チモシーおよびオーチャードグラス付着乳酸菌発酵液の添加がサイレージの発酵品質に及ぼす影響

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Effect of Addition of Fermented Juice of Epiphytic Lactic Acid Bacteria Prepared from Timothy and Orchardgrass on Fermentation Quality of Silages

Takayoshi MASUKO, Yoji HARIYAMA, Yuki TAKAHASHI, Li-Man CAO*, Masakazu GOTO* and Mitsuaki OHSHIMA**

Faculty of Bioindustry, Tokyo University of Agriculture, Abashiri 099-2493, Japan
* Faculty of Bioresources, Mie University, Tsu 514-8507, Japan
** Faculty of Agriculture, Meijo University, Nagoya 468-0073, Japan

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Synopsis

MASUKO, T., Y. HARIYAMA, Y. TAKAHASHI, L. CAO, M. GOTO and M. OHSHIMA (2002) : Effect of addition of fermented juice of epiphytic lactic acid bacteria prepared from timothy and orchardgrass on fermentation quality of silages. *Grassland Science* 48, 120-125.

Two experiments were made to investigate the organic acid composition and microbial flora of fermented juice of epiphytic lactic acid bacteria (FJLB) prepared from timothy and orchardgrass. Changes in the composition and microorganisms with days of fermentation and dilution rates were investigated. Two experiments were made to investigate the effect of adding FJLB on the fermentation quality of silages. FJLB was used at various dilution rates and various volumes.

In experiments 1 and 2, FJLB organic acids included lactic acid and acetic acid, and did not include butyric acid in both of the two grass FJLBs. The lactic acid bacteria level in both FJLBs had increased to the range of 10^8-10^{10} cfu ml^{-1} by day 1-2 after incubation. In both grasses, FJLB with a low pH and a high lactic acid bacteria level was prepared even a high dilution rate. Among microorganisms adherent to the grass materials, lactic acid bacteria predomonantly grew in FJLB using sugars contained in the grass materials and glucose added to the culture as the substrate, and FJLB contained lactic acid and acetic acid as the fermentation products.

In experiments 3 and 4, the untreated silages of timothy and orchardgrass were badly preserved. All the groups with treatment with FJLB regardless of the dilution rate in both timothy and orchardgrass were well preserved. Despite the addition of FJLBs of different volumes, all FJLB treated silages for both grasses were well preserved. When FJLB was added, the fermentation quality of the silages improved. The V score was high even when FJLB prepared at a high dilution rate (FJLB×10) was added and even when the additive volume of FJLB (1 ml kg^{-1} of FM) was reduced. These effects may have been due to lactic acid bacteria contained in FJLB that grew during the silage fermentation and produced a large amount of lactic acid, inhibiting butyric acid fermentation.

Key words : Fermentation quality, Fermented juice of epiphytic lactic acid bacteria, Silage, Silage additives.

Introduction

Fermented juice of epiphytic lactic acid bacteria (FJLB) is prepared by culturing microorganisms adherent to the grass materials before preparation of silages and the grown microorganisms are used as a starter of silage fermentation. OHSHIMA *et al.*[7-9] reported that in a laboratory scale study, silages with good fermentation quality can be made from direct cut alfalfa by adding fermented juice of epiphytic lactic acid bacteria (FJLB), a novel additive. Thus an improvement of fermentation quality is expected by the use of FJLB. The pH value of FJLB prepared from Italian ryegrass and alfalfa with adding sugar was in the range of 3.60-4.07, lactic acid bacteria level was in the range of 1.23-2.67×10^8 cfu ml^{-1} [8].

Although commercial inoculant contains excellent species of lactic acid bacteria, FJLB is prepared by culturing microorganisms adherent to the grass materials. Only a few detailed data were available regarding the composition of FJLB. The purpose of this study was to investigate the usefulness of FJLBs containing many different organic acid composition and species of domestic lactic acid bacteria as silage additives.

Outline of this work was presented at the 52th meeting (March 1997) of the Japanese Society of Grassland Science and the 9th Congress of the Asian-Australasian Association of Animal Production (July 2000). This work was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan (No. 07306013).

Table 1. The chemical compositions and viable counts of grass.

	Timothy	Orchardgrass
Growth stage	Heading	Early heading
Cutting time	June 24	June 17
Moisture ($g\ kg^{-1}$ FM)	757	735
Crude protein ($g\ kg^{-1}$ DM)	108	125
WSC[1] ($g\ kg^{-1}$ DM)	70	113
Lactic acid bacteria (cfu g^{-1} DM)	2.3×10^5	6.8×10^6
Aerobic bacteria (cfu g^{-1} DM)	4.5×10^7	6.0×10^{10}
Yeasts (cfu g^{-1} DM)	nd[2]	nd
Moulds (cfu g^{-1} DM)	2.9×10^3	3.4×10^4

1) Water soluble carbohydrate. 2) Not detected.

Materials and Methods

Plant materials

First cut timothy (*Phleum pratense* L.) harvested at heading stage on 24 June 1996, and first cut orchardgrass (*Dactylis glomerata* L.) at early heading on 17 June 1996 were used. The grass was cut into about 2 cm length before ensiling. The chemical compositions and viable counts of grass are shown in Table 1.

Fermented juice preparation

Experiment 1

FJLB was prepared from timothy and orchardgrass by the following method. Briefly 100 g of fresh grass were macerated with 500 ml of water using a blender. The macerate was filtered through double cheese cloth and 500 ml portions of the filtrate were collected into a 900 ml glass bottle to which 20 gl^{-1} of glucose was added. The bottle was fitted with a gas trap and preserved anaerobically at 30℃ for 4 days.

Experiment 2

FJLB was prepared from timothy and orchardgrass. One hundred grams of fresh grass were macerated with 300 (FJLB×3), 500 (FJLB×5) or 1000 (FJLB×10) ml of water using a blender. Twenty grams l^{-1} of glucose were added to a glass bottle containing the blended mixture and preserved anaerobically at 30℃ for 2 days.

Silage preparation

Experiment 3

Timothy and orchardgrass were ensiled in 900 ml laboratory silos with gas traps with water, and FJLB ×3, FJLB×5 or FJLB×10 at a level of 2 $ml\,kg^{-1}$ of fresh chopped grass. The silos were maintained at 25℃ and opened after 45 days. Three replicate silos were prepared for each treatment.

Experiment 4

Timothy and orchardgrass were ensiled in the same silos with water at a level of 10 $ml\,kg^{-1}$ or FJLB ×5 at a level of 1, 2, 5 or 10 $ml\,kg^{-1}$ of fresh chopped grass. The silos were maintained at 25℃ and opened after 45 days. Three replicate silos were prepared for each treatment.

Chemical analysis

The chemical composition of the grass and silage were determined using ground samples oven-dried at 60℃ for 48 hours. The fermentation quality of silage was determined using water extracts prepared from macerated materials. Fifty grams of each silage was macerated with 100 ml of water using a blender. Dry matter contents of grass and silage were determined by oven-drying at 135℃ for 2 hours. Crude protein was determined using the Kjeldahl method. WSC content was estimated colorimetrically using anthrone[12]. The pH value was determined with a pH meter. Lactic acid content was determined using the colorimetric method of BARKER and SUMMERSON[12]. Ammonia nitrogen (N) was measured using steam distillation[12]. Volatile fatty acids (VFA) was measured using gaschromatography (GC-12A, Shimadzu Co., Ltd.)[2]. V score was evaluated using the values of organic acids and ammonia N ($g\,kg^{-1}$ TN)[11].

Microbiological analysis

Ten grams of silage were shaken with 90 ml of sterilizer 0.85% NaCl solution, and 10^{-1}–10^{-9} serial dilutions were made in sterile 0.85% NaCl solution. The plating media were GYP-calcium carbonate agar[3] for lactic acid bacteria, bouillon agar for aerobic bacteria and YM agar[1] containing 0.5% lactic acid for moulds and yeasts. The plates for lactic acid bacterial and aerobic bacterial counts were incubated for 5 days at 35℃ and plates for mould and yeast counts were incubated for 6 days at 25℃. The viable colonies were counted using the dilution plate method.

Statistical analysis

Statistical analysis was performed using one way layout design and mean values were compared using Fisher's least significant difference test.

Results

Organic acid and microbiological composition of FJLB

Experiment 1

The changes in pH value, organic acid and viable counts of FJLB are shown in Table 2. The pH value of timothy and orchardgrass FJLBs decreased to 3.6 by day 1 and 3.2 by day 4 after incubation. FJLB organic acids included lactic acid and acetic acid, and did not include butyric acid in both of the two grass FJLBs. The lactic acid bacteria level in both FJLBs had increased to the range of 10^8–10^{10} $cfu\,ml^{-1}$ by day 1–2 after incubation and were at least kept a high level 10^{10} $cfu\,ml^{-1}$ thereafter. The aerobic bacteria

Table 2. The changes in pH value, organic acid and viable counts of FJLB (Experiment 1).

	pH	Lactic acid (gl^{-1})	Acetic acid (gl^{-1})	Butyric acid (gl^{-1})	Lactic acid bacteria (cfu ml^{-1})	Aerobic bacteria (cfu ml^{-1})	Yeasts (cfu ml^{-1})	Moulds (cfu ml^{-1})
Timothy								
0[1)]	4.70	—	—	—	8.0×10^3	2.8×10^5	nd[2)]	2.8×10^2
1	3.67	4.03	3.09	0	3.5×10^{10}	5.3×10^6	4.5×10^3	nd
2	3.31	6.69	2.12	0	1.5×10^{10}	2.1×10^7	7.6×10^5	nd
3	3.25	2.65	0.87	0	1.7×10^{10}	4.1×10^9	1.4×10^5	nd
4	3.21	3.52	0.70	0	5.2×10^{10}	4.3×10^{10}	5.3×10^4	nd
Orchardgrass								
0	5.31	—	—	—	4.4×10^3	4.4×10^5	nd	nd
1	3.63	4.75	2.89	0	8.6×10^8	1.0×10^7	nd	nd
2	3.48	3.18	1.97	0	2.0×10^9	1.0×10^5	1.1×10^3	nd
3	3.23	4.06	1.50	0	1.2×10^{10}	1.1×10^6	3.0×10^5	nd
4	3.13	5.69	1.24	0.02	1.9×10^{10}	5.3×10^6	7.1×10^6	1.5×10^2

1) The days of incubating in fermented juices. 2) Not detected.

levels in timothy FJLB increased with time after incubation, but that of orchardgrass FJLB did not markedly increase during incubation. Yeasts were not detected at the beginning of incubation but were noted during incubation. Moulds were detected at a level of 10^2 cfuml^{-1} at the beginning of incubation, but were not detected or levels were low thereafter.

Experiment 2

The pH value, organic acid and viable counts in FJLB prepared from different dilution of juices are shown in Table 3. The pH value in timothy and orchardgrass FJLBs were in the range of 3.3–3.7 regardless of the dilution of juices. The production of lactic acid was highest in 5-fold dilution (FJLB×5) of timothy FJLB and 3-fold dilution (FJLB×3) of orchardgrass FJLB. Butyric acid was not produced in all FJLB dilutions. Overall, lactic acid bacteria levels were 10^8 cfuml^{-1}, aerobic bacteria was 10^4–10^7 cfuml^{-1}, yeasts were 10^3–10^5 cfuml^{-1} and moulds were $<10^3$ cfuml^{-1} in both of the two grass FJLBs.

Fermentation quality of silages

Experiment 3

The effect of adding FJLBs of different dilutions on fermentation quality of silages are shown in Table 4. The untreated silages of timothy and orchardgrass were badly preserved, with a high pH value, butyric acid and ammonia N contents, low lactic acid content and V score. All the groups with treatment with FJLB regardless of the dilution rate in both timothy and orchardgrass were well preserved, the pH value, butyric acid and ammonia N contents were significantly ($P<0.05$, $P<0.01$) lower, and the lactic acid content and V score were significantly ($P<0.05$, $P<0.01$) higher than those of the untreated silages. Compared with silage prepared at various dilution rates of FJLBs, 5-fold dilu-

Table 3. The pH value, organic acid and viable counts in FJLB (Experiment 2).

	pH	Lactic acid (gl^{-1})	Acetic acid (gl^{-1})	Butyric acid (gl^{-1})	Lactic acid bacteria (cfu ml^{-1})	Aerobic bacteria (cfu ml^{-1})	Yeasts (cfu ml^{-1})	Moulds (cfu ml^{-1})
Timothy								
FJLB×3[1)]	3.48	3.70	2.70	0	2.6×10^9	1.5×10^5	5.3×10^3	3.5×10^2
FJLB×5[2)]	3.31	6.69	2.12	0	1.5×10^{10}	2.1×10^7	7.6×10^5	nd[4)]
FJLB×10[3)]	3.69	3.48	2.88	0	6.4×10^9	9.0×10^6	1.3×10^4	1.1×10^3
Orchardgrass								
FJLB×3	3.53	4.38	2.56	0	3.0×10^9	8.0×10^5	1.9×10^5	5.0×10^2
FJLB×5	3.48	3.18	1.97	0	2.0×10^9	1.0×10^5	1.1×10^3	nd
FJLB×10	3.49	2.70	2.26	0	4.9×10^8	1.0×10^4	1.4×10^4	nd

1) One hundred grams of fresh grass were macerated with 300 ml of a 20 gl^{-1} glucose solution.
2) One hundred grams of fresh grass were macerated with 500 ml of a 20 gl^{-1} glucose solution.
3) One hundred grams of fresh grass were macerated with 1000 ml of a 20 gl^{-1} glucose solution.
4) Not detected.

Table 4. The effect of adding FJLBs of different dilutions on fermentation quality of silages (Experiment 3).

	Timothy silage					Orchardgrass silage				
	Untreated	FJLB[1)] ×3[3)]	FJLB ×5[4)]	FJLB ×10[5)]	SEM[6)]	Untreated	FJLB[2)] ×3	FJLB ×5	FJLB ×10	SEM
pH	4.97^{A} [7)]	3.80^{B}	3.80^{B}	3.81^{B}	0.153	5.40^{A}	3.97^{B}	3.89^{B}	4.04^{B}	0.192
Lactic acid (g kg^{-1} DM)	24.4^{d}	60.0^{c}	99.6^{ab}	81.0^{bc}	9.31	14.4^{d}	68.6^{bc}	100.0^{a}	47.0^{c}	10.22
Acetic acid (g kg^{-1} DM)	18.9^{a}	7.1^{b}	11.5^{ab}	10.8^{ab}	1.93	17.5	15.6	17.0	12.3	1.07
Propionic acid (g kg^{-1} DM)	39.4^{A}	2.5^{B}	1.2^{B}	1.0^{B}	5.71	37.8^{A}	1.2^{B}	1.2^{B}	0.7^{B}	4.80
Butyric acid (g kg^{-1} DM)	20.3^{a}	0^{b}	0^{b}	0.1^{b}	3.26	6.5^{A}	0^{B}	0^{B}	0^{B}	0.95
Ammonia N (g kg^{-1} TN)	171^{A}	73^{B}	67^{B}	62^{B}	13.7	204^{A}	69^{B}	63^{B}	70^{B}	18.0
V score	29.0^{B}	93.7^{A}	95.0^{A}	96.0^{A}	8.76	33.3^{B}	94.0^{A}	95.3^{A}	95.7^{A}	8.10

1) FJLB was prepared from timothy. 2) FJLB was prepared from orchardglass. 3), 4) and 5) see footnote of Table 2. 6) Standard error of the means. 7) Means with different superscripts in a row for treatments of each grass species are significantly different (a, b, c, d ; $P<0.05$, A, B ; $P<0.01$).

tion (FJLB×5) treatment increased the lactic acid content significantly ($P<0.05$) in both grasses but the V score was similar.

Experiment 4

The effect of adding FJLBs of different volumes on fermentation quality of silages are shown in Table 5. The untreated timothy and orchardgrass silages were poorly preserved, with a high pH value, low lactic acid content, high butyric acid and ammonia N contents and low V score. Despite the addition of FJLBs of different volumes, all FJLB treated silages for both grasses were well preserved, the pH value, butyric acid and ammonia N contents were significantly ($P<0.01$) lower, and the lactic acid content and V score were significantly ($P<0.05$, $P<0.01$) higher than those of the untreated silages. Compared with silages prepared at various volume FJLB, the pH value, butyric acid and ammonia N contents and V score showed similar level.

Discussion

Characteristics of FJLB

Using FJLBs prepared from timothy and orchardgrass, the authors investigated changes in the composition with days of fermentation and dilution rate. In both grasses, lactic acid and acetic acid had already been produced on day 1 of fermentation, and the pH decreased to 3.6. No or a minute of butyric acid was produced. The lactic acid bacteria levels markedly increased, reaching 10^{8} cfu ml^{-1} or higher on day 1 of fermentation. The aerobic bacteria levels ranges from 10^{5} to 10^{10} cfu ml^{-1}, and the levels increased with days of fermentation in timothy FJLB. The yeast levels ranges 10^{3} to 10^{6} cfu ml^{-1}, and yeasts tended to increase with days of fermentation in orchardgrass FJLB. The growth of moulds was inhibited. FJLB were stable for four days after the initiation of fermentation, but based on the

Table 5. The effect of adding FJLBs of different volumes on fermentation quality of silages (Experiment 4).

	Timothy silage						Orchardgrass silage					
	Untreated	1 ml kg^{-1} [1)]	2 ml kg^{-1}	5 ml kg^{-1}	10 ml kg^{-1}	SEM[3)]	Untreated	1 ml kg^{-1} [2)]	2 ml kg^{-1}	5 ml kg^{-1}	10 ml kg^{-1}	SEM
pH	4.83^{A} [4)]	3.81^{B}	3.78^{B}	3.78^{B}	3.77^{B}	0.113	5.55^{A}	3.80^{B}	3.77^{B}	3.77^{B}	3.74^{B}	0.191
Lactic acid (g kg^{-1} DM)	24.6^{c}	65.8^{a}	58.0^{ab}	48.7^{b}	53.7^{ab}	4.08	6.9^{B}	69.5^{A}	69.9^{A}	62.1^{A}	71.8^{A}	7.03
Acetic acid (g kg^{-1} DM)	15.7	19.3	16.9	10.8	10.4	1.21	16.7^{a}	15.2^{ab}	12.6^{ab}	12.4^{b}	13.4^{ab}	0.67
Propionic acid (g kg^{-1} DM)	17.6^{A}	1.7^{B}	0.7^{B}	0.7^{B}	0.6^{B}	1.83	39.6^{A}	1.7^{B}	0.7^{B}	0.7^{B}	0.6^{B}	4.16
Butyric acid (g kg^{-1} DM)	4.7^{A}	0.1^{B}	0^{B}	0^{B}	0^{B}	0.51	16.8^{A}	0.3^{B}	0^{B}	0^{B}	0^{B}	1.79
Ammonia N (g kg^{-1} TN)	230^{A}	83^{B}	83^{B}	89^{B}	83^{B}	15.6	206^{A}	78^{B}	70^{B}	70^{B}	75^{B}	14.4
V score	39.3^{B}	91.7^{A}	92.0^{A}	91.0^{A}	91.0^{A}	5.57	15.3^{B}	93.0^{A}	95.0^{A}	94.7^{A}	93.3^{A}	8.42

1) FJLB was prepared from timothy. 2) FJLB was prepared from orchardglass. 3) Standard error of the means. 4) Means with different superscripts in a row for treatments of each grass species are significantly different (a, b, c ; $P<0.05$, A, B ; $P<0.01$).

growth state of aerobic bacteria and yeasts, it was considered appropriate to use FJLB within two days of fermentation.

To obtain a large volume of FJLB, the authors prepared FJLBs at various dilution rates. In orchardgrass FJLB, when the dilution rate was high, the amount of lactic acid production and the lactic acid bacteria level decreased, but a lactic acid bacteria level of 10^8 cfu ml^{-1} was maintained. In both grasses, FJLB with a low pH and a high lactic acid bacteria level was prepared even a high dilution rate. These values were close to pH 3.88 and 1.8×10^8 cfu g^{-1} lactic acid bacteria in alfalfa FJLB prepared at 20 -fold dilution by OHSHIMA *et al.*[7)].

Based on these findings, among microorganisms adherent to the grass materials, lactic acid bacteria predominantly grew in FJLB using sugars contained in the grass materials and glucose added to the culture as the substrate, and FJLB contained lactic acid and acetic acid as the fermentation products.

Fermentation quality of silages

To investigate the effect of adding FJLB on the fermentation quality of silages, the authors examined various dilution rates and various volumes of FJLB. In FJLB×10, which was prepared at the highest dilution rate, the lactic acid bacteria levels were 6.4×10^9 cfu ml^{-1} and 4.9×10^8 cfu ml^{-1} in timothy and orchardgrass, respectively. In the 1 ml kg^{-1} group to which the lowest volume of FJLB was added, the lactic acid bacteria level were 1.5×10^{10} cfu ml^{-1} and 2.0×10^9 cfu ml^{-1} in timothy and orchardgrass, respectively. Therefore, based on the volume of FJLB added at the preparation of the silage, 9.8×10^5–1.5×10^7 cfu of lactic acid bacteria per 1 g of FM grass materials was added. This lactic acid bacteria level was equal to the level added using a commercial lactic acid bacteria preparation, and this level met the amount of 10^6 cfu g^{-1} required for facilitating lactic acid fermentation[5,6)].

The quality of silage was poor in the groups without treatment with FJLB in both timothy and orchardgrass. The WSC contents were 70 g kg^{-1} of DM and 113 g kg^{-1} of DM the timothy material and orchardgrass material, respectively. The epiphytic lactic acid bacteria levels were 2.3×10^5 cfu g^{-1} of DM and 6.8×10^6 cfu g^{-1} of DM, respectively. These values were close to the conditions for the preparation of good silages : WSC content : 100 g kg^{-1} of DM[4)], lactic acid bacteria level : 10^6 cfu g^{-1} [5,6)]. However, the silage in the group without treatment with FJLB was poor even through the level of lactic acid bacteria adherent to the grass material was 10^5–10^6 cfu g^{-1} of DM, suggesting that there may have been problems in the growth ability and lactic acid productivity of lactic acid bacteria.

When FJLB was added, the fermentation quality of the silages improved. The V score was high even when FJLB prepared at a high dilution rate (FJLB× 10) was added and even when the additive volume of FJLB (1 ml kg^{-1} of FM) was reduced. Therefore, fermentation quality improved regardless of the dilution rate and the additive volume of FJLB. These effects may have been due to lactic acid bacteria contained in FJLB that grew during the silage fermentation and produced a large amount of lactic acid, inhibiting butyric acid fermentation. The lactic acid bacteria level reached 10^6 cfu g^{-1} of FM even when the level in the FJLB was low, suggesting that addition of lactic acid bacteria higher than a certain level improves fermentation quality even if the dilution rate and additive volume of FJLB vary. OHSHIMA *et al.*[7-9)] reported that the addition of FJLB improved the fermentation quality of alfalfa silage, and the effect of the addition of FJLB was also observed in the legumes in addition to grasses tested in this study.

Other factors involved in the effect of adding FJLB include organic acids in FJLB. Lactic acid and acetic acid were the major components, and the maximum contents were 6.69 g l^{-1} and 2.70 g l^{-1}, respectively. The lactic acid content in the highest additive volume 10 ml kg^{-1} of FM was 66.9 mg kg^{-1} of FM. The pH reducing ability of lactic acid is high next to formic acid[10)], and it was reported that the amount of lactic acid required to reduce the pH to 4.5 was 6.3 g kg^{-1} of FM in alfalfa. Since lactic acid contained in FJLB was only 1.1% of the amount required for reducing the pH, lactic acid may not reduce the pH.

In addition to lactic acid bacteria, aerobic bacteria and yeasts are found in FJLB, and these microorganisms are causative of aerobic deterioration after the silo is opened. Therefore, it is possible that the addition of FJLB induces aerobic deterioration, but this remains to be investigated. To elucidate the characteristics of lactic acid bacteria in FJLB, it is necessary to identify and investigate the species of lactic acid bacteria, and compare the effect of adding FJLB with commercial lactic acid bacterial preparations.

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* : In Japanese with English summary.

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要　旨

増子孝義・張山陽司・高橋由紀・曹　力蔓・後藤正和・大島光昭 (2002)：チモシーおよびオーチャードグラス付着乳酸菌発酵液の添加がサイレージの発酵品質に及ぼす影響．日草誌 **48**, 120-125. 東京農業大学生物産業学部（099-2493 北海道網走市字八坂 196）

実験1と2では，チモシーおよびオーチャードグラスから作製された牧草付着乳酸菌発酵液（FJLB）を供試して，発酵日数と希釈倍率による成分変動を調べた。実験3と4では，FJLB の添加がチモシーサイレージおよびオーチャードグラスサイレージの発酵品質に及ぼす影響を調べるために，希釈倍率を変えて作製した FJLB と異なる添加量の FJLB を供試した。

FJLB は両草種ともに，発酵1日目ですでに乳酸や酢酸が生成され，pH は 3.6 まで低下した。酪酸は生成しないか，生成されても微量であった。乳酸菌の増殖は著しく，1日目に 10^8 cfu ml^{-1} 以上に達した。FJLB は4日目を経過しても，安定的であったが，好気性細菌と酵母菌の増殖経過から判断すると，発酵2日目をめどに使用するのが望ましいと考えられた。FJLB の量を確保し，多量の添加に備えたいと考え，希釈倍率を変えて FJLB の作製を行った。両草種ともに，希釈倍率を高くしても pH が低く，乳酸菌数の多い FJLB が作製された。FJLB は材料草に付着する微生物群のうち，乳酸菌種が材料草の糖や培養時に添加したグルコースを基質として，優勢に増殖したものであり，その発酵生産物として乳酸や酢酸を含んでいることが明らかとなった。

サイレージ調製時に添加された FJLB 量から，新鮮物1g当たり $9.8\times10^5-1.5\times10^7$ cfu の乳酸菌が添加されたことになる。無添加区のサイレージの発酵品質は，チモシーとオーチャードグラスともに劣質であった。FJLB を添加すると，サイレージの発酵品質が改善された。希釈倍率を高めた FJLB（FJLB×10）を添加しても，添加量を少なくしても（1 ml kg^{-1}），V スコアーが高かった。すなわち，FJLB の希釈倍率や添加量にかかわらず改善効果が認められた。このような添加効果は，FJLB 中の乳酸菌がサイレージ発酵過程で増殖し，多量の乳酸生成をもたらし，酪酸発酵が抑制されたことにより発現されたものと考えられた。

キーワード：サイレージ，サイレージ添加物，乳酸菌発酵液，発酵品質，牧草付着．