Effects of Isolated and Commercial Lactic Acid Bacteria on the Silage Quality, Digestibility, Voluntary Intake and Ruminal Fluid Characteristics

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ABSTRACT : Silage is a major component of cattle rations, so the improvement of silage quality by the inoculation of lactic acid bacteria is of great interest. In this study, commercially distributed *Lactobacillus plantram* and *Lactobacillus rhamnousas* NGRI 0110 were used for ensilaging of guinea grass. The four treatments used were a control silage, a silage with cellulase addition, a silage with cellulose+*L. plantram* addition, and a silage with cellulose + NGRI 0110 addition. Silage quality, voluntary intake, nutrient digestibility, and the characteristics of ruminal fluid of wethers were investigated. Silage to which lactic acid bacteria were added showed low pH and acetic acid concentration and the highest lactic acid content. Dry matter and organic matter digestibility were significantly ($p<0.05$) increased by cellulase addition and significantly ($p<0.05$) higher values were observed in *L. plantram*- and NGRI 0110-added silage. Voluntary intake of NGRI 0110-added silage was the highest and that of control silage was the lowest. We concluded that the observed ability of NGRI 0110 to tolerate low pH and to continue lactic acid fermentation in high lactic acid concentration had also occurred in actual ensilaging. The results indicate that the addition of lactic acid bacteria might improve silage quality and increase digestibility and voluntary intake. The potential for improvement by NGRI 0110 was higher than that to be gained by the use of commercially available lactic acid bacteria. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 3 : 386-389)***

Key Words : Silage, Lactic-acid Bacteria, Guinea Grass, Cellulase

INTRODUCTION

Silage is a major component of the ration of cattle. The use of high-quality silage (that is, silage with low pH, high lactic and low butyric acid content) may improve cattle performance by the conservation of nutrients and the probiotic effect of lactic acid bacteria (Guan Wu-Tai et al., 2003; Weinberg et al., 2004a, b). Consequently, there have been many attempts to use lactic acid bacteria as silage inoculants to improve silage fermentation quality (Oyama et al., 1971; Oyama et al., 1973; Wolford et al., 1984). The ability to produce lactic acid and tolerance to low pH are important criteria to consider when selecting the strains of lactic acid bacteria for inoculants. *Lactobacillus rhamnousas,* NGRI 0110 was found on Okinawa island. It has the ability to produce lactic acid and is tolerant of low pH (Tanaka et al., 1994), which appears to make it a good candidate as a silage inoculant. In this study, to clarify the potential of NGRI 0110 as a silage inoculant, the effects of adding NGRI 0110 and a commercially distributed *Lactobacillus plantram* on guinea grass silage quality were investigated. Furthermore, voluntary intake and nutrient digestibility of the experimental silages by wethers and the characteristics of ruminal fluid of wethers were investigated.

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MATERIALS AND METHODS

Lactic acid bacteria

Lactobacillus rhamnousas NGRI 0110 and commercially distributed *Lactobacillus plantram* (Yukijirusi-syubyou Co Ltd.) were used. Lactobacillus inoculants were prepared in MRS broth (Difco Laboratories) in 1-liter bottles by incubation at 37°C for 48 h. Each lactobacillus culture was then added to 200 kg of the plant materials at 10^7 cells/g with a sprinkling can.

Fodder plant

Guinea grass (seeded in May 15, harvested in August 21) dried for 24 h at ambient temperature (dry matter 18.7%, average particle size 10 mm) was used.

Ensilaging

The following four treatments were prepared : No addition; Cellulase (ACS-2, origin from *Acremonium celluloyticus* at addition rate of 0.02% of fresh matter and containing 850 U/g avicelase, 27,000 U/g

Table 1. Fermentation quality of silages

	Control	Cellulase	L. *plantarum*	NGRI0110
pH	5.30	4.34	4.21	4.10
Lactic acid (% DM)	0.56	1.24	1.67	1.66
Acetic acid (% DM)	0.50	0.28	0.50	0.17
Butyric acid (% DM)	0.03	0.01	0.00	0.00
Total (% DM)	1.09	1.53	2.17	1.83
VBN (% DM)	0.09	0.06	0.07	0.04

Table 2. Chemical composition of silages

	Control	Cellulase	L. *plantarum*	NGRI0110
Dry matter (%)	18.2	18.0	18.3	18.8
Organic matter (% DM)	86.6	86.6	86.5	86.7
Crude protein (% DM)	14.1	14.6	15.8	15.2
Ether extract (% DM)	2.8	3.3	3.6	3.4
Crude fiber+NFE (% DM)	69.6	68.7	68.1	68.1
ADF (% DM)	38.4	33.0	32.5	32.4
NDF (% DM)	32.6	54.4	53.9	55.3

carboxymethylcellulase, 13,000 U/gxylanase and 206 U/g pectinase); Cellulase + *L. plantram*; and Cellulase + NGRI 0110. After mixing guinea grass with the supplements, the mixtures were ensiled in 200 L drums which were tightly sealed by steel holders. Ten silos were prepared for each treatment. Silages were stored for 45 days and then used for the measurement of silage quality, voluntary intake, and digestibility. Samples (1%) of silage were stored at -20°C until analysis.

Measurement of voluntary intake and digestibility

Four silages were allotted to a 4×4 latin square using four Suffolk wethers (average body weight 55 kg). Wethers were kept in metabolic cages from which faeces could be collected. One period consisted of three weeks (one week for adaptation, one week for measurement of voluntary intake, one week for measurement of digestibility). For the measurement of voluntary intake, silages were offered until there was 10% refusal of the amount fed once a day (9:00). For the measurement of digestibility, silages equivalent to 500 g of dry matter were fed once a day (9:00). All faeces were collected and dried at 60°C for 48 h. On the last day of the digestion trial, rumen fluid was collected via oral catheter.

Analysis of silages, faeces, and rumen fluid

VFA and lactic acid of silages were measured by HPLC (Ohmomo et al., 1993) and volatile basic nitrogen (VBN) was detected by the Phenol-Hypochloride reaction method (Weatherburn, 1967). ADF and NDF of silages and faeces were analyzed by the method of Van Soest (1991). Analyses of ash, crude protein, and ether extract were conducted following the method of AOAC (1996). VFA and VBN contents of rumen fluid were measured by the same method used for silage.

RESULTS

Silage quality and chemical composition

Table 1 shows the quality of silages. A lower pH value was observed in cellulase-added silage than in silage without cellulase. The pH of *L. plantram*- and NGRI 0110-added silage was lower than that in cellulase-added silage. Lactic acid content was higher in the cellulase-added silage than in silage without cellulase. Lactic acid content of *L. plantram*- and NGRI 0110-added silage was higher than in cellulase-added silage. Acetic acid content of the NGRI 0110-added silage was lower than that of the other three silages. Butyric acid was not detected in *L. plantram*- or NGRI 0110-added silage. VBN content of NGRI 0110-added silage was lower than that of the other three silages. Table 2 shows the chemical composition of silages. CP and EE contents did not differ among silages. NDF and ADF contents were reduced by cellulase addition.

Nutrient digestibility and voluntary intake

Table 3 shows the digestibility and voluntary intake of silages. Dry matter and organic matter digestibility was significantly ($p<0.05$) increased by cellulase addition. Furthermore, significantly ($p<0.05$) higher values were observed in *L. plantram*- and NGRI 0110-added silage. CP digestibility of *L. plantram*- and NGRI 0110-added silage was significantly ($p<0.05$) higher than in control and cellulase-added silage. Voluntary intake of NGRI 0110-added silage was the highest. Voluntary intake of control silage was the lowest. A significant ($p<0.01$) difference was observed between the two silages.

Table 4 shows the pH and VFA content of ruminal fluid. Ruminal pH tended to be higher in the wethers fed *L. plantram*- and NGRI 0110-added silages. Ruminal acetic acid content of wethers fed control silage tended to be

Table 3. Digestibility and Voluntary intake of silages

	Control	Cellulase	L. *plantarum*	NGRI0110
Digestibility				
Dry matter (%)	59.4±0.9a	62.7±1.1b	64.1±0.8c	64.4±0.7c
Organic matter (% DM)	60.2±1.2a	63.5±0.7b	64.9±1.5b	65.0±1.3b
Crude protein (% DM)	62.6±3.1A	61.0±2.3A	72.7±2.4B	69.9±1.9B
Ether extract (% DM)	58.9±2.5A	67.4±2.7B	67.5±1.9B	67.5±2.6B
Crude fiber+NFE (% DM)	63.0±3.2	64.4±2.3	63.4±3.8	68.1±1.9
ADF (% DM)	60.1±2.3	58.8±2.5	58.8±2.9	60.3±2.4
NDF (% DM)	61.6±2.6	59.1±3.6	58.0±1.7	60.1±2.1
Voluntary Intake (g DM/day)	638.0±20.3A	846.0±19.3B	885.0±17.6B	928.0±15.6C

Table 4. Characteristics of ruminal fluid

	Control	Cellulase	L. *plantarum*	NGRI0110
pH	6.74±0.3	6.77±0.2	6.96±0.4	6.90±0.3
Total VFA (mM)	85.8±5.6	86.1±6.5	86.2±5.2	84.0±4.8
Acetic acid (%)	72.2±3.9	67.4±6.2	67.5±4.3	68.2±4.6
Propionic acid (%)	19.0±2.5	22.7±3.1	24.1±4.5	23.0±3.7
Isobutyric acid (%)	1.1±0.4	1.0±0.5	1.0±0.4	1.0±0.3
Butyric acid (%)	5.2±0.7	5.9±1.0	5.2±0.8	5.3±0.9
Isovaleric acid (%)	1.4±0.6	1.4±0.5	1.2±0.4	1.3±0.5
Vareric acid (%)	1.0±0.3	1.4±0.4	0.9±0.5	1.1±0.3
Capronic acid (%)	0.1±0.1	0.2±0.1	0.1±0.1	0.1±0.1

higher than other silages. Conversely, ruminal propionic acid content of the control group tended to be lower than that of wethers fed other silages.

DISCUSSION

In previous laboratory studies, it was reported that NGRI 0110 had a good ability to tolerate low pH and to continue lactic acid fermentation in high lactic acid concentration (Tanaka et al., 1994). In this study, NGRI 0110-added silage showed low pH and acetic acid concentration and high lactic acid content, which indicates that the ability of NGRI 0110 to tolerate low pH and to continue lactic acid fermentation in high lactic acid concentration had also occurred in actual ensilaging. It was reported previously that feeding silage inoculated with lactic acid bacteria improved feed intake and feed efficiency (Kung et al., 2003). In this study, the addition of lactic acid bacteria increased the digestibility of dry matter, organic matter, and crude protein and also voluntary intake. These results support those reported earlier. Ruminal acetic acid content of wethers fed the control silage tended to be higher than that of wethers fed other silages, and ruminal propionic acid content of control wethers tended to be lower than that of wethers fed other silages. This result shows that silage to which lactic acid bacteria were added contained more fermentable matter, which might be the reason for the increased digestibility and voluntary intake. On the other hand, it was reported that lactic acid bacteria have a probiotic effect (Yoon and Stern, 1995; Weinberg et al., 2004a, b), so the increase of digestibility and voluntary intake by the addition of lactic acid bacteria may be a result of its probiotic effect.

Given all of these results, it can be concluded that the addition of lactic acid bacteria might improve silage quality and increase digestibility and voluntary intake. The ability for improvement resulting from the addition of NGRI 0110 was higher than that gained by the use of commercially available lactic acid bacteria.

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