Effect of lactic acid bacteria inoculant and beet pulp addition on fermentation characteristics and in vitro ruminal digestion of vegetable residue silage

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ABSTRACT

The objective of this study was to determine the effect of beet pulp (BP) and lactic acid bacteria (LAB) on silage fermentation quality and in vitro ruminal dry matter (DM) digestion of vegetable residues, including white cabbage, Chinese cabbage, red cabbage, and lettuce. Silage was prepared using a small-scale fermentation system, and treatments were designed as control silage without additive or with BP (30% fresh matter basis), LAB inoculant Chikuso-1 (*Lactobacillus plantarum*, 5 mg/kg, fresh matter basis), and BP + LAB. In vitro incubation was performed using rumen fluid mixed with McDougall's artificial saliva (at a ratio of 1:4, vol/vol) at 39°C for 6 h to determine the ruminal fermentability of the vegetable residue silages. These vegetable residues contained high levels of crude protein (20.6–22.8% of DM) and moderate levels of neutral detergent fiber (22.7–33.6% of DM). In all silages, the pH sharply decreased and lactic acid increased, and the growth of bacilli, coliform bacteria, molds, and yeasts was inhibited by the low pH at the early stage of ensiling. The silage treated with BP or LAB had a lower pH and a higher lactic acid content than the control silage. After 6 h of incubation, all silages had relatively high DM digestibility (38.6–44.9%); in particular, the LAB-inoculated silage had the highest DM digestibility and the lowest methane production. The vegetable residues had high nutritional content and high in vitro DM digestibility. Also, both the addition of a LAB inoculant and moisture adjustment with BP improved the fermentation quality of the vegetable residue silages. In addition, LAB increased DM digestibility and decreased ruminal methane production.

Key words: beet pulp, in vitro digestibility, lactic acid bacteria, vegetable residue silage

INTRODUCTION

Commercial processing of vegetables results in many residues, but most are burned and dumped into landfills or used as compost, which is a waste of resources and leads to possible environmental problems because of unsuitable disposal. Demand for efficient use of food byproducts is increasing because of economic and environmental concerns. Residues from vegetables such as white cabbage, Chinese cabbage, red cabbage, and lettuce are high in nutrients such as vitamins, minerals, and vegetable fiber (Council for Science and Technology, 2005), and large quantities of these vegetables are produced annually in many countries, including Japan. However, these vegetable residues (V) perish easily because of their high moisture content. Technologies to create high-quality animal feed from V and to provide long-term storage of the resulting silage need to be developed. In regions where V are the main food byproduct, the use of V silage as animal feed has proven economically viable, not only as a way of disposing of V but also as an alternative livestock feed.

The self-sufficiency rate of food cereals in Japan is low. Therefore, the goal is to raise the roughage self-sufficiency rate and to move from a concentrate-centered feed structure to a roughage-centered structure (Sato, 2005). It is increasingly common in Japan to ensile high-moisture byproducts with dry feeds as a fermented byproduct mix. When high-moisture food byproducts are ensiled as fermented byproduct mix with dry feeds, the risk of effluent production can be minimized, rumen function stabilized, and self-selection by animals avoided (Coppock et al., 1981; Imai, 2000). Furthermore, unpalatable byproducts may be incorporated into feed if odors and flavors are altered through fermentation during ensiling (Cao et al., 2009b). Although we investigated natural populations of lactic acid bacteria isolated from V and silage fermentation (Yang et al., 2010), the moisture of the V was not adjusted to prepare good quality silage, and the digestive characteristics were not studied. However, before preparing fermented byproduct mix as ruminant feed, it is important to investigate the digestive characteristics of these V.

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The objective of this experiment was to determine the chemical and microorganism composition of V, and the influence of lactic acid bacteria addition and moisture adjustment on fermentation quality and the in vitro ruminal fermentability of silages.

MATERIALS AND METHODS

Vegetable Residue Samples

Residues of white cabbage (*Brassica campestris* L. var. *capitata*), Chinese cabbage (*Brassica rapa* L. var. *glabra* Regel), red cabbage (*Brassica oleracea* var. *capitata* F. *rubra*), and lettuce (*Lactuca sativa* L.) (3 samples of each) were collected from a local commercial vegetable factory (Fujiyama factory, Matsuya Foods Company, Limited, Fujinomiya, Shizuoka, Japan). The V comprised the outside leaf part of white cabbage, Chinese cabbage, red cabbage, and lettuce with no added bacteria.

Microbiological Analysis

Vegetable residue samples (10 g) were blended with 90 mL of sterilized water, and serially diluted from 10^{-1} to 10^{-5} in sterilized water. The number of lactic acid bacteria (**LAB**) were measured by plate count on Lactobacilli de Man, Rogosa, Sharpe (MRS) agar (Difco Laboratories, Inc., Detroit, MI) incubated at 30°C for 48 h under anaerobic conditions (Anaerobic box; TEHER Hard Anaerobox, ANX-1; Hirosawa Ltd., Tokyo, Japan). Coliform bacteria were counted on blue light broth agar (Nissui Ltd., Tokyo, Japan), incubated at 30°C for 48 h; mold and yeast were counted on potato dextrose agar (Nissui Ltd.), incubated for 24 h at 30°C. Yeasts were distinguished from molds or bacteria by colony appearance and observation of cell morphology. Bacilli and aerobic bacteria were distinguished by the colony shape and counted on nutrient agar (Nissui Ltd.) incubated for 24 h at 30°C under aerobic conditions. Colonies were counted as viable numbers of microorganisms [cfu/g of fresh matter (**FM**)].

Experimental Treatments and Laboratory Silage Preparation

Experimental treatments included control silage without additive or with LAB, beet pulp [**BP**; DM, 90.7%; OM, 94.9%; CP, 8.4%; ether extract (**EE**), 0.7%; ADF, 25.6%; NDF, 52.1%; water-soluble carbohydrates (**WSC**), 2.1% of DM], and BP + LAB. The strain FG1 (*Lactobacillus plantarum* Chikuso-1; Snow Brand Seed, Sapporo, Japan) isolated from a commercial inoculant was used. The MRS broth was inoculated with

strain FG1 and incubated overnight. After incubation, the optical density of the suspension at 700 nm was adjusted to 0.42 using sterile 0.85% NaCl solution. The inoculum size of LAB was 1 mL of suspension per kilogram of FM. The inoculated LAB number was 1.0×10^5 cfu/g of FM. The addition ratio of BP was 300 g/kg of FM. Silages were prepared using a small-scale system of silage fermentation (Cai et al., 1998). Approximately 100-g portions of forage material, chopped into about 20-mm length, were mixed well and packed into plastic bags (Hiryu KN type, 180 × 260 cm; Asahikasei, Tokyo, Japan). The bags were sealed with a vacuum sealer (BH 950; Matsushita, Tokyo, Japan). The plastic silo bags were stored in a room at 25°C. Ten silo bags were used per treatment. One silo bag per treatment was opened on d 3, 5, and 7, and 3 bags per treatment were opened on d 30 and 60. Samples were dried in a forced-air oven at 60°C for 48 h, ground to pass through a 1-mm screen with a Wiley mill (ZM200; Retch GmbH & Co. KG, Haan, Germany), and used for chemical analysis and in vitro digestibility measurements.

Cultures and Incubations

Two adult wethers (average initial BW, 78.5 kg) fitted with rumen cannulas were used as donors of ruminal fluid. The wethers were fed a basal diet of 50% reed canary grass (*Phalaris arundinacea* L.) hay and 50% commercial feed concentrate (Koushi-Ikusei-Special; Kitanihon Kumiai Feed Co., Ltd., Miyagi, Japan) at maintenance energy level (2.0% DM of their BW) and had free access to clean drinking water. They were fed once daily at 0900 h. Wethers were cared for in accordance with the animal care and use institutional guidelines for animal experiments at the Faculty of Agriculture, Yamagata University (Tsuruoka, Japan).

Rumen fluid was collected through the rumen cannulas 2 h after feeding and diverted to plastic bottles. The fluid was filtered through 4 layers of cheesecloth and combined on an equal volume basis. The combined filtrate was mixed with CO₂-bubbled McDougall's artificial saliva (pH 6.8; McDougall, 1948) at a ratio of 1:4 (vol/vol). Then 50 mL of buffered rumen fluid was transferred to 128-mL serum bottles containing 0.5 g of sample, and flushed with O₂-free CO₂. Tubes were capped with a butyl rubber stopper and sealed with an aluminum cap. Incubations were performed in triplicate at 39°C for 6 h (Mohammed et al., 2004) in a water bath with a reciprocal shaker (100 strokes/min).

Chemical Analysis

Dry matter, CP, EE, and OM were analyzed according to AOAC Methods 934.01, 976.05, 920.39, and 942.05,

respectively (AOAC, 1990). The ADF and NDF were analyzed by the methods of Van Soest et al. (1991). Sodium sulfite and heat-stable amylase were used in the analysis of NDF. Fermentation products of the V silages were determined from cold water extracts. Wet silage (10 g) was homogenized with 90 mL of sterilized distilled water (Cai et al., 1999b). The pH was measured with a glass electrode pH meter (MP230; Mettler Toledo, Greifensee, Switzerland), and ammonia-N concentration was determined by steam distillation of the filtrates (Cao et al., 2010b). The organic acid contents were measured by HPLC (Jasco Corp., Tokyo, Japan,) according to the methods described by Cai et al. (1999b). The WSC, including glucose, sucrose, and fructose were determined by HPLC (Jasco Corp.). The analytical conditions were as follows: Shodex Sugar SC1011 column (8.0 mm $\times$ 30 cm; Shoko, Tokyo, Japan); 80°C oven temperature; water mobile phase; and 1.0 mL/min detector (Jasco RI-1530; Jasco Corp.).

***In Vitro* Ruminal DM Digestibility and Fermentation Products**

To terminate fermentation at the end of incubation, 25 μ L of formaldehyde solution (35%) was injected into serum bottles, which were immediately sealed and cooled at room temperature. Gas samples were collected by air syringe from the serum bottles and injected into a gas chromatograph (GC323; GL Sciences Inc., Tokyo, Japan) equipped with a thermal conductivity detector and a stainless steel column (WG-100 SUS, 1.8 m $\times$ 6.35 mm OD), and the methane production in each serum bottle was measured. The analytical conditions were as follows: 50°C column oven temperature, 50°C injector temperature at, and 50°C detector temperature.

Separate subsamples of the supernatant were taken to determine VFA and ammonia-N concentration using the same methods as for the silage filtrates. Finally, the contents of the bottles were transferred to previously weighed filter papers, and the incubation residue was washed with 50 mL of hot (50°C) distilled water. These filter papers containing incubation residue were then oven dried for 48 h at 60°C, and the digestibility of the residual DM was determined (Mohammed et al., 2004; Cao et al., 2010a).

Statistical Analyses

Data on the fermentative characteristics of each vegetable silage opened on d 30 were analyzed by one-way ANOVA. Data on chemical compositions, fermentative characteristics, *in vitro* ruminal DM digestibility, and fermentation products after 6-h incubation of silages

opened on d 60 were analyzed using a completely randomized design with a 4 $\times$ 4 [V $\times$ additive treatment (A)] factorial treatment structure. The GLM procedure of SAS version 9.0 (SAS Institute Inc., Cary, NC) was used for the analysis, and the model included the main effects of V and A, and their interactions. Sealing time and ensiling duration were excluded from the model because the 60-d silages, processed wastes, and silos were made only one time. The Tukey test was used to identify differences ($P < 0.05$) between means.

RESULTS

Chemical Composition of Vegetable Residues and Microbiological Composition of Silages

The chemical composition of V is shown in Table 1. Dry matter of the 4 types of V was less than 6%. Their OM contents were more than 70% of DM, and the CP and NDF contents were approximately 20% and 30% of DM, respectively. The WSC (including sucrose, glucose, and fructose) contents ranged from 8.4 to 21.7% of DM; the highest and lowest values were observed in white cabbage and lettuce residues, respectively.

Changes in the microbiological composition of the V silages on d 0, 3, 5, 7, and 30 after ensiling are shown in Table 2. Before ensiling (d 0), overall, 10^4 to 10^7 (cfu/g of FM) LAB and coliform bacteria, 10^2 to 10^4 bacilli, 10^6 to 10^7 aerobic bacteria, 10^3 to 10^4 molds, and 10^3 to 10^5 yeasts were found in the 4 types of V, but clostridia were too few to count in all of the V. After 3 d of fermentation, the numbers of LAB increased rapidly and reached values of 10^8 cfu/g of FM in the 4 types of V silage. At 5 and 7 d of fermentation, they remained at this level, and by d 30, 10^6 to 10^7 cfu/g of FM were found. The numbers of bacilli, coliform bacteria, and molds decreased to a low level or were too few to count after 3 d of fermentation in almost all silages. The numbers of aerobic bacteria also decreased to a low level ($\leq 10^4$ cfu/g of FM) by d 3. Yeasts were not detected in white cabbage and Chinese cabbage silages after 3 d of fermentation, but 10^3 to 10^5 cfu/g of FM were detected in red cabbage and lettuce silages from d 5 to 30. The numbers of clostridia were not detected in any of the vegetable silages at any of the periods.

Changes in Fermentation Quality of Silage Treated with LAB and BP

Changes in pH, lactic acid, acetic acid, and ammonia-N of V silages without or with LAB and BP are shown in Table 3. The pH values of all silages decreased sharply after 3 d of fermentation; the lowest value was observed at d 5 or 7, and from then to d 30 the val-

Table 1. Chemical composition¹ of vegetable residues

Item	White cabbage	Chinese cabbage	Red cabbage	Lettuce
DM, %	3.6 ± 0.02	2.2 ± 0.01	5.2 ± 0.02	2.0 ± 0.01
OM, % of DM	81.6 ± 0.30	70.8 ± 0.08	87.3 ± 0.24	75.4 ± 0.09
CP, % of DM	21.9 ± 0.01	20.6 ± 0.21	22.8 ± 0.32	21.1 ± 0.41
Ether extract, % of DM	2.8 ± 0.07	1.5 ± 0.02	1.7 ± 0.14	4.9 ± 0.07
ADF, % of DM	31.0 ± 0.20	26.5 ± 1.49	31.6 ± 0.42	29.4 ± 0.14
ADL, ² % of DM	2.3 ± 0.19	5.8 ± 0.02	3.2 ± 0.03	5.9 ± 0.30
NDF, % of DM	32.8 ± 0.56	27.7 ± 0.24	33.6 ± 0.40	31.4 ± 0.01
Sucrose, % of DM	12.1 ± 0.15	1.1 ± 0.02	6.3 ± 0.10	3.1 ± 0.13
Glucose, % of DM	4.3 ± 0.04	3.6 ± 0.07	2.5 ± 0.01	1.3 ± 0.04
Fructose, % of DM	5.3 ± 0.12	3.8 ± 0.05	3.7 ± 0.10	4.0 ± 0.18

¹Values are means ± standard deviations.²Acid detergent lignin.

ues did not change significantly in any silage except untreated Chinese cabbage silage; the pH values of all of the LAB-treated silages and BP- and LAB + BP-treated Chinese cabbage and lettuce silages were lower ($P < 0.05$) than those of control silages after 30 d of fermentation. The lactic acid contents of all silages rose rapidly at the early stage of fermentation, whereas from d 5 to 7 a slight decrease was observed in some silages, such as LAB-, LAB + BP-treated, and untreated white cabbage residue silages, BP-treated Chinese cabbage silages, LAB- and LAB + BP-treated red cabbage silages, and BP- and LAB + BP-treated lettuce silages. When storage was prolonged up to 30 d, a slow increase or no change was observed in some silages; after 30 d of ensiling, the lactic acid content of all A silages, except for

LAB-treated red cabbage silage, was higher ($P < 0.05$) than that of control silages. Ammonia-N contents of the majority of silages increased rapidly after ensiling. From d 7 to 30, little change was observed in the white and red cabbage silages, but in the 2 control silages of Chinese cabbage and lettuce residue, the ammonia-N content tended to rise and was significantly higher ($P < 0.05$) than in the other treatment silages.

Chemical Composition and Fermentation Quality of LAB- and BP-Treated Silages After 60 d of Fermentation

The chemical composition and fermentative quality of V silages treated with LAB and BP after 60 d of

Table 2. Changes in counts of viable microorganisms of vegetable residues and silages during fermentation¹

Vegetable residue	Ensiling days	Microorganism (cfu/g of fresh matter)					
		LAB ²	Bacilli	Coliform bacteria	Aerobic bacteria	Molds	Yeasts
White cabbage	0	4.6×10^5	6.2×10^4	4.9×10^6	1.4×10^7	3.6×10^4	1.3×10^5
	3	4.0×10^8	1.0×10^3	ND ³	2.4×10^4	ND	ND
	5	1.6×10^8	ND	5.0×10^2	4.0×10^3	ND	ND
	7	2.2×10^8	5.0×10^2	ND	3.0×10^3	ND	ND
	30	9.0×10^7	5.0×10^2	ND	4.0×10^3	ND	ND
Chinese cabbage	0	3.4×10^4	5.0×10^2	4.4×10^4	2.9×10^6	3.0×10^3	9.5×10^3
	3	1.4×10^8	ND	ND	1.5×10^3	ND	ND
	5	2.3×10^7	ND	ND	ND	ND	ND
	7	1.4×10^8	ND	ND	2.0×10^3	ND	ND
	30	3.0×10^7	ND	ND	5.0×10^3	ND	ND
Red cabbage	0	1.8×10^7	6.0×10^3	2.0×10^7	2.1×10^7	1.5×10^3	3.0×10^3
	3	2.7×10^8	1.0×10^3	ND	1.9×10^4	ND	ND
	5	5.2×10^8	ND	ND	1.1×10^4	ND	2.0×10^3
	7	4.0×10^8	ND	ND	6.0×10^3	ND	1.4×10^4
	30	5.0×10^6	ND	ND	2.5×10^3	ND	2.0×10^5
Lettuce	0	3.2×10^4	8.0×10^3	1.4×10^6	1.2×10^7	1.0×10^3	2.5×10^4
	3	3.7×10^8	1.0×10^3	3.2×10^4	1.9×10^4	ND	ND
	5	2.4×10^8	ND	5.0×10^2	2.0×10^3	ND	2.5×10^3
	7	2.2×10^8	ND	ND	4.5×10^3	ND	7.2×10^3
	30	7.5×10^7	ND	ND	5.5×10^3	ND	8.0×10^4

¹Clostridia were not detected.²Lactic acid bacteria.³Not detected.

fermentation are shown in Tables 4 and 5, respectively. Vegetable residue, A, and their interaction ($V \times A$) influenced ($P < 0.001$) OM, CP, EE, ADF, NDF, and WSC. The DM did not differ among the 4 types of vegetable silages ($P = 0.249$); the highest OM content ($P < 0.001$) was found in red cabbage, followed by white cabbage, lettuce, and Chinese cabbage. The CP content ranged 14 to 17.3%, with the highest content ($P < 0.001$) found in white cabbage; the EE content was low, ranging from 1.4 to 4.1%; the ADF and NDF contents ranged from 24.7 to 31.3% and from 35.6 to 41.6%, respectively. The WSC content was low, ranging from 0.3 to 2.0%. Compared with the control silages, both LAB- and BP-treated silages showed increased ($P < 0.001$) OM, but decreased ($P < 0.001$) WSC content; furthermore, the LAB-treated silage showed increased CP content, whereas BP-treated silage showed decreased CP, EE, and ADF, but increased NDF.

The pH, lactic acid, acetic acid, and ammonia-N were affected not only by V, but also by A and $V \times A$. Comparison among the 4 types of vegetable silages revealed that the pH was the lowest ($P < 0.001$) in silage with white cabbage residue, followed by red cabbage, Chinese cabbage, and lettuce silages. The lactic acid content was highest in white cabbage silage ($P < 0.001$), whereas the acetic acid content was highest in lettuce silage ($P < 0.001$), followed by red cabbage, white cabbage, and Chinese cabbage silages. Propionic and butyric acids were not detected among the 4 types of vegetable silages; the ammonia-N concentration of white cabbage, red cabbage, and lettuce silages was lower ($P < 0.001$) than that of Chinese cabbage silage. Comparison of the treated silages showed that all silages treated with LAB or BP had lower ($P < 0.001$) pH values and ammonia-N concentrations, but higher ($P < 0.001$) lactic acid contents compared with the control silage.

***In Vitro* Ruminant Digestive Characteristic of Vegetable Residue Silage**

Dry matter digestibility, methane production, VFA, and ammonia-N concentrations of vegetable-residue silage after 6 h of incubation *in vitro* are shown in Table 6. Although DM digestibility was not influenced by V, it was influenced by A and by $V \times A$; methane production, VFA, and ammonia-N were influenced by V, A, and $V \times A$. Dry matter digestibility did not differ among silages. However, ruminal methane production of white and Chinese cabbage silages was lower ($P < 0.001$) than that of red cabbage and lettuce silages, and the total VFA production of red cabbage and lettuce residue silages was higher ($P = 0.014$) than that of Chinese cabbage silage. The acetic acid pro-

duction of the lettuce silage was higher ($P < 0.001$) than that of the white cabbage silage. The propionic acid production of white cabbage was the highest ($P < 0.001$) among the 4 V, followed by lettuce, which showed higher propionic acid production ($P < 0.001$) than did red or Chinese cabbage; the last 2 silages did not differ in this regard. Red cabbage had higher ($P < 0.001$) butyric acid production than Chinese cabbage and lettuce, and butyric acid production was higher in white cabbage ($P < 0.001$) than in Chinese cabbage. The acetic acid:propionic acid ratio of white cabbage silages was the lowest ($P < 0.001$) among the 4 types of vegetable silages. The highest and lowest ($P < 0.001$) ammonia-N production was found in white cabbage and lettuce silages, respectively. The LAB-treated silage had a higher ($P < 0.001$) DM digestibility than BP- and BP + LAB-treated silages; it also had the highest ammonia-N production, but the lowest ($P < 0.001$) methane production. Together with the control silage, LAB-treated silage had lower ($P < 0.001$) total VFA, acetic acid, and propionic acid production, but higher ($P < 0.001$) butyric acid production and acetic acid:propionic acid ratio compared with BP- and LAB + BP-treated silages.

DISCUSSION

The 4 types of V had high moisture content (more than 95%) and higher CP and WSC, but lower OM and NDF content than do some types of fresh forage such as alfalfa, Italian ryegrass, or Timothy grass (Cai et al., 1999a; Xu et al., 2007).

It is well established that LAB play an important role in silage fermentation, and LAB values have become a significant factor in predicting the adequacy of silage fermentation and in determining whether to apply bacterial inoculants to silage materials. Generally, when LAB reaches at least 10^5 (cfu/g of FM), silage can be well preserved (Cai et al., 1999a). However, as shown in Table 2, the LAB values below 10^5 and aerobic bacteria values above 10^6 present in most V suggest that high-quality silage fermentation may need to be controlled using certain inoculants. The inoculant strain used in this study was *Lactobacillus plantarum* Chikuso-1; this strain promotes lactic acid fermentation and can grow well in low-pH environments. Therefore, silage prepared using this strain can promote the propagation of LAB, decrease pH, inhibit the growth of clostridia and aerobic bacteria, and improve silage quality (Cai et al., 1999a).

The factors involved in fermentation quality include not only the physiological properties of epiphytic bacteria but also the chemical composition of the silage material (Cai et al., 1999a). In this study, the 4 types

Table 3. Changes in pH and fermentation products of vegetable residue silages ensiled for 0, 3, 5, 7, and 30 d

Item	pH					Lactic acid (g/kg of FM ¹)					Acetic acid (g/kg of FM)					Ammonia-N (g/kg of FM)				
	0	3	5	7	30 ²	0	3	5	7	30	0	3	5	7	30	0	3	5	7	30
White cabbage																				
Control	6.03	4.12	4.00	4.02	3.63 ^b	ND ³	5.2	7.2	6.3	12.2 ^c	ND	5.8	6.1	4.2	6.6 ^b	0.11	0.29	0.30	0.35	0.26
LAB ⁴	6.03	3.72	3.59	3.54	3.47 ^c	ND	10.1	12.1	11.0	14.6 ^b	ND	0.4	0.9	0.5	2.6 ^c	0.11	0.27	0.21	0.19	0.22
BP ⁵	6.03	4.38	4.10	3.93	3.68 ^{ab}	ND	5.1	8.1	8.8	20.3 ^a	ND	4.3	5.9	4.5	11.0 ^a	0.11	0.33	0.24	0.34	0.28
BP + LAB	6.03	3.79	4.02	3.59	3.71 ^a	ND	11.4	16.0	12.9	20.0 ^a	ND	0.8	1.9	0.8	4.0 ^c	0.11	0.22	0.24	0.23	0.25
SEM	—	—	—	—	0.02	—	—	—	—	0.51	—	—	—	—	0.40	—	—	—	—	0.01
Chinese cabbage																				
Control	5.76	4.29	4.08	3.93	4.62 ^a	ND	4.4	5.3	6.0	4.2 ^c	ND	2.7	2.0	1.8	9.8 ^a	0.05	0.20	0.17	0.21	0.58 ^a
LAB	5.76	3.86	3.84	3.74	4.0 ^b	ND	7.1	8.2	8.2	8.8 ^b	ND	0.6	0.8	0.7	3.9 ^c	0.05	0.17	0.14	0.14	0.22 ^b
BP	5.76	4.31	3.86	3.84	3.77 ^{bc}	ND	7.4	17.0	14.3	19.0 ^a	ND	2.0	2.7	2.1	6.8 ^b	0.05	0.23	0.18	0.24	0.24 ^b
BP + LAB	5.76	3.85	3.71	3.61	3.65 ^c	ND	11.0	13.9	15.8	19.6 ^a	ND	0.5	0.6	0.7	3.0 ^c	0.05	0.22	0.17	0.19	0.19 ^b
SEM	—	—	—	—	0.07	—	—	—	—	0.46	—	—	—	—	0.24	—	—	—	—	0.07
Red cabbage																				
Control	6.21	4.26	3.89	3.91	3.67 ^b	ND	6.0	10.2	10.2	10.4 ^b	ND	0.8	0.7	0.5	4.1 ^b	0.13	0.34	0.37	0.30	0.28
LAB	6.21	3.83	3.51	3.80	3.57 ^c	ND	5.5	13.5	10.4	11.3 ^b	ND	0.2	0.4	2.6	2.4 ^c	0.13	0.09	0.23	0.25	0.19
BP	6.21	4.39	4.10	4.02	3.84 ^a	ND	4.6	7.4	10.1	14.9 ^a	ND	0.7	0.9	7.0	5.8 ^a	0.13	0.26	0.31	0.28	0.28
BP + LAB	6.21	3.87	3.56	3.96	3.77 ^{ab}	ND	10.4	16.9	13.7	14.6 ^a	ND	0.5	0.4	3.4	4.1 ^b	0.13	0.22	0.23	0.26	0.22
SEM	—	—	—	—	0.03	—	—	—	—	0.33	—	—	—	—	0.20	—	—	—	—	0.04
Lettuce																				
Control	5.98	4.56	4.20	4.19	4.6 ^a	ND	3.2	7.1	6.8	6.8 ^c	ND	2.0	2.9	1.8	11.9 ^a	0.03	0.10	0.14	0.13	0.35 ^a
LAB	5.98	3.87	3.65	3.68	3.98 ^b	ND	6.0	9.3	8.8	9.4 ^b	ND	0.8	0.4	0.9	3.6 ^c	0.03	0.09	0.07	0.08	0.14 ^b
BP	5.98	4.36	4.03	3.99	3.75 ^b	ND	6.4	14.7	11.2	21.3 ^a	ND	3.9	6.0	4.2	7.9 ^b	0.03	0.12	0.14	0.15	0.18 ^b
BP + LAB	5.98	3.74	3.74	3.77	3.68 ^b	ND	13.9	19.8	17.1	21.5 ^a	ND	0.3	0.4	1.0	3.2 ^c	0.03	0.09	0.10	0.13	0.13 ^b
SEM	—	—	—	—	0.14	—	—	—	—	0.58	—	—	—	—	0.66	—	—	—	—	0.03

^{a-c}Means within columns with different superscript letters differ ($P < 0.05$).¹Fresh matter.²Values are means of three silage samples.³Not detected.⁴*Lactobacillus plantarum* (Chikuso-1; Snow Brand Seed Co. Ltd., Sapporo, Japan).⁵Beet pulp.

Table 4. Chemical composition (% of DM) of vegetable residue silage prepared with LAB¹ and BP² after 60 d of storage

Item	DM	OM	CP	EE ³	ADF	NDF	WSC ⁴
White cabbage							
Control	6.3	86.3	23.8	3.6	28.0	28.7	2.9
LAB	5.3	86.5	24.2	3.9	30.1	33.0	1.5
BP	29.5	91.4	10.3	0.5	26.1	44.0	1.6
BP + LAB	29.3	90.5	10.8	0.5	27.1	45.6	1.9
Chinese cabbage							
Control	1.6	70.9	16.5	3.4	22.4	22.9	0.0
LAB	3.7	84.4	20.7	3.7	21.7	32.8	0.5
BP	32.4	90.0	10.8	0.5	27.1	44.7	0.8
BP + LAB	30.7	89.7	10.9	0.4	27.5	46.0	0.2
Red cabbage							
Control	6.9	89.8	20.8	2.7	31.7	32.6	1.8
LAB	7.3	89.2	22.7	2.2	30.3	32.9	1.0
BP	31.5	91.0	10.5	0.3	27.2	45.4	0.2
BP + LAB	31.2	91.1	10.6	0.4	28.3	45.7	0.1
Lettuce							
Control	3.5	83.6	19.7	7.8	34.9	38.8	0.3
LAB	3.3	83.8	22.5	7.6	35.5	36.4	0.3
BP	30.3	90.3	9.9	0.5	27.1	45.5	0.5
BP + LAB	27.4	90.4	10.6	0.6	27.8	45.8	0.0
SEM	2.2	0.12	0.29	0.12	0.36	0.56	0.22
Vegetable residue means							
White cabbage	17.6	88.7 ^b	17.3 ^a	2.1 ^b	27.8 ^c	37.8 ^b	2.0 ^a
Chinese cabbage	17.1	83.7 ^d	14.0 ^c	2.0 ^b	24.7 ^c	35.6 ^c	0.4 ^c
Red cabbage	19.2	90.3 ^a	16.1 ^b	1.4 ^c	29.4 ^b	39.2 ^b	0.8 ^b
Lettuce	16.1	87.0 ^c	15.7 ^b	4.1 ^a	31.3 ^a	41.6 ^a	0.3 ^c
Additive treatment means							
Control	4.6 ^b	82.7 ^c	20.2 ^b	4.4 ^a	29.2 ^a	30.8 ^a	1.3 ^a
LAB	4.9 ^b	86.0 ^b	22.5 ^a	4.3 ^a	29.4 ^a	33.8 ^a	0.8 ^b
BP	30.9 ^a	90.7 ^a	10.4 ^c	0.5 ^b	26.9 ^b	44.9 ^b	0.8 ^b
BP + LAB	29.7 ^a	90.4 ^a	10.7 ^c	0.5 ^b	27.8 ^b	45.8 ^b	0.6 ^b
Significance of main effects and interactions							
Vegetable residues (V)	0.249	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Additive treatment (A)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
V × A	0.841	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

^{a-d}Means within columns with different superscript letters differ ($P < 0.05$).

¹*Lactobacillus plantarum* (Chikuso-1; Snow Brand Seed Co. Ltd., Sapporo, Japan).

²Beet pulp.

³Ether extract.

⁴Water-soluble carbohydrates.

of V had relatively high WSC contents; the epiphytic LAB transformed WSC into organic acids during the ensiling process, and as a result, the pH was decreased, which inhibited the growth of some microorganisms, such as bacilli, coliform bacteria, aerobic bacteria, yeasts, and molds. When silage was treated with LAB or BP, the fermentation tended to ensure rapid and vigorous results with the faster accumulation of lactic acid (Table 3) and lower pH values at earlier stages of ensiling, and it also inhibited the production of acetic acid and ammonia-N during silage fermentation and, thus, improved vegetable-residue conservation. The transitional behavior of the VFA in the silage during fermentation indicated sharp decreases in pH, and corresponding increases in lactic acid contents at earlier stages (7 d of ensiling) were typical of a good fermentation process and were in agreement with previous studies (Woelford, 1984; Okine et al., 2007). Subsequently,

a steady reduction in pH depicted stability, whereas lactic acid contents gradually stabilized after a decrease during storage. Some studies (Woelford, 1984; Okine et al., 2007) have shown that the development of LAB peaks in the first 7 d in parallel with the rise in lactic acid concentration of silage, and this is followed by decreases in LAB numbers; however, no apparent decrease in LAB numbers was observed in this study. The d 60 LAB-treated silage had higher OM and CP, but lower WSC than did control silages. During silage fermentation, LAB could effectively utilize WSC to produce sufficient lactic acid to decrease pH and inhibit the growth of harmful bacteria; therefore, the resulting silage was of good quality. These results are in agreement with our previous studies (Cai et al., 1998, 1999a; Cai, 1999). Furthermore, the moisture content of silage material is also a major factor influencing silage fermentation (Garcia et al., 1989). An intrinsic

Table 5. Fermentation profile of vegetable residue silages prepared with LAB¹ and BP² after 60 d of storage³

Item	Moisture (%)	pH	Lactic acid (g/kg of FM ⁴)	Acetic acid (g/kg of FM)	Ammonia-N (g/kg of FM)
White cabbage					
Control	93.7	3.57	11.1	1.9	0.28
LAB	94.7	3.46	13.7	0.8	0.22
BP	70.5	3.72	29.8	2.4	0.31
LAB + BP	70.7	3.63	29.1	1.0	0.25
Chinese cabbage					
Control	98.4	5.72	8.3	1.1	1.07
LAB	96.3	3.85	11.2	1.3	0.18
BP	67.6	3.75	11.4	1.4	0.19
LAB + BP	69.3	3.74	12.3	1.2	0.20
Red cabbage					
Control	93.1	3.73	8.8	2.0	0.32
LAB	92.7	3.64	10.7	1.7	0.23
BP	68.5	3.86	15.0	2.7	0.26
LAB + BP	68.8	3.74	16.3	1.7	0.22
Lettuce					
Control	96.5	4.83	4.5	4.6	0.43
LAB	96.7	4.85	7.6	3.6	0.40
BP	69.7	3.75	14.1	3.5	0.18
LAB + BP	72.6	3.63	15.3	1.5	0.13
SEM	2.49	0.05	1.45	0.27	0.02
Vegetable residue means					
White cabbage	82.4	3.59 ^c	20.9 ^a	1.5 ^c	0.26 ^b
Chinese cabbage	82.9	4.26 ^a	10.8 ^b	1.3 ^c	0.41 ^a
Red cabbage	80.8	3.74 ^b	12.7 ^b	2.0 ^b	0.26 ^b
Lettuce	83.9	4.27 ^a	10.4 ^b	3.3 ^a	0.28 ^b
Additive treatment means					
Control	95.4 ^a	4.46 ^a	8.2 ^c	2.4 ^a	0.52 ^a
LAB	95.1 ^a	3.95 ^b	10.8 ^b	1.9 ^b	0.26 ^b
BP	69.1 ^b	3.77 ^c	17.6 ^a	2.5 ^a	0.23 ^{bc}
LAB + BP	70.3 ^b	3.68 ^c	18.3 ^a	1.4 ^b	0.20 ^c
Significance of main effects and interactions					
Vegetable residues (V)	0.249	<0.001	<0.001	<0.001	<0.001
Additive treatment (A)	<0.001	<0.001	<0.001	<0.001	<0.001
V × A	0.841	<0.001	<0.001	<0.001	<0.001

^{a-c}Means within columns with different superscript letters differ ($P < 0.05$).

¹*Lactobacillus plantarum* (Chikuso-1; Snow Brand Seed Co. Ltd., Sapporo, Japan).

²Beet pulp.

³Propionic and butyric acids were not detected.

⁴Fresh matter.

characteristic of V is their very high moisture content (95 to 98% of FM), and this is a major limitation to its use as livestock feed. Although dried V can easily be incorporated into rations, the energy cost associated with drying wet V has been increasing. Moreover, the risk of effluent production is high because of the low DM content. Therefore, pressed V have been preferred for adjusting moisture with other feed to ensile. Garcia et al. (1989) reported that high-moisture silage is more beneficial to lactic acid fermentation and has less risk of heat damage than low-moisture silage. In this study, according to our preliminary experiment and taking into consideration the cost of feed, the moisture of BP-treated silage was adjusted to 70%, and most BP-treated silages had lower pH and ammonia-N and higher lactic acid content compared with control silage. It is possible that this is because the addition of BP

not only adjusted the moisture content of the V but also increased the WSC content; therefore, silages with added BP could greatly contribute to better lactic acid fermentation. Furthermore, we used a small-scale system of silage fermentation; all silages stored well and maintained high quality without aerobic deterioration in this study. We will study the aerobic stability of silage prepared in flexible containers in the future.

The present in vitro experiment was conducted to determine the ruminal fermentative characteristics of the 4 types of V silages and the effects of addition of LAB and BP on the ruminal digestive characteristic of these silages. After 6 h incubation in vitro, all vegetable-residue silages had high DM digestibility (38.6–44.9%), similar to that of other forage feeds incubated for 6 h in vitro, such as alfalfa hay and corn silage (Eun and Beauchemin, 2007). Although no significant difference

Table 6. Measurements of DM digestibility, methane production, VFA concentration, and ammonia-N concentration after 6-h in vitro incubation with rumen fluid of vegetable residue silage

Item	DM digestibility (%)	CH ₄ (L/kg of DDM ¹)	Total VFA (mmol/L)	Acetic acid (mmol/L)	Propionic acid (mmol/L)	Butyric acid (mmol/L)	A/P ²	Ammonia-N (mg/L)
White cabbage								
Control	50.3	3.5	39.0	22.7	8.0	8.3	2.8	181.3
LAB ³	53.4	2.2	40.9	23.1	8.3	9.5	2.8	183.7
BP ⁴	36.2	5.3	45.6	27.0	9.6	9.0	2.8	41.7
BP + LAB	39.2	4.0	46.5	26.4	10.5	9.5	2.5	44.0
Chinese cabbage								
Control	29.2	5.9	36.1	23.5	6.0	6.7	3.9	70.3
LAB	43.7	1.9	39.5	26.1	6.8	6.7	3.8	189.7
BP	39.8	3.9	45.0	28.3	8.1	8.6	3.5	46.0
BP + LAB	41.6	3.6	46.9	34.2	8.6	4.1	4.0	47.3
Red cabbage								
Control	52.3	4.0	46.6	29.5	4.8	12.3	6.1	149.0
LAB	52.1	3.3	41.7	23.7	5.3	12.7	4.5	147.7
BP	36.7	8.7	45.2	28.5	8.5	8.2	3.4	38.0
BP + LAB	36.0	8.7	45.6	28.2	8.3	9.0	3.4	32.7
Lettuce								
Control	33.5	4.6	38.7	20.8	5.2	12.7	4.0	89.0
LAB	40.8	2.2	46.8	30.0	5.8	10.9	5.1	135.3
BP	46.6	9.6	46.7	33.2	10.1	3.4	3.3	35.3
BP + LAB	45.7	7.8	46.6	32.8	9.2	4.5	3.6	40.7
SEM	3.31	0.85	1.61	1.85	0.34	0.92	0.25	2.56
Vegetable residue means								
White cabbage	44.9	3.8 ^b	43.0 ^{ab}	24.8 ^b	9.1 ^a	9.1 ^{ab}	2.7 ^b	112.7 ^a
Chinese cabbage	38.6	3.8 ^b	41.9 ^b	28.0 ^{ab}	7.4 ^{bc}	6.5 ^c	3.8 ^a	88.3 ^b
Red cabbage	44.3	6.2 ^a	44.8 ^a	27.5 ^{ab}	6.7 ^c	10.6 ^a	4.3 ^a	91.8 ^b
Lettuce	41.6	6.1 ^a	44.7 ^a	29.2 ^a	7.6 ^b	7.9 ^{bc}	4.0 ^a	75.1 ^c
Additive treatment means								
Control	41.3 ^{ab}	4.5 ^b	40.1 ^b	24.1 ^b	6.0 ^b	10.0 ^a	4.2 ^a	122.4 ^b
LAB	47.5 ^a	2.4 ^c	42.2 ^b	25.7 ^b	6.5 ^b	10.0 ^a	4.1 ^a	164.1 ^a
BP	39.8 ^b	6.9 ^a	45.6 ^a	29.3 ^a	9.1 ^a	7.3 ^b	3.2 ^b	40.3 ^c
BP + LAB	40.6 ^b	6.0 ^{ab}	46.4 ^a	30.4 ^a	9.2 ^a	6.8 ^b	3.4 ^b	41.2 ^c
Significance of main effects and interactions								
Vegetable residues (V)	0.056	<0.001	0.014	0.014	<0.001	<0.001	<0.001	<0.001
Additive treatment (A)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
V × A	<0.001	0.002	0.009	0.01	<0.001	<0.001	<0.001	<0.001

^{a-c}Means within columns with different superscript letters differ ($P < 0.05$).

¹Digestible DM.

²Acetic acid/propionic acid ratio.

³*Lactobacillus plantarum* (Chikuso-1; Snow Brand Seed Co. Ltd., Sapporo, Japan).

⁴Beet pulp.

was observed among residues, Chinese cabbage silage tended to decrease DM digestibility. According to the chemical composition (Table 4) and fermentative quality (Table 5), some of the CP in Chinese cabbage silage was degraded to produce ammonia-N, which decreased the OM content and resulted in some nutrient loss during the ensiling processes, possibly resulting in a decrease in the DM digestibility of the Chinese cabbage silage; this finding is consistent with a previous study (Sanon et al., 2008).

Two known mechanisms exist for the conversion of lactic acid or pyruvic acid to propionic acid (Leng, 1970). When lactic acid is secondarily fermented in the rumen by lactate-utilizing bacteria such as *Megasphaera elsdenii*, *Selenomonas ruminantium*, and *Veillonella parvula*, propionate is generally produced (Dawson et al., 1997; Russell and Wallace, 1997). This

can reduce methanogenesis because electrons are used during propionate formation. If hydrogen is then used to convert lactic acid to propionic acid in the rumen (Moss et al., 2000), the hydrogen will decrease, which, in turn, will inhibit the conversion of hydrogen and CO₂ to methane. Our recent study (Cao et al., 2009a, 2010a,b) also found that silage with a high lactic acid content decreased methane production in the rumen. In this study, both white and Chinese cabbage silages decreased ruminal methane production after 6 h of in vitro incubation. Our finding that white cabbage silage with a high lactic acid content decreased ruminal methane production seems to be consistent with the results of a previous study (Cao et al., 2009a, 2010a,b). However, we cannot explain why the Chinese cabbage silage also decreased the production of ruminal methane in this study. We suspect that the low OM content or DM

digestibility of the Chinese cabbage silage may have led to the decrease in ruminal methane production. In the present study, the lettuce silage with a high ADF content resulted in high ruminal acetic acid production after 6 h incubation in vitro; white cabbage silage with high CP and WSC contents had high ruminal propionic acid and ammonia-N production; however, we cannot explain why red cabbage had the highest production of butyric acid and the lowest production of propionic acid. Weinberg et al. (2007) and Filya et al. (2007) reported that LAB inoculants affected the in vitro digestibility of alfalfa hay and corn silage, respectively, after 48 h incubation. In the present study, LAB inoculants not only increased ($P = 0.0098$) the silage DM digestibility after 6 h in vitro incubation but also decreased ruminal methane production, which decreases the energy loss of feed (Cao et al., 2010b). Furthermore, LAB inoculants improve the fermentation quality of vegetable silage, which might decrease the degradation of CP in the silage; therefore, LAB-treated silage had a high concentration of ruminal ammonia-N. Adding BP to vegetable silage did not affect the DM digestibility after 6 h in vitro incubation, but did increase the production of acetic acid, propionic acid, and even methane, while decreasing the production of butyric acid and ammonia-N. We cannot explain the mechanism of these effects. More research is needed to elucidate the probiotic effect of adding LAB or BP to V silage in ruminants.

Furthermore, in this study, the interactions of V and A not only influenced the majority of chemical composition, pH, organic acids, and ammonia-N, but also influenced ruminal fermentation characteristics of the silages. We propose that, as described in a previous study (Cai et al., 1999a, 2010b), the factors involved in fermentation quality include chemical composition, particularly the WSC content of the silage material and the physiological properties of epiphytic bacteria. The addition of BP (containing high WSC) or LAB to silage can improve the fermentation quality, decrease some fermentation loss, and influence the ruminal digestion characteristics of the silage. However, the effects of added LAB are influenced by the silage materials; that is, when the V contains high WSC during silage fermentation, LAB can produce sufficient lactic acid to decrease pH, inhibit the growth of harmful bacteria, and decrease some fermentation loss, resulting in silage of good quality.

CONCLUSIONS

Vegetable residues have high nutritive value and in vitro DM digestibility and good potential as vegetable protein sources for ruminants. Moisture adjustment

with BP can improve the fermentation quality of V silages. Lactic acid bacteria inoculants have the potential to increase DM digestibility and to decrease ruminal methane production.

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