Chapter 15

Ruminal Digestibility and Quality of Silage Conserved via Fermentation by Lactobacilli

Yang Cao, Yimin Cai and Toshiyoshi Takahashi

Additional information is available at the end of the chapter

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1. Introduction

The utilization of whole crop rice (WCR) as an animal feed has proven economically viable, not only as a way of disposing of rice straw residues but also as a real alternative for feeding livestock in regions where rice is the main crop (Han et al., 1974). As a result, in Japan and other rice-producing countries, rice is no longer grown exclusively for human consumption but increasingly as a valuable forage crop. Forage rice is in fact believed to be an ideal alternative crop, not only in helping farmers adjust grain rice production but also in preserving the soil, leading to long-term utilization of the paddy field. Yet a major drawback of forage rice is that it yields low-quality silage, due to poor digestibility of nutrients, mostly crude proteins (Cai et al., 2003). Several processes have been developed to improve the fermentation and nutritional value of whole-crop silage from forage paddy rice. Breeding programs are carried out, and newly developed rice varieties with increased yield and amount of digestible nutrients are being grown and tested. Also, harvesting, preparation, and storage techniques are constantly being improved. However, WCR is usually insufficient in sugars and lactic acid bacteria (LAB), and may produce silages rich in ethanol rather than lactic acid and volatile fatty acid (VFA) (Cai et al., 2003). This could be attributed to the structure of the rice plant; the hollow stem may increase the air in a silo, facilitating yeast WCR is usually insufficient in sugars and lactic acid bacteria (LAB) (Cai et al., 2003), and may produce silages rich in ethanol rather than lactic acid and VFA (Yamamoto et al., 2004). This could be attributed to the structure of the rice plant; the hollow stem may increase the air in a silo, facilitating yeast growth especially in the early ensiling period. Furthermore, most of the processes used to date still rely on heavy chemical treatments with ammonia and sodium hydroxide and were reported to reduce the palatability of silage to ruminants (Cai et al., 2003; Enishi et al., 1998). Of the many factors that can affect silage fermentation, the type of microorganisms that dominate the process often dictates the final quality of the silage. For instance, homolactic fermentation by LAB is more desirable than

other types of fermentation because the theoretical recoveries of dry matter and energy are greatest. During this type of fermentation, LAB utilizes water-soluble carbohydrates to produce lactic acid, the primary acid responsible for decreasing the pH in silage. In contrast, other fermentations are less efficient. Natural populations of LAB on plant material are often low in number and heterofermentative. Thus, the concept of using a microbial inoculant to silage involves adding fast-growing homofermentative LAB in order to dominate the fermentation, thereby producing higher-quality silage. Some of the commonly used homofermentative LAB in silage inoculants include *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Pediococcus acidilactici*, and *Enterococcus faecium*. Commercially available microbial inoculants contain one or more of these bacteria that have been selected for their ability to dominate the fermentation.

Food by-product such as tofu cake is high in crude protein and fatty acids (Xu et al. 2001). Not only could the by-products be utilized as a source of nutrients for ruminants, but using them to replace imported commercial feedstuffs could save energy in transportation, and possibly reduce the environmental impact of burning them as waste of burying them landfills. Preparing total mixed ration (TMR) silage is one practice whereby food by-products are stored and utilized as animal feeds in Japan (Imai, 2001). Our previous study (Cao et al., 2009b) showed that TMR silage with 30% dried tofu cake had the higher lactic acid content than that with rice bran or green tea waste. Alli et al. (1984) reported that as molasses can provide fermentable sugars for the production of organic acids, it has been used extensively as a fermentation aid, and that silage prepared with molasses may show a lower pH, higher residual water-soluble carbohydrates levels, greater quantities of lactic acid, lower levels of volatile basic nitrogen, and decreased dry matter (DM) loss compared to silage without molasses. Weinberg et al. (2003) also reported a high lactic acid content in silage ensiled with straw and molasses.

However, even if there is plenty of glucose as a substrate, if insufficient in lactic acid bacteria, to preparing good quality silage is difficult. Cai et al. (1999) reported that the factors involved in fermentation quality include chemical composition, particularly the water-soluble carbohydrates content of the silage material and the physiological properties of epiphytic bacteria. Conservation of forage crops by ensiling is based on natural fermentation in which epiphytic LAB convert sugars into lactic acid under anaerobic conditions. As a result, the pH decreases and the forage is preserved. The fermentation quality of silage is influenced by the size, diversity and activity of epiphytic LAB. The population density of LAB has been reported to range from 10–10^3 CFU/g fresh matter (FM) on standing forage crops to 10^3–10^7 CFU/g FM on chopped ones entering the silo. Generally, When LAB reaches at least 10^5 (CFU/g of FM), silage can be well preserved; if LAB values below 10^5 high-quality silage fermentation may need to be controlled using certain inoculants. *Lactobacillus plantarum* Chikuso-1 is shown to have great potential as an inoculant for WCR silage (Cai et al., 1999, 2003; Cai, 2001; Cao et al., 2011).

Furthermore, 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 due to unsuitable disposal. Demand for efficient

use of food by-products is increasing due to 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, and large quantities of these vegetables are produced annually in many countries, including Japan. However, these vegetable residues perish easily because of their high moisture content. Technologies to create high-quality animal feed from vegetable residues and to provide long-term storage of the resulting silage need to be developed. In regions where vegetable residues are the main food by-product, the use of vegetable-residue silage as animal feed has proven economically viable, not only as a way of disposing of vegetable residues but also as an alternative livestock feed. To prepare fermented by-product mix as ruminant feed, it is important to investigate the digestive characteristics of these vegetable residues.

The purpose of this experiment was to examine the effects of lactic acid bacteria inoculant on the quality of fermentation and ruminal digestibility and fermentation characteristics of ruminant fed silage, and to determine the chemical and microorganism composition of vegetable residues, and the influence of lactic acid bacteria addition and moisture adjustment on fermentation quality and the in vitro ruminal fermentability of silages.

2. Materials and methods

Experiments were conducted with permission from the Committee of Animal Experimentation, and according to the animal care and use institutional guidelines for animal experiments at the Faculty of Agriculture, Yamagata University.

2.1. Silage preparation

Whole crop rice (Haenuki) was cultivated using transplant cultivating methods in a paddy field on an experimental farm at Yamagata University, Japan, and harvested at the full-ripe stage with a length of 2 cm. As shown in Table 1, TMR was prepared using compound feed (Kitanihon-Kumiai Feed, Yamagata, Japan), WCR, dried beet pulp, a vitamin-mineral supplement (Snow Brand Seed, Iwate, Japan), dried tofu cake (Zenno, Tsuruoka, Japan), molasses (sugarcane; Dai-Nippon Meiji Sugar Co., Tokyo, Japan) and LAB (*Lactobacillus plantarum* Chikuso-1, Snow Brand Seed, Sapporo, Japan). Experimental treatments included control silage without additive, or with molasses, (4% FM basis), LAB (5 mg kg^{-1} FM basis), and molasses+LAB. Moisture was adjusted with water to approximately 65%. Silages were prepared using a small-scale system of silage fermentation. Approximately 1 kg TMR was packed into plastic film bags (Hiryu BN-12 type, 270 mm × 400 mm, Asahikasei, Tokyo, Japan), and the bags were sealed with a vacuum sealer (SQ303, Sharp, Osaka, Japan). Three silos per treatment were prepared and stored in a room at 20–25°C for 60 days.

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.) (three samples of each) were collected from a local commercial vegetable factory (Fujiyama factory, Matsuya Foods Company, Limited, Fujinomiya,

Shizuoka, Japan). The vegetable residue comprised the outside leaf part of white cabbage, Chinese cabbage, red cabbage, and lettuce with no added bacteria. Experimental treatments included control silage without additive or with LAB, beet pulp (DM, 90.7%; organic matter, 94.9%; crude protein, 8.4%; ether extract, 0.7%; acid detergent fiber, 25.6%; neutral detergent fiber, 52.1%; WSC, 2.1% of DM), and beet pulp+LAB. The strain FG1 (Lactobacillus plantarum Chikuso-1; Snow Brand Seed, Sapporo, Japan) isolated from a commercial inoculant was used. The de Man Rogosa Sharpe agar 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 beet pulp was 300 g per kg of FM. Silages were prepared using a small-scale system of silage fermentation (Cai et al., 1999). 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-bag silos were stored at a room at 25°C. There were ten bag silos per treatment. One bag of silo per treatment was opened on d 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.

2.2. Chemical analyses

The TMR silages, vegetable and its silages were dried in a forced draft oven at 60°C for 48 h and ground into a 2-mm powder with a sample mill (Foss Tecator; Akutalstuku, Tokyo, Japan). Moisture, ash, crude protein, ether extract, and crude fiber contents were determined by general methods. Analyses of neutral detergent fiber and acid detergent fiber contents were made following Van Soest et al. (1991). Heat-stable amylase and sodium sulfite were used in the neutral detergent fiber procedure, and the results were expressed without residual ash. Nonfibous carbohydrate was calculated by the formula as: Nonfibous carbohydrate = organic matter − crude protein − nonfibous carbohydrate − ether extract. The fermentation products of silages were determined using cold-water extracts. Wet silage (50 g) was homogenized with 200 ml sterilized distilled water and stored at 4°C overnight (Cai et al. 1999). The pH of the silages was determined using a glass electrode pH meter (D-21, Horiba, Kyoto, Japan). Lactic acid was analyzed using the methods of Cai et al. (1999). Ammonia- N was determined as described by (Cai et al. 2003). To measure total VFA, silage and ruminal fluid were steam-distilled and titrated using sodium hydroxide. Dried VFA salt was separated and quantified using gas chromatography (G-5000A, Hitachi, Tokyo, Japan) equipped with a thermal conductivity detector and a stainless column (Unisole F-200, 3.2 mm × 2.1 m). The analytical conditions were as follows: column oven temperature, 140°C; injector temperature, 210°C; detector temperature, 250°C. V-score, which was used to assess silage quality, was determined from the proportion ammonia-N in the total nitrogen and VFA contents in the silage.

2.3. Cultures and incubations

Two adult wethers (average initial body weight, 78.5 kg) fitted with rumen cannulae 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, Miyagi, Japan) at maintenance energy level (2.0% DM of their body weight) and had free access to clean drinking water. They were fed once daily at 09:00 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 cannulae 2 h after feeding and diverted to plastic bottles. The fluid was filtered through four layers of cheesecloth and combines on an equal volume basis. The combined filtrate was mixed with CO_2-bubbled McDougall's artificial saliva (pH 6.8) at a ratio of 1:4 (vol/vol). Then 50 mL buffered rumen fluid was transferred to 128-mL serum bottles containing 0.5 g sample, and flushed with O_2-free CO_2. 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).

2.4. Analysis of fermentation products

To terminate fermentation at the end of incubation, 25 μL of formaldehyde solution (35%) were 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, Tokyo, Japan) equipped with a thermal conductivity detector and a stainless steel column (WG-100 SUS, 1.8 m × 6.35 mm OD), and the methane production in each serum bottle was measured. The analytical conditions were as follows: column oven temperature at 50°C, injector temperature at 50°C, and detector temperature at 50°C.

2.5. *In vitro* DM digestibility, and methane and VFA production

Separate sub-samples of the supernatant were taken to determine the pH and VFA concentration. The bottles were rinsed with warm water to remove all solid residues, which were then oven-dried at 60°C and stored for further analyses. In total, 2 g of dried residue were oven-dried at 135°C and stored to determine DM digestibility.

2.6. Statistical analyses

Analyses were performed using the general linear model procedure (SAS institute, Cary, NC, USA). Data on fermentative characteristics, in vitro DM digestibility, and ruminal methane and VFA production of TMR silages were subjected to one-way analysis of variance Tukey's test was used to identify differences ($P < 0.05$) between means. Data on the fermentative characteristics of each vegetable silage opened on d 30 were analyzed by one-

way analysis of variance. 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 × 4 (vegetable residues × additive treatment) factorial treatment structure. The general linear model procedure of SAS version 9.0 (SAS Institute, Inc., Cary, NC) was used for the analysis, and the model included the main effects of vegetable residues and additive treatment, and their interactions. Sealing time and ensiling duration were excluded from the model because the 60-d silages, wastes processed, and silos were made only one time. The Tukey test was used to identify differences ($P < 0.05$) between means.

3. Results and discussion

3.1. Chemical composition of materials and silage

The contents of DM, crude protein, ether extract, nonfibous carbohydrate, ash, and neutral detergent fiber in molasses were 72.7, 4.3, 0.7, 83.6, 11.4, and 0%, respectively (Table 1). The contents of organic matter and crude protein in WCR were 86.5 and 5.3%, respectively. The contents of crude protein, nonfibous carbohydrate, and neutral detergent in the tofu cake were 30.1, 15.8, and 37.7%, respectively. And the chemical composition of vegetable residues is shown in Table 2. The DM of the four types of vegetable residues was less than 6%. Their OM contents were more than 70% of DM, and the crude protein and neutral detergent fiber contents were approximately 20% and 30% of DM, respectively. The water-soluble carbohydrates (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.

Preparing TMR silage is one practice whereby food by-products are stored and utilized as ruminant feeds in Japan. It can avoid energy costs associated with drying, and may improve odors and flavors of unpalatable feed resources through fermentation in a silo (Cao et al., 2009a; Imai 2001; Wang & Nishino 2008). We have performed a number of experiments to investigate the effects of food by-products including tofu cake, rice bran, and

	WCR[1]	Concentrate[2]	Beet pulp	TC[3]	Molasses[4]
DM[5] (%)	36.0	88.2	90.7	91.3	72.7
CP[6] (% DM)	5.3	16.7	8.4	30.1	4.3
EE[7] (% DM)	2.2	3.8	0.7	12.2	0.7
NFC[8] (% DM)	32.1	60.1	33.7	15.8	83.6
Ash (% DM)	13.5	5.1	5.1	4.3	11.4
ADF[9] (% DM)	30.2	8.7	25.6	22.2	–
NDF[10] (% DM)	48.0	14.4	52.1	37.7	0

[1]Whole crop rice; [2]Formula feed ("Koushi Ikusei Special Mash" made by Zenno with 120g kg^{-1} crude protein in fresh matter); [3]Tofu cake; [4]Wang & Goetsch (1998); [5]Dry matter; [6]Crude protein; [7]Ether extract; [8]Non-fibrous carbohydrate (100 – crude protein – ether extract – neutral detergent fiber – ash); [9]Acid detergent fiber; [10]Neutral detergent fiber.

Table 1. Chemical composition of WCR, concentrate, beet pulp and tofu cake used in total mixed ration silages (Cao et al., 2010b)

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, % 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

[1]Values are means ± SD.

Table 2. Chemical composition1 of vegetables residues (Cao et al., 2011)

	Treatment				SEM[3]
	Control	M[1]	LAB[2]	M+LAB	
Ingredient					
LAB (mg kg^{-1} FM[4])	-	-	5	5	
M (% FM)	-	4	-	4	
Whole crop rice (% DM[5])	30	30	30	30	
Concentrate[6] (% DM)	25	25	25	25	
Vitamin-mineral supplement[7] (% DM)	1.5	1.5	1.5	1.5	
Dried beet pulp (% DM)	13.5	13.5	13.5	13.5	
Tofu cake (% DM)	30	30	30	30	
Chemical composition					
DM (%)	35.9	36.2	36.4	37.0	1.98
Organic matter (% DM)	92.6	92.5	92.6	92.3	0.25
Crude protein (% DM)	15.3	14.6	15.4	15.1	0.38
Ether extract (% DM)	5.1	5.3	5.5	5.6	0.24
Nitrogen free extract (% DM)	57.1	58.4	58.4	57.5	0.40
Crude fiber (% DM)	15.7	14.9	14.2	14.5	0.88
NFC[8] (% DM)	30.9	32.0	29.5	32.0	1.54
Crude ash (% DM)	7.4	7.5	7.4	7.7	0.25
Acid detergent fiber (% DM)	19.0	20.0	18.9	20.2	1.21
Neutral detergent fiber (% DM)	41.4	40.6	42.2	39.6	1.12

[1]Molassess; [2]Lactic acid bacteria (Lactobacillus plantarum); [3]Standard error of means; [4]Fresh matter; [5]Dry matter; [6]Formula feed ("Koushi Ikusei Special Mash" made by Zenno; total digestible nutrients, 70.0%; crude protein, 12.0% in fresh matter); [7]Commercial vitamin-mineral supplement product (Snow brand seed, Iwate, Japan); [8]Non-fibrous carbohydrate (100 – crude protein – ether extract – neutral detergent fiber – ash).

Table 3. Ingredient and chemical composition of total mixed ration silages (Cao et al., 2010b)

wet green tea waste on fermentation quality of WCR-containing TMR silage. Our previous study (Cao et al., 2009a, b) showed that silages with 30% tofu cake had higher lactic acid content, compared to those with rice bran and green tea waste. Therefore, we prepared TMR silage using tofu cake, and in order to investigate if adding LAB or molasses can further increase lactic acid content of the silages with tofu cake, LAB and molasses were added into these silages in this study. LAB can increase the lactic acid content of a silage (Cai, 2001; Cai et al., 2003), and was well used to prepare silage. Molasses is a fermentable carbohydrate (Maiga & Schingoethe, 1997) and many researchers (Alli et al., 1984) have reported its successful use with grass silage. In addition, molasses is a food by-product of sugar beet and sugarcane production. Molasses with high water-soluble carbohydrates is used as a major energy source for meat or milk production (Araba et al., 2002; Granzin & Dryden, 2005; Sahoo & Walli, 2008; Wang & gotsch, 1998).

3.2. Fermentation quality

As indicated by the low pH value (around 4.0) and ammonia-N/total N content (2.83–2.97%), high lactic acid content (2.49–2.87%), and V-score (99.8) for the silages, the four TMR silages were well preserved (Table 4). Although the levels of moisture, pH, acetic acid, propionic acid, butyric acid, and ammonia-N/total N and V-score did not differ significantly, lactic acid contents for the silages with LAB and molasses+LAB were higher ($P = 0.005$) than the control and molasses silages.

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 2001; Cai et al., 1999; Cai et al., 2003). However, the LAB values below 10^5 and aerobic bacteria values above 10^6 present in most WCR 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., 1999).

	Treatment				SEM[3]	*P*-value
	Control	M[1]	LAB[2]	M+LAB		
pH	3.99	3.92	4.01	4.03	0.0391	0.585
Lactic acid (% FM[4])	2.49[a]	2.52[a]	2.84[b]	2.87[b]	0.0880	0.005
Acetic acid (% FM)	0.09	0.09	0.10	0.09	0.0032	0.934
Propionic acid (% FM)	0.003	0.003	0.001	0.001	0.0009	0.574
Butyric acid (% FM)	0.002	0.003	0.003	0.003	0.0001	0.776
ammonia-N/total-N (%)	2.97	2.91	2.92	2.83	0.1775	0.956
V-score	99.8	99.8	99.8	99.8	0.0090	0.970

[1]Molassess; [2]Lactic acid bacteria (Lactobacillus plantarum); [3]Standard error of means; [4]Fresh matter; Means within a row with different letters (a, b) differ ($P < 0.05$).

Table 4. Fermentative characteristics of total mixed ration silages (Cao et al., 2010b)

The pH, lactic acid, acetic acid, and ammonia-N were affected not only by vegetable, but also by addition and vegetable × addition. Comparison among the four 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 four 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.

Alli et al. (1984) assessed the effects of molasses on the fermentation of chopped whole-plant Leucaena. Silages were treated with molasses at a rate of 2.25% or 4.5% fresh weight at the time of ensiling, which led to increased rates of lactic acid production, lower pH, decreased DM loss, and reduced levels of ammonia-N compared to Leucaena to which no molasses was added. In the present experiment, WCR-containing TMR silages were treated with or without molasses at the rate of 4% fresh weight. Although the addition of molasses did not significantly influence the chemical composition, it increased DM and Non-fibrous carbohydrate contents by 3.06 and 3.56%, respectively. Adding molasses did not increase lactic acid content significantly, Adding LAB and molasses+LAB, however, increased lactic acid content significantly. This is probably because that, even if no molasses, there was enough fermentable sugars in TMR silage, and the LAB may have converted more fermentable sugars to lactic acid (Cai 2001; Cai et al., 2003). This study showed the advantage of LAB over molasses. Alli et al. (1984) reported that adding molassesf reduced the ammonia-N of silage, although in present study, ammonia-N did not differ among the four silages.

Furthermore, our previous study (Cao et al., 2011) has determined the effect of LAB inoculant and beet pulp addition on silage fermentation quality and *in vitro* ruminal DM digestion of vegetable residues. The silage treated with LAB or beet pulp had a lower pH and a higher lactic acid content than the control silage (Table 4). After 6 h of incubation, the LAB-inoculated silage had the highest DM digestibility and the lowest methane production. Weinberg et al. (2003) 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 ($P < 0.01$) 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., 2010a). Furthermore, LAB inoculants improve the fermentation quality of vegetable silage, which might decrease the degradation of crude protein in the silage; therefore, LAB-treated silage had a high concentration of ruminal ammonia-N. Adding beet pulp 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 beet pulp to vegetable silage in ruminants.

Item	Moisture	pH	Lactic acid	Acetic acid	Ammonia-N
	%		g/kg of FM		
Vegetable residue means					
White cabbage	82.4	3.59^{c}	20.9^{a}	1.5^{c}	0.26^{b}
Chinses 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

[1]Means within columns with different letters (a-c) differ ($P < 0.05$); [2]Propionic and Butyric acids were not detected; [3]Lactobacillus plantarum (Chikuso-1, Snow Brand Seed, Sapporo, Japan); [3]Beet pulp.

Table 5. Fermentation profile of vegetable residue silages prepared with LAB and BP after 60 days of storage (Cao et al., 2011)

The pH, lactic acid, acetic acid, and ammonia-N were affected not only by vegetable, but also by addition and vegetable × addition （Table 5）. Comparison among the four 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 four 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.

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., 1999). In this study, the four types of vegetable residues had relatively high water-soluble carbohydrates contents; the epiphytic LAB transformed water-soluble carbohydrates into organic acids during the ensiling process, and as a result, the pH was reduced, 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. Subsequently, a steady reduction in pH depicted stability, while lactic acid contents gradually stabilized after a decrease during storage. Some studies (Cai, 2001; Cai et al., 1999; Cai et al., 2003) 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 organic matter and crude protein, but lower water-soluble carbohydrates than did control silages. During silage fermentation, LAB could effectively utilize water-soluble carbohydrates to produce sufficient lactic acid to reduce pH and inhibit the growth of harmful bacteria; therefore, the resulting silage was of good quality. Furthermore, the moisture content of silage material is also a major factor influencing silage fermentation (Cai et al., 2003). An intrinsic characteristic of vegetable residues 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 vegetable residues can easily be incorporated into rations, the energy cost associated with drying wet vegetable residues has been increasing. Moreover, the risk of effluent production is high because of the low DM content. Therefore, pressed vegetable residues have been preferred for adjusting moisture with other feed to ensile. Cai et al. (1999) 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 beet pulp-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 vegetable residues but also increased the water-soluble carbohydrates 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.

3.3. *In vitro* DM digestibility, and methane and VFA production

After in vitro 6 h incubation, DM digestibility, total VFA, acetic acid, isovaleric acid, valeric acid, and the acetic to propionic acid ratio did not differ significantly among the treatments (Table 6). However, methane production for the LAB silage and the molasses silage tended (P = 0.065) to decrease and increase, respectively, propionic acid for the LAB silage tended (P = 0.061) to increase, and butyric acid for the control silage was higher (P = 0.008) than the other silages.

	Treatment				SEM[3]	*P*-value
	Control	M[1]	LAB[2]	M+LAB		
DM[4] digestibility (%)	42.2	44.5	44.8	44.5	1.03	0.313
Methane production (L kg^{-1} DDM[5])	10.5	11.2	9.6	10.2	0.30	0.065
Total VFA (mmol 100 ml^{-1})	5.3	5.8	5.9	5.7	0.16	0.340
Acetic acid (A) (mol %)	37.0	38.9	38.0	38.8	0.55	0.142
Propionic acid (P) (mol %)	39.9	40.4	41.8	40.5	0.35	0.061
Butyric acid (mol %)	19.9[a]	17.8[b]	17.0[b]	17.8[b]	0.34	0.008
Isovaleric acid (mol %)	0.4	0.3	0.3	0.3	0.06	0.844
Valeric acid (mol %)	2.8	2.5	2.5	2.6	0.10	0.416
A/P	0.9	1.0	0.9	1.0	0.02	0.271

[1]Molassess; [2]Lactic acid bacteria (Lactobacillus plantarum); [3]Standard error of means; [4]Dry matter; [5]Digestible dry matter; Means within a row with different letters (a, b) differ ($P < 0.05$).

Table 6. *In vitro* dry matter digestibility, methane production and volatile fatty acid concentration after 6 hours incubation of total mixed ration silages (Cao et al., 2010b)

DM digestibility, VFA, and ammonia-N concentrations of vegetable-residue silage after 6 h incubation in vitro are shown in Table 7. Although DM digestibility was not influenced by vegetable, it was influenced by addition and by vegetable × addition; VFA, and ammonia-N were influenced by vegetable, addition, and vegetable × addition. DM digestibility did not differ among silages. However, ruminal CH_4 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 production 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 four vegetable residues, followed by lettuce, which showed higher propionic acid production ($P < 0.001$) than did red or Chinese cabbage; the last two 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 A:P ratio of white cabbage silages was the lowest ($P < 0.001$) among the four 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 beet pulp+LAB-treated silages; it also had the highest ammonia-N 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 ratio compared with beet pulp- and LAB+beet pulp-treated silages.

In vitro DM digestibility was higher in silage with LAB than without LAB because LAB reduces DM loss in silage fermentation (Cai 2001; Cai et al., 2003). Furthermore, although there are some reports that adding molasses has no effect on DM digestibility (Granzin & Dryden 2005; Wang & Goetsch 1998), many more studies (Shellito et al., 2006; Sahoo & Walli 2008) have reported that diets with molasses have higher ruminal DM digestibility.

In the present experiment, there was a non-significant increasing trend in DM digestibility with molasses, LAB and molasses+LAB. Ruminal methane production and the molar proportion of propionic acid for silage with LAB decreased by 8.6% and increased by 4.8%, respectively. These might be because that adding LAB increased lactic acid content in the silage, when the silage containing high lactic acid content was incubated in vitro, there are two known mechanisms for the conversion of lactic acid or pyruvic acid to propionic acid, and when lactate acid is secondarily fermented by lactate-utilizing bacteria such as Megasphaera elsdenii, Selenomonas ruminantium, and Veillonella parvula, propionate is generally produced as a major product (Dawson et al., 1997) and this can reduce methanogenesis because electrons are used during propionate formation. But adding molasses, which has a high sugar content, may augment methane production in the rumen (Hindrichsen et al., 2005), perhaps because of which, molasses per se canceled (compensated) the effect of lactic acid content on methane production. A further research is necessary about the effect of molasses and the complex effects of molasses and LAB concerning the methane production in TMR silage. Furthermore, it is not yet clear why adding molasses or LAB decreased in vitro ruminal butyric acid in this study.

Item	DM digestibility	Total VFA	Acetic acid	Propionic acid	A/P[1]	Ammonia-N
	%	mmol/L				mg/L
Vegetable residue means						
White cabbage	44.9	43.0^{ab}	24.8^{b}	9.1^{a}	2.7^{b}	112.7^{a}
Chinese cabbage	38.6	41.9^{b}	28.0^{ab}	7.4^{bc}	3.8^{a}	88.3^{b}
Red cabbage	44.3	44.8^{a}	27.5^{ab}	6.7^{c}	4.3^{a}	91.8^{b}
Lettuce	41.6	44.7^{a}	29.2^{a}	7.6^{b}	4.0^{a}	75.1^{c}
Additive treatment means						
Control	41.3^{ab}	40.1^{b}	24.1^{b}	6.0^{b}	4.2^{a}	122.4^{b}
LAB	47.5^{a}	42.2^{b}	25.7^{b}	6.5^{b}	4.1^{a}	164.1^{a}
BP	39.8^{b}	45.6^{a}	29.3^{a}	9.1^{a}	3.2^{b}	40.3^{c}
BP+LAB	40.6^{b}	46.4^{a}	30.4^{a}	9.2^{a}	3.4^{b}	41.2^{c}
Significance of main effects and interactions						
Vegetable residues (V)	0.056	0.014	0.014	<0.001	<0.001	<0.001
Additive treatment (A)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
V × A	<0.001	0.009	0.01	<0.001	<0.001	<0.001

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

[1]Acetic acid/propionic acid ratio.

[2]Digestible dry matter.

[3]Lactobacillus plantarum (Chikuso-1, Snow Brand Seed, Sapporo, Japan).

[4]Beet pulp.

Table 7. Measurements of dry matter digestibility, methane production, VFA concentration and ammonia-N after 6-h in vitro incubation with rumen fluid of vegetable residue silage (Cao et al., 2011)

4. Conclusions

The results of the present study show that adding LAB increased the lactic acid content of silage, had the potential to increase DM digestibility and to decrease ruminal methane production.

Author details

Yang Cao
College of Animal Science & Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing, China

Yimin Cai
Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

Toshiyoshi Takahashi
Faculty of Agriculture, Yamagata University, Tsuruoka, Japan

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