viride* and is a world-famous one as a silage additive. The other was Acremonium cellulase (AC) which is derived from a selected strain of *Acremonium cellulolyticus*[24] and is a new enzyme preparation. The enzymic activity of AC and MC, provided by the manufacturer, are as follows : CMCase, AC 27,000 U/g, MC 9,800 U/g ; avicelase, AC 784 U/g, MC 721 U/g ; β-glucosidase, AC 780 U/g, MC 761 U/g and xylanase, AC 13,000 U/g , MC 10,800 U/g. Hence, AC had a greater activity than MC.

Straw preparation and silage making

The naked barley (*Hordeum vulgare* L. emand Lam) was grown on the experimental field of the Agricultural College of Ehime University. The crop was harvested with a combine harvester on May 25, 1995, and its straw was collected and chopped to approximately 1cm by a forage chopper and immediately transported to the laboratory. After thorough mixing, samples were taken for analysis of chemical composition and LAB counts. Batches of well mixed straw were spread over polyethylene sheets, and the additives diluted with distilled water were applied using a hand sprayer. Since combined application of LAB with cellulases can further improve the fermentation of this straw silage due to their synergism[25], and the number of LAB detected on the straw was extremely small, therefore, a LAB inoculant was added to all treatments.

Experiment 1 was designed to examine effects of temperature and cellulase additives on the fermentation and structural carbohydrates of silages. A two-way layout design with five incubation temperature levels (10, 20, 30, 40 and 50℃) and three types of the cellulase addition (Control without cellulase, AC and MC) was used. To enhance enzymic effects, the moisture content of the straw was adjusted to approximately 65% with distilled water containing LAB inoculant alone or together with AC or MC. The LAB and cellulases were applied at levels of 1×10^5 cfu(colony forming unit)$\cdot g^{-1}$ fresh material and 0.02% of the weight of the straw material, respectively. A certain amount (100 g fresh matter, FM) of treated straw then were packed into plastic film bag[19] (Asahi Kasei, Hilyu KN type, 18×26 cm, Tokyo, Japan) in triplicate, and the film bag was sealed with vacuum sealer (Matushita, BH 950, Osaka, Japan). Thereafter, they were kept in incubator according to the desired temperatures. The silos were opened after ensiling for 45 days and the fermentation quality and chemical composition of the silages were determined.

Experiment 2 was designed to examine effects of moisture contents and cellulase additives on the fermentation quality and structural carbohydrates of silages. The following treatments were applied to the straw : four moisture levels (approximately 50, 60, 70 and 75%) and three types of the cellulase additive (Control, AC and MC). In this experiment, the application levels of the inoculant and cellulases on the basis of dry matter (DM) were 2×10^5 cfu$\cdot g^{-1}$ and 0.04%, respectively. The materials were identically packed into the bags as in experiment 1 and kept at room temperature (13.3–29.4℃). The

Table 1. Characteristics of the naked barley straw before ensiling.

Measurement	Straw
Dry matter (%)	37.6
Organic matter (% DM)	92.0
Crude protein (% DM)	2.3
Crude fat (% DM)	1.5
Crude fiber (% DM)	42.0
NFE (% DM)	46.2
Crude ash (% DM)	8.0
NDF (% DM)	78.7
ADF (% DM)	46.5
ADL (% DM)	7.6
Cellulose (% DM)	38.9
Hemicellulose (% DM)	32.2
WSC (% DM)	2.7
Lactic acid bacteria (cfu$\cdot g^{-1}$ fresh matter)	$<1.0\times10^2$
Lactic buffering capacity (lactic acid mg$\cdot g^{-1}$ DM)	25.2

NFE : Nitrogen free extract.
NDF : Neutral detergent fiber.
ADF : Acid detergent fiber.
ADL : Acid detergent lignin.
WSC : Water-soluble carbohydrates.
cfu : Colony forming unit.

silos were opened after ensiling for 45 days and the fermentation quality and chemical composition of the silages were determined.

Analytical methods

LAB of the straw was measured with GYP-$CaCO_3$ agar[6]. The lactic buffering capacity of the straw was determined as described by McDONALD *et al.*[10].

When the silos were opened, each treatment was mixed thoroughly, and 25 g of sample was taken from each silo and added with 100 g of distilled water and stored in the refrigerator at 5℃ for 18 h. Then, the material was filtered using a filter paper (Toyo No. 5 A) and the filtrate was used for pH, volatile basic nitrogen (VBN) and organic acid determinations. DM content of the silages was determined by oven drying at 80℃ for 48 h. Crude fat, crude fiber, crude ash, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to AOAC[22] procedures. Hemicellulose was calculated as the difference between NDF and ADF while cellulose as the difference between ADF and ADL. Total nitrogen (TN) was determined using the high sensation N.C. analyzer (Sumigraph Model NC-80, Sumika Chemical Analysis Service Ltd., Osaka, Japan). VBN was measured by the micro-diffusion method[13]. The pH of fresh silages was measured with a glass electrode meter. The content of WSC was estimated colorimetrically using anthrone[13]. The organic acid contents were measured by a high-performance liquid chromatography[17].

Statistical analysis was performed using the analysis of variance and TUKEY's multiple range test was used to test differences between treatment means.

Results

Straw material characteristics

From the characteristics of the initial barley straw materials in experiments 1 and 2 given in Table 1, it was apparent that the straw contained very low WSC (2.7% DM), low population of lactic acid bacteria (under 10^2 cfu·g^{-1} FM) and high contents of NDF

Table 2. Effects of temperature and cellulases on the fermentation quality of silages in experiment 1.

Treatment	pH	Organic acids (% DM)						Lactic/ Acetic ratio	VBN (% TN)	FLIEG'S score[1)]
		Acetic	Butyric	Lactic	Propionic	Valeric	Total			
10℃										
Control	4.40^{CD}	0.60^{ABC}	0.00^{C}	1.77^{EFG}	0.00	0.00	2.37^{FG}	3.0^{EF}	4.6^{C}	88^{ABC}
AC	3.84^{GH}	0.49^{ABC}	0.00^{C}	4.17^{B}	0.00	0.00	4.66^{BC}	9.6^{BC}	4.5^{C}	100^{A}
MC	4.10^{E}	0.63^{ABC}	0.00^{C}	2.91^{D}	0.00	0.00	3.54^{DE}	4.6^{DE}	4.3^{C}	95^{AB}
20℃										
Control	4.39^{CD}	0.69^{AB}	0.23^{C}	2.00^{EF}	0.00	0.00	2.92^{EF}	2.9^{EF}	5.4^{BC}	68^{CDE}
AC	3.71^{H}	0.49^{ABC}	0.00^{C}	5.37^{A}	0.00	0.00	5.86^{A}	11.1^{A}	5.2^{BC}	100^{A}
MC	3.91^{FG}	0.66^{ABC}	0.17^{C}	3.71^{BC}	0.00	0.00	5.54^{A}	5.7^{D}	5.4^{BC}	80^{ABCD}
30℃										
Control	4.61^{B}	0.77^{A}	0.46^{BC}	1.03^{G}	0.02	0.01	2.29^{FG}	1.3^{F}	6.3^{AB}	40^{F}
AC	3.82^{GH}	0.34^{C}	0.94^{AB}	4.23^{B}	0.00	0.02	5.53^{AB}	12.3^{A}	6.7^{A}	70^{BCDE}
MC	4.26^{DE}	0.46^{ABC}	1.23^{A}	2.11^{E}	0.00	0.00	3.80^{CDE}	4.6^{DE}	6.4^{AB}	50^{EF}
40℃										
Control	4.57^{BC}	0.54^{ABC}	0.09^{C}	1.26^{FG}	0.04	0.00	1.93^{FGH}	2.3^{F}	5.6^{ABC}	70^{BCDE}
AC	3.88^{GH}	0.63^{ABC}	0.20^{C}	5.31^{A}	0.00	0.00	6.14^{A}	8.5^{C}	5.2^{BC}	85^{ABCD}
MC	4.07^{EF}	0.57^{ABC}	0.26^{C}	3.31^{CD}	0.00	0.00	4.14^{CD}	5.8^{D}	5.5^{ABC}	80^{ABCD}
50℃										
Control	4.84^{A}	0.40^{BC}	0.20^{C}	0.63^{G}	0.00	0.00	1.23^{H}	1.6^{F}	6.2^{AB}	45^{EF}
AC	4.58^{BC}	0.49^{ABC}	0.09^{C}	0.91^{G}	0.00	0.00	1.49^{GH}	1.9^{F}	5.9^{AB}	60^{DEF}
MC	4.65^{B}	0.40^{BC}	0.14^{C}	0.86^{G}	0.00	0.00	1.40^{GH}	2.1^{F}	5.9^{AB}	65^{DEF}
Analysis of variance										
Cellulases	**	NS	**	**	NS	NS	**	**	NS	**
Temperature	**	**	**	**	NS	NS	**	**	**	**
Interaction	**	**	**	**	NS	NS	**	**	NS	**

1) Calculated according to the FLIEG'S evaluation test which is based upon the relative ratio of lactic, acetic and butyric acids.

AC : Acremonium cellulase, MC : Meicelase, VBN : Volatile basic nirogen, TN : Total nitrogen.

NS : Not significant, ** and columns with different superscripts are significant at P<0.01.

(78.7% DM) and ADF (46.5% DM). It was also of very low crude protein content (2.3% DM) and of low lactic buffering capacity (25.2 mg lactic acid g^{-1} DM).

Experiment 1

The fermentation quality and chemical composition of silages were shown in Tables 2 and 3. The temperature, cellulase and their interaction had significant ($P<0.01$) effects on the pH values, the contents of butyric, lactic and total organic acids, and the ratio of lactic to acetic acid and FLIEG's score. Temperature significantly ($P<0.01$) affected acetic acid and VBN contents, but cellulase additives did not so. All silages incubated below 40℃ were of a more extensive fermentation compared to that at 50℃. The enzyme-treated silages had higher concentrations of lactic acid and residual WSC, higher ratios of lactic to acetic acid and higher FLIEG's score than the controls. Moreover, AC was superior to MC in most parameters under all temperatures. As the temperature was increased from 20℃ to 50℃ except 30℃ where all silages showed peculiar results due to the high butyric acid contents, the silages showed a tendency of higher pH values and lower lactic acid contents. In addition, the lactate fermentation at 10℃ was restricted slightly, but it was not so much as that at 50℃. The cellulase additives did not reduce the butyric acid contents in the silages kept at 30 and 40℃. Cellulase application reduced NDF, ADF and cellulose concentrations (Tables 3), but it was not significant under all temperatures.

Experiment 2

The fermentation quality and chemical composition of silages are presented in Tables 4 and 5. Differences in moisture content and in cellulase additives had marked effects on the fermentation quality of silages (Table 4). Higher pH values were observed in the silages with highest (75%) and lowest (50%) moisture contents, although their pH values in cellulase-treated silages were slightly lower than the control silages. It also showed that the cellulases had more striking effects on pH values, the contents of lactic acid and total acids at the moisture content of 60% and 70%. The butyric acid content and VBN content of silages tended to be increased as the moisture content increased, both with and without cellulase additives. Two cellulases significantly (P

Table 3. Effects of temperature and cellulases on the chemical composition of silages in experiment 1 (% DM).

Treatment	Organic matter	Crude protein	Crude fat	Crude fiber	NFE	Crude ash	NDF	ADF	ADL	Cellulose	Hemi-cellulose	WSC
10℃												
Control	91.8	1.8	1.7	42.4	45.9	8.2	80.2	49.6	8.3	41.3	30.6	1.2^{GH}
AC	91.8	1.9	1.7	40.7	47.6	8.2	78.0	48.1	8.1	40.0	29.9	2.9^{B}
MC	92.0	1.8	1.7	42.4	46.1	8.0	78.5	48.3	8.4	39.9	30.2	2.2^{D}
20℃												
Control	91.8	1.8	1.7	42.2	46.1	8.2	80.6	49.8	8.2	41.6	30.8	1.2^{GH}
AC	91.6	1.8	2.0	39.6	48.2	8.4	77.0	46.6	8.1	38.5	30.4	2.1^{DE}
MC	91.3	1.9	1.8	42.3	45.3	8.7	79.3	48.7	8.3	40.4	30.6	1.8^{DEF}
30℃												
Control	91.1	1.8	1.9	42.9	44.5	8.9	80.5	49.6	8.2	41.4	30.9	0.9^{H}
AC	91.4	1.9	2.1	40.4	47.0	8.6	77.1	47.5	8.0	39.5	29.6	2.3^{CD}
MC	91.2	1.7	1.8	41.7	46.0	8.8	78.2	48.5	8.4	40.1	29.7	1.7^{EFGH}
40℃												
Control	91.9	1.8	1.8	42.2	46.3	8.1	81.0	50.0	8.3	41.7	31.0	1.5^{FGH}
AC	91.6	1.7	1.9	40.7	47.3	8.4	77.1	47.4	8.0	39.4	29.7	2.8^{BC}
MC	91.7	1.8	1.9	41.2	46.8	8.3	78.9	48.8	8.2	40.6	30.1	2.0^{DEF}
50℃												
Control	91.8	1.7	1.9	42.7	45.5	8.2	80.9	49.8	8.5	41.3	31.1	1.6^{EFGH}
AC	91.7	1.8	1.7	40.0	48.2	8.3	77.5	47.4	8.3	39.1	30.1	4.2^{A}
MC	92.0	1.8	1.8	41.4	47.2	8.0	78.8	48.2	8.0	40.2	30.6	3.0^{B}
Analysis of variance												
Cellulases	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**
Temperature	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**
Interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**

AC : Acremonium cellulase, MC : Meicelase. NFE, NDF, ADF, ADL and WSC : See Table 1.
NS : Not significant, ** and columns with different superscripts are significant at $P<0.01$.

Table 4. Effects of moisture contents and cellulases on the fermentation qualiy of silage in experjment 2.

Treatment	pH	Organic acids (% DM)						Lactic/ Acetic ratio	VBN (% TN)	FLIEG'S score[1)]
		Acetic	Butyric	Lactic	Propionic	Valeric	Total			
Moisture content of 50%										
Control	4.39^AB	0.54^B	0.02^D	1.40^E	0.00	0.00	1.96^F	2.6^C	4.0^C	83^AB
AC	4.16^BCD	0.38^B	0.02^D	2.02^CD	0.00	0.00	2.42^EF	5.3^AB	4.0^C	90^A
MC	4.35^AB	0.40^B	0.02^D	1.88^CD	0.00	0.00	2.30^EF	3.2^BC	4.0^C	90^A
Moisture content of 60%										
Control	4.30^ABC	0.65^B	0.15^CD	1.95^CD	0.00	0.10	2.85^DEF	3.0^BC	4.8^BC	73^ABC
AC	3.88^DE	0.60^B	0.10^CD	4.15^AB	0.03	0.08	4.96^B	5.9^A	4.5^BC	90^A
MC	4.01^CDE	0.50^B	0.13^CD	2.95^BC	0.00	0.08	3.65^CD	5.9^A	4.7^BC	85^AB
Moisture content of 70%										
Control	4.36^AB	0.67^B	0.67^BCD	1.67^CD	0.03	0.13	3.17^DE	2.5^CD	5.0^BC	45^CD
AC	3.77^E	0.67^B	0.37^CD	5.00^A	0.03	0.03	6.10^A	7.5^A	5.0^BC	80^AB
MC	4.12^BCD	0.50^B	0.93^BC	3.07^BC	0.00	0.07	4.57^BC	5.1^AB	5.7^B	58^BC
Moisture content of 75%										
Control	4.44^A	1.28^A	1.40^AB	0.16^E	0.08	0.04	2.96^DE	0.1^D	7.1^A	5^E
AC	4.25^ABC	0.60^B	2.04^A	0.96^DE	0.04	0.04	3.68^CD	1.6^CD	7.5^A	20^DE
MC	4.40^AB	0.64^B	2.08^A	0.72^DE	0.04	0.04	3.52^C	1.1^CD	7.3^A	15^DE
Analysis of variance										
Cellulases	**	**	NS	**	NS	NS	**	**	NS	**
Moisture	**	**	**	**	NS	NS	**	**	**	**
Interaction	**	**	NS	**	NS	NS	**	NS	NS	NS

1) Calculated according to the FLIEG'S evaluation test which is based upon the relative ratio of lactic, acetic and butyric acids.
AC : Acremonium cellulase, MC : Meicelase, VBN : Volatile basic nirogen, TN : Total nitrogen.
NS : Not significant, ** and columns with different superscripts are significant at $P<0.01$.

<0.01) reduced the acetic acid content of silage at the moisture content of 75%. Cellulase additives reduced NDF, ADF and cellulose concentrations (Tables 5), but it was not significant under all moisture contents. The contents of residual WSC did not differ between silages except the silage at moisture content of 50% where AC significantly increased the residual WSC content compared with the control and MC.

Comparing AC with MC, AC had more advantage than MC at any moisture content.

Discussion

Effects of temperature, cellulases and their interaction

A number of workers have reported that low temperature could enhance the lactate fermentation and depress butyrate fermentation[3,15,16], on the contrary, high temperature could depress the lactate fermentation but stimulate the butyrate fermentation[5,11,14,20]. Nevertheless, as mentioned above, the silages incubated at 30℃ produced high butyric acid contents and lower lactic acid contents. This agrees with WIERINGA's results[21] that among the temperatures ranging from 20℃ to 60℃ the silage at the temperature of 35℃ produced butyric acid content the most. This also agrees with the findings by OHYAMA *et al.*[16] to some extent that the fermentation quality was better at 15℃ than that at 30℃. This may be related to the eco-physiological properties of the microflora. LANIGAN[8] and OHYAMA *et al.*[16] reported that during the ensiling period the ambient temperature of about 30℃ was not favorable for lactic acid bacteria maintaining their vigor compared with lower temperatures. For the present experiment, it was however hardly said whether the result was attributed to a similar reason.

Although cellulase additives had significantly improved the fermentation quality of silages as indicated by lower pH, higher lactic acid content and higher FLIEG's score, it could not prevent the production of high butyric acid content at 30℃. According to LANIGAN[8] and OHYAMA *et al.*[16], molasses or glucose addition could overcome the deteriorating effect at high temperature, and lactic acid contents in silages were consistent irrespective of temperature when soluble carbohydrates were applied to an adequate degree. However, in the present experiment, the increase of WSC associated with cellulase additives could not diminish the deteriorating effect at 30℃.

Table 5. Effects of moisture and cellulases on the chemical composition of silages in experiment 2 (% DM).

Treatment	Organic matter	Crude protein	Crude fat	Crude fiber	NFE	Crude ash	NDF	ADF	ADL	Cellulose	Hemi-cellulose	WSC
Moisture content of 50%												
Control	91.4	1.9	2.1	42.7	44.7	8.6	80.0	50.2	8.0	42.2	29.8	1.4^{CD}
AC	91.4	1.6	2.2	42.0	45.6	8.6	78.2	49.1	8.2	40.9	29.1	2.2^{A}
MC	91.6	1.8	1.9	42.0	45.9	8.4	78.9	49.6	7.9	41.7	29.3	1.3^{D}
Moisture content of 60%												
Control	91.7	1.8	2.0	42.5	45.4	8.3	79.6	50.5	8.1	42.4	29.1	1.6^{BCD}
AC	91.6	1.8	1.9	41.7	46.2	8.4	78.2	49.1	7.8	41.3	29.1	1.9^{ABC}
MC	91.8	1.9	1.9	42.3	45.7	8.2	78.8	49.6	8.0	41.6	29.2	1.4^{CD}
Moisture content of 70%												
Control	91.7	1.6	1.8	43.1	45.2	8.3	80.0	50.3	8.3	42.0	29.7	1.2^{D}
AC	91.7	1.7	1.8	41.8	46.4	8.3	78.3	49.3	8.0	41.3	29.0	1.5^{CD}
MC	91.7	1.7	1.8	42.0	46.2	8.3	79.0	49.6	7.9	41.7	29.4	1.7^{BCD}
Moisture content of 75%												
Control	91.9	1.7	2.0	43.0	45.2	8.1	80.5	50.8	8.2	42.6	29.7	1.6^{BCD}
AC	91.5	1.7	1.9	42.4	45.5	8.5	78.2	49.1	8.1	41.0	29.1	2.1^{AB}
MC	91.4	1.6	1.7	42.2	45.9	8.6	78.8	49.2	7.9	41.3	29.6	1.3^{D}
Analysis of variance												
Cellulases	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**
Moisture	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**

AC, MC, NFE, NDF, ADF, ADL and WSC : See Table 3.
NS : Not significant, ** and columns with different superscripts are significant at $P<0.01$.

This difference may be caused by insufficient cellulase application or difference between cellulase and sugar additives. It would take much time to produce sugars by cellulase addition, unlike glucose additive which could be used immediately after applying. At about 30℃ cellulase additives markedly increased the butyric acid content in the silages. This result may be attributed to different fermentation conditions. The critical pH value or WSC content, below or above which butyrate fermentation is inhibited, varies directly depending on temperature, moisture and so forth. Ensiling in extremely disadvantageous conditions for lactate fermentation, such as undesirable temperature and high moisture contents, seems to encourage a butyrate fermentation, unless soluble carbohydrate levels are exceptionally high. WIERINGA[21] reported that sugar additive could stimulate both lactate and butyrate fermentation if total sugars are below the essential amount, since WSC is a main nutrition for clostridia as well as lactic acid bacteria. When WSC in material exceeds the critical content, the butyrate fermentation will be limited. This may be because of reduced pH value of silage and reduced water activity, which was associated with high density of sugars. The lactate fermentation of all silages at 50℃ became very weak as demonstrated by high pH and low lactic acid content. It may be explained that high temperature is less fit for lactic acid bacteria, rather than low sugar concentrations, as indicated by high residual WSC content in Table 3.

Two cellulases used in this study could not significantly reduce the concentrations of NDF, ADF, cellulose and hemicellulose. Based on the results of previous report[25], it is considered that the rate of cellulases applied in present experiment is enough to improve silage fermentation quality but it is insufficient for degrading the structural polysaccharides of the straw with a solid cell wall.

From the results of Tables 2 and 3, AC was more effective than MC as silage additive, in good agreement with its greater cellulolytic enzyme activity. On the whole, temperature had greater effect on the silage fermentation than on the activity of cellulases and under the condition of 20℃ the fermentation quality was the best for the control and cellulase-treated silages.

Effects of moisture contents and cellulases and their interaction

Moisture content had marked effect on the silage fermentation quality. Lactic acid content was low under moisture contents of 50% and 75%, which was consistent with general findings previously reported[5,11,14,20]. The silage fermentation quality was improved by cellulase additive, particularly under moisture contents of 60% and 70%. Results indicated that a low moisture content of 50% restricted lactate fermentation, while high moisture content of 75% encouraged butyrate fermentation. Both cellulase additives tended to increase the butyric acid content as the moisture content of silages was 75%, while below this level all cellulase-treated silages except for the MC-treated silage at the moisture content of 70% were of lower butyric acid content than the control silages, although it was not significant. The VBN contents in silages were not significantly affected by cellulase additives. This implies that for the naked barley straw the butyrate fermentation became stronger as the moisture content increased in the range employed in this study, and when it exceeded 75% both cellulases at present rate could not restrict the butyrate fermentation which was similar to the control.

The contents of NDF, ADF and cellulose in silages treated with cellulases had no differences under different moisture conditions. This is likely since low moisture content restricted the enzymic activity, while higher moisture content reduced the cellulase concentration.

In this experiment, AC was also better than MC as silage additives throughout various moisture conditions. Differences in moisture contents had stronger effect on the silage fermentation than on the activity of cellulases, and at moisture content of about 60% the fermentation quality was excellent for the control and cellulase-treated silages.

It is suggested in this paper that the addition of cellulase at ensilage, which degrades the structural polysaccrides to a certain extent, can increase the extent of fermentation of naked barley straw silages and make them well-preserved under adequate temperature (below 30℃) and medium moisture (about 60%) conditions.

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References

1) BAYORBOR, T.B., S. KUMAI, R. FUKUMI and I. HATTORI (1993) Effects of acremonium cellulase and lactic acid bacteria inoculant on the fermentation quality and digestibility of guineagrass silages. *J. Japan Grassl. Sci.* **39**, 317–325.

2) GIBSON, T., A.C. STIRLING, R.M. KEDDIE and R.F. ROSENBERGER (1958) Bacteriological changes in silage made at controlled temperatures. *J. General Microbiogy* **19**, 112–119.

3) HENDERSON, A.R., P. MCDONALD and D.H. ARDERSON (1983) The effect of silage additives containing formaldehyde on the fermentation of ryegrass ensiled at different dry matter levels and on the nutritive value of direct-cut silage. *Animal Feed Sci. Tech.* **7**, 303–314.

4) HUHTANEN, P., K. Hiss, S. JAAKKOLA and E. POUTIAINEN (1985) Enzymes as silage additives : Effect on fermentation quality, digestibility in sheep, degradability in sacco and performance in growing cattle. *J. Agric. Sci. Finl.* **57**, 285–291.

5) KIBE, K., E. NODA and Y. KARASAWA (1981) Effect of moisture level of material grass on silage fermentation. *Shinshu Univ., J. Faculty Agric.* **18**, 145–154.

6) KOSAKI, M. (1992) Experimental Manual of Lactic Acid Bacteria**. Kyobundo. Tokyo. pp. 15–16.

7) KUMAI, S., T. KIMURA, R. FUKUMI, Y. CAI and L.F. QUINITIO (1990) Effect of inoculation of lactobacilli at ensiling on the fermentative quality of silage and changes in microflora during ensilage*. *J. Japan. Grassl. Sci.* **36**, 231–237.

8) LANIGAN, G.W. (1961) Studies on ensilage. 1. A comparative laboratory study of molasses and sodium metabisulphite as aids to the conservation of lucerne. *Aust. J. Agric. Res.* **12**, 1023–1038.

9) MASAKI, S. and Y. OHYAMA (1979) Changes in sugars during ensilage production of lactic acid and volatile fatty acids. *Jap. J. Zootech Sci.* **50**, 280–287.

10) MCDONALD, P. and A.R. HENDERSON (1962) Buffering capacity of herbage samples as a factor in ensilage. *J. Sci. Fd. Agric.* **13**, 395–400.

11) MCDONALD, P., A.R. HENDERSON and A.W. MACGREGOR (1968) Chemical changes and losses during the ensilage of wilted grass. *J. Sic. Fd. Agric.* **19**, 125–132.

12) MCDONALD, P., A.R. HENDERSON and S.J.E. HERON (1991) The Biochemistry of Silage. Chalcombe Publ., Marlow, pp. 9–137.

13) MORIMOTO, H. (1971) The Experimental Methods for Animal Nutrition**. Yokendo. Tokyo. pp. 293–422.

14) MUCK, R.E. (1990) Dry matter level effects on alfalfa silage quality. II. Fermentation products and starch hydrolysis. *ASAE. Transaction* **33**, 373–380.

15) MURDOCH, J.C. (1960) The effect of temperature on silage fermentation. Proc. 8th Int. Grassl. Congr. 502–505.

16) OHYAMA, Y., S. MASAKI and T. MORICHI (1973) Effects of temperature and glucose addition on the process of silage fermentation. *Jap. J. Zootech. Sci.* **44**, 59–67.

17) OHMOMO, S., O. TANAKA and H. KITAMOTO (1993) Analysis of organic acids in silage by high-performance liquid chromatography*. *Bull. Natl. Grassl. Res. Inst.* **48**, 51–56.

18) SELMER-OLSEN, I., A.R. HENDERSON, S. ROBERTSON and R. MCGINN (1993) Cell wall degrading enzymes for silage. 1. The fermentation of enzyme-treated ryegrass in laboratory silos. *Grass & Forage Sci.* **48**, 45–54.

19) TANAKA, O. and S. OHMOMO (1995) A simple method of laboratory silage fermentation by using a plastic pouch for packaging. *Grassl. Sci.* **41**, 55–59.

20) WHITTENBURY, R., P. MCDONALD and D.G. BRYAN-JONES (1967) A short review of some biochemical and microbiological aspects of ensilage. *J. Sci. Fd. Agric.* **18**, 441–444.

21) WIERINGA, G.W. (1961) Some factors affecting silage fermentation. Proc. 8th Int. Grassl. Congr. 497–502.

22) WILLIAMS, S. (1984) Official Methods of Analysis. Association of Official Analytical Chemists. Arlington. Virginia. pp. 152–164.

23 VAN VURREN, A. M., K. M. BERGSMA, F. KROL-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 silages. *Grass & Forage Sci.* **44**, 223–230.

24) YAMANOBE, T., Y. MITSUISHI and Y. TAKASAKI (1987) Isolation of a cellulolytic enzyme producing microorganism, culture conditions and some properties of the enzyme. *Agric. Biol. Chem.* **51**, 65–74.

25) ZHANG, J.-G., 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. *Grassland Science* **43**, 88–94.

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要　　旨

張　建国・熊井清雄・福見良平 (1997)：環境温度，水分含量およびセルラーゼの添加がハダカムギ麦稈サイレージの発酵品質と化学成分に及ぼす影響．*Grassland Science* **43**, 95–102. 愛媛大学農学部 (790 愛媛県松山市樽味 3-5-7)

麦稈サイレージの品質を改善するために，環境温度，水分含量およびセルラーゼ添加の影響を検討した。実験1においては対照，アクレモニウムセルラーゼ（AC）およびメイセラーゼ（MC）の各処理麦稈をそれぞれ10，20，30，40および50℃のサイロ環境温度条件下で調製した。実験2では，水分含量を50，60，70および75%に調整した麦稈にACとMCを加え対照区とともにサイレージを調製した。

実験1では，環境温度50区のサイレージは発酵が抑制されたが，それ以下の温度では，セルラーゼ添加サイレージはpHが低くなり，乳酸が増加し，発酵品質は向上した。特にACはMCよりも改善効果が高かった。また，30℃区では酪酸含量が特異的に高くなった。実験2では，60%区は全てのサイレージの発酵品質が優れ，それ以外の対照区はpHが上昇し，乳酸含量が低下した。70%区においてはAC添加のみ，サイレージの発酵品質が優れた。75%区では全てのサイレージの酪酸含量が高くなった。

実験1および2の結果，セルラーゼ添加によってNDF，ADFおよびセルロースの含量が僅かに低下し，有意差は認められなかった。セルラーゼがサイレージの発酵品質の改善に及ぼす効果については，ACがMCより優れていた。

キーワード：温度，水分含量，セルラーゼ，発酵品質，麦稈サイレージ．