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Research Article

Effect of Lactic Acid Bacteria Treatment on Nutritive Value and *In Vitro* Ruminal Fermentation of Italian Ryegrass (*Lolium multiflorum* L.) Silage

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ABSTRACT

This study was conducted to evaluate the effect of lactic acid bacteria (LAB) inoculation to domestically-cultivated Italian ryegrass (IRG) on silage fermentation and *in vitro* ruminal fermentation. There were six treatments based on the LAB inoculants: 1) no addition of LAB (negative control: NC), additions of 2) commercially-available LAB (positive control: PC), 3) *Lactobacillus plantarum* (LPL), 4) *L. paracasei* (LPA), 5) *L. acidophilus* (LA), and 6) *L. pentosus* (LPT). All treatments were inoculated at a concentration of 10^6 CFU/g and ensiled for 3, 7, 21, and 42 days in triplicate and analyzed for nutritive values when ensiling was terminated. Day 42 silage from all treatments were also examined for *in vitro* ruminal fermentation. After 42 days, LAB-inoculated silages had higher (P<0.05) lactic acid concentration compared to the NC. In terms of nutritive values, the silages treated with LPA, LA, and LPT showed higher (P<0.05) crude protein and lower (P<0.05) neutral detergent fiber and acid detergent fiber content compared to the rest of the treatment. *In vitro* ruminal dry matter degradability was not affected by LAB addition. However, LAB-treated IRG had shown higher (P<0.05) ammonia-N compared with that of the NC. LPA had shown the highest (P<0.05) volatile fatty acid concentration among the LAB examined. In conclusion, the addition of a single strain of LAB appeared to produce a quality IRG silage compared with the NC and the PC. Among the strains examined, LPA seemed to be superior to the others.

(**Key words**: Italian ryegrass, Silage, Lactic acid bacteria, Additives)

Ⅰ. INTRODUCTION

Italian ryegrass (*Lolium multiflorum* L.) occupies 52% of the domestic fodder crop cultivation area and is widely cultivated in livestock farms as a high-quality winter crop feed crop (Kim et al., 2011). The varieties of Italian ryegrass (IRG) bred in Korea are particularly advantageous for cultivation as it has a high content of water-soluble carbohydrates, and when fed to ruminants (Kim et al., 2011), and it is known as an excellent feed resource because of its palatability and digestibility. In Korea, IRG is mainly manufactured in the form of silage and supplied to livestock (Kim et al., 2011; Kim et al., 2015).

Silage is stabilized through several stages of fermentation, and it is very important to create an environment that can help the growth of microorganisms involved in the fermentation process, especially lactic acid bacteria, in the early stages of fermentation after preparation (Bolsen et al., 1996). Several methods, such as the addition of silage additives, have been reported. They included the addition of water-soluble carbohydrates (i.e., molasses), fiber-degrading enzymes (i.e., cellulase, hemicellulase), organic acids (i.e., formic acid) and lactic acid bacteria (LAB) to induce rapid initial fermentation (Muck et al., 2018). In terms of LAB, several studies have shown that the addition of LAB not only promotes lactic acid fermentation but also improves the quality of the fermentation, reduces the loss of dry matter, and suppresses aerobic spoilage (Kim et al., 2008; Nsereko et al., 2008; Santos et al., 2013). Lactic acid bacteria are mainly effective in improving silage quality in storage, reducing the rate of dry matter loss and reducing microbial protein degradation, and helps stabilize fermentation by lowering the pH quickly (Seale, 1986). Besides, it has been reported that LAB affects improving the palatability and feed intake of livestock by reducing the butyrate production (Buchanan-Smith, 1990), and butyrate may be associated with the stench in silage, which is usually

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the decreasing causes of silage palatability and feed intake.

In the rumen, particular silage inoculant LAB (i.e., *L. pentosus* and *L. plantarum*) could compete with rumen microflora and affect the rumen pH and enhanced VFA production (Weinberg et al., 2004). The LAB mostly produced high lactic acid during the ensiling period, resulted in propionate production in the rumen (Huhtanen et al., 1997). Silage inoculated by LAB showed the potential to enhance the digestibility and ruminant performance (Weinberg et al., 2007). In this study, we hypothesized that the inoculation of specific LAB strains to IRG could improve the silage quality. Therefore, this study was conducted to investigate the effect of inoculation of various lactic acid bacteria on nutrient values and *in vitro* rumen fermentation characteristics of IRG silage.

Ⅱ. MATERIALS AND METHODS

1. Experimental design

This experiment used IRG (*Lolium multiflorum* L., cultivar: kowinearly) grown and harvested from the field of a commercial Hanwoo farm in Cheongni-myeon (36.335869, 128.121090), Sangju-si, Gyeongsangbuk-do on May 19, 2014. Immediately harvesting rice grain, the IRG was seeded at 60 kg/ha in early October with 400 kg/ha of fertilizer at the ratio of 21:17:17 for N:P:K, respectively. Approximately 300 kg of fresh and wilted (over a day) IRG was divided into total six treatments: negative control (NC, without additives), positive control (PC, inoculation of commercially-available silage additives), and the strains of several Lactobacillus isolated and identified by Genebiotech Co., Ltd. including *Lactobacillus plantarum* (LPL), *L. paracasei* (LPA), *L. acidophilus* (LA), and *L. pentosus* (LPT). The commercially available silage additive inoculated on PC contained *L. acidophilus* (4.4×10^8 CFU/g), *L. plantarum* (9.5×10^8 CFU/g), *Pediococcus acidilactici* (8.9×10^{10} CFU/g), and was inoculated so that the same number as other strains at the time of inoculation.

2. Ensiling process

After harvesting, aliquot 600 kg (fresh weight) of Italian ryegrass was cut into 5-6 cm lengths using a multipurpose shredder (Model GS-2000, Samsung Guemsan Precision, Korea), separated by treatment, and inoculated by LAB, which was already diluted in water. The inoculation strain of the LAB treatment was diluted to 1×10^6 CFU/g and inoculated at 1 mL/kg. The NC treatment was inoculated with primary distilled water. The inoculated IRG was packed into a 10 L plastic container, vacuumed and sealed. The compressed samples were stored and incubated at room temperature for 3, 7, 21, and 42 days in triplicate.

3. Sample collection and analyses

After the ensiling period, the plastic container containing the silage was opened, and a representative sample was collected by using the coning and quartering method. Aliquot 200 g of sample was dried at 65°C for 3 days to remove the excess moisture. The dried sample was ground using an ultra-high-speed grinder ZM 200 (Retch, Germany) equipped with a 1 mm mesh, and then stored for the proximate analysis. Extra samples in fresh form were stored at -20°C and thawed during analysis. The dry matter (DM), organic matter (OM), and crude protein (CP) of the silages were analyzed according to AOAC (2005). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest et al. (1991).

An aliquot 100 g of frozen sample was thawed at room temperature and homogenized together with 900 mL of distilled water using (Stomacher, Bag mixer 400, Interscience, France) for four min. The supernatant collected and pH was immediately measured (pH meter, Thermo Sci, Korea). The supernatant was utilized to analyze lactic acid, volatile fatty acid (VFA), and ammonia-N concentration in the silage. The ammonia-N analysis was analyzed using the method developed by Chaney and Marbach (1962). For lactic acid and VFA analyses, the supernatant was filtered using 0.45 μm (Rephile, RjN1345NH, China) syringe filter and was analyzed by high-performance liquid chromatography (Prostar, Varian, USA) equipped with a SUPELCOGEL C-610H (30 cm × 7.8 mm internal diameter) column (SUPELCO, Sigma- Aldrich, USA) and UV/VIS detector (210 nm). The column temperature was maintained at 30℃, and the mobile phase was 0.1% phosphoric acid (H_3PO_4) at a flow-rate of 0.5 mL/min (Ryu et al., 2017).

4. *In vitro* rumen fermentation characteristics

In vitro rumen fermentation was conducted by employing Tilley and Terry (1963) method. The rumen inoculum was obtained from three cannulated Holstein steers, 3 h after morning feeding. The rumen fluid was filtered using eight layers of muslin to

remove the feed particle and mixed with McDougall's buffer (McDougall, 1948) on a 1:4 ratio.

Aliquot 0.5 g of 42 d ensiled IRG silages was used as substrate and mixed with 50 mL of rumen-buffer solution, sealed and incubated for 6 and 24 h at 39°C. At the end of each fermentation period, the gas production was measured using a glass syringe (Fortuna®, Germany). The substrate was filtered using a 5 × 10 cm nylon bag (ANKOM Technology, USA) and utilized to determine the dry matter degradability. The supernatant was used for determining the pH, ammonia-N, and VFA production. The ammonia-N was conducted according to the method developed by Chaney and Marbach (1962). The VFA concentration was analyzed using gas chromatography (GC), according to Erwin et al. (1961). Aliquot 0.2 mL of metaphosphoric acid (Wako, Japan) was added to 1 mL supernatant, centrifuged for 10 min at 11,200 × g, filtered, and injected to GC (450-GC, Bruker Inc., Germany) equipped with the BR-Wax fame column (BR87503, Germany). The VFA standard solution (Sigma-Aldrich, USA) was used as an external standard. The temperature of the injector and the detector (FID) was set to 250°C, while the oven temperature was 100°C. The airflow of nitrogen, hydrogen, and high-purity air was set to 29 mL/min, 30 mL/min, and 300 mL/min.

5. Statistical analysis

The results of the nutritive values of IRG silages and their *in vitro* fermentation characteristics were subjected to analysis of variance. The comparison among the means, if existed, was conducted by Duncan's multiple range test. The statistical analysis was performed with 95% significance level, using SPSS software (Ver. 23, IBM, USA).

Ⅲ. RESULTS AND DISCUSSION

1. Fermentation and nutritive value of silage

The effect of different LAB on the concentrations of pH, lactic acid, and acetate of IRG silage was presented in Table 1. The silage pH showed significant differences among treatments from 3 to 42 days ensiling period ($P<0.05$). On the 21st and 42nd day of the ensiling period, the LPA treatment has shown the lowest ($P<0.05$) pH (4.03 and 3.87, respectively) compared with the rest of the treatments. A study by Weinberg and Muck (1996) reported that a large amount of lactate in the silage affected reducing the pH, and our results were in agreement with their study.

The lactate and acetate concentrations were not different on the 3rd day of silage preparation. However, there were significant differences ($P<0.05$) in the 7th, 21st and 42nd day of the ensiling. The LPA treatment showed a higher ($P<0.05$) lactic acid concentration than the other treatments from the 7th d to 42nd day of the ensiling period, which explained the low pH of LPA treatment. Winters et al. (1998) also reported that *L. paracasei* produced enormous amounts of lactic acid, mainly from fructans, which mainly accounted for up to 90% in grasses. Silage is often evaluated through the content of lactate and acetate. More than 70% of lactate and less than 22% of acetate are evaluated as good quality silage, and the ratio of lactate and acetate should be three or more (Mohd-Setapar et al., 2012). In the 42nd day treatment group to which LPA was added, lactate was 125.4 (g/kg DM), and acetate was 7.25 (g/kg DM).

There was a significant difference ($P<0.05$) during the entire ensiling period among treatments on ammonia-N concentration. The LPA showed lower ($P<0.05$) ammonia-N concentration compared with the other treatments. It has been reported that the addition of LAB can rapidly decrease pH and inhibits protein degradation (McDonald et al., 1991; Weinberg and Muck, 1996).

The effect of different LAB on the chemical composition (%) of IRG silage was shown in Table 2. The DM composition of IRG silage was significantly different from the 3rd ensiling d to 42nd ensiling days. At the 3rd, 7th, and 21st ensiling days, all the treatments were shown higher ($P<0.05$) DM compared to NC. However, at the 42nd ensiling days, the higher DM belonged to PC, LA and LPT treatments.

At the 42nd ensiling day, the CP was significantly higher ($P<0.05$) in the treated groups with LPA, LA, and LPT compared to the control group. This result was synchronized with the ammonia-N results in Table 1. The low pH during the ensiling process reduced the activity of protein degradability, and this was likely to affect overall protein degradation during ensiling so that CP of LAB treated silages was higher than the control group. Contreras-Govea et al. (2011) also mentioned that the LAB could preserve CP during ensiling periods.

The NDF was the lowest ($P<0.05$) in LPA treatment, and ADF was also significantly lower in LPA treatment ($P<0.05$). The crude fat content was 7.59% in the LPA treatment group, showing the highest result ($P<0.05$). Morrison (1979) mentioned that the natural fiber loss during ensiling happened due to silage microflora, which produces some fiber degrading enzymes. In

Table 1. Effect of different lactic acid bacteria on the concentrations of pH, lactate, acetate, and ammonia-N of Italian ryegrass silage

Days of ensiling	Treatments						SEM	Sig
	NC	PC	LPL	LPA	LA	LPT		
pH								
3	5.40^{a}	4.98^{d}	5.45^{a}	5.28^{b}	4.60^{e}	5.15^{c}	0.071	*
7	4.94^{a}	4.42^{d}	4.88^{a}	4.73^{c}	4.33^{e}	4.81^{b}	0.056	*
21	4.62^{a}	4.17^{c}	4.62^{a}	4.03^{d}	4.15^{c}	4.32^{b}	0.056	*
42	4.45^{a}	4.12^{b}	4.47^{a}	3.87^{c}	4.10^{b}	4.10^{b}	0.051	*
Lactate (g/kg DM)								
3	68.59	66.29	80.35	75.98	66.54	66.11	2.467	NS
7	71.62^{ab}	73.86^{ab}	62.02^{b}	80.66^{a}	79.85^{a}	65.10^{b}	2.221	*
21	70.64^{d}	84.55^{b}	60.45^{d}	107.13^{a}	79.73^{bc}	76.61^{bc}	3.752	*
42	71.74^{d}	86.20^{c}	74.65^{cd}	125.43^{a}	84.63^{c}	105.02^{b}	4.708	*
Acetate (g/ kg DM)								
3	5.04	4.22	10.50	11.23	11.95	9.04	1.261	NS
7	8.56^{c}	3.20^{c}	6.33^{bc}	7.35^{bc}	14.46^{a}	7.77^{b}	0.933	*
21	8.64^{bc}	4.29^{c}	5.62^{bc}	7.55^{bc}	17.34^{a}	11.18^{b}	1.210	*
42	10.32^{bc}	4.51^{d}	10.95^{bc}	7.25^{cd}	20.83^{a}	12.77^{b}	1.305	*
Ammonia-N (% of total-N)								
3	5.07^{bc}	5.63^{a}	4.67^{c}	3.14^{d}	3.01^{d}	5.50^{ab}	0.262	*
7	5.67^{a}	6.05^{a}	5.90^{a}	4.03^{b}	3.67^{b}	5.64^{a}	0.237	*
21	7.37^{ab}	7.79^{a}	7.14^{b}	4.42^{d}	5.47^{c}	7.07^{b}	0.294	*
42	7.98^{b}	8.41^{a}	8.13^{ab}	4.80^{d}	6.39^{c}	7.95^{b}	0.314	*

NC = Negative control (without any additives); PC = Positive control (with a commercial silage additive); LPL, LPA, LA and LPT=Italian ryegrass silage treated with *L. plantarum, L. paracasei, L. acidophilus and L. pentosus*, respectively.
SEM = Standard error of the mean; NS = not significant; *= $P<0.05$
^{a,b,c,d} Superscripts within rows significantly differ at $P<0.05$

the study conducted by Weinberg et al. (2007), several strains of LAB could reduce the NDF concentration in the silage.

2. *In vitro* rumen fermentation characteristics

Table 3 presented the effect of day 42 ensiled IRG silage on *in vitro* rumen fermentation characteristics. No difference was observed between the NC and the other treatments at 6 and 24 h incubation period. The pH of the incubation bottle content significantly differed ($P<0.05$) at 6 and 24 h period. Previous studies reported that the pH suitable for rumen microbial activity was 5.80-7.0 (Hiltner and Dehority, 1983; Ørskov, 2000). In this study, the pH of the entire incubation period was observed around 6.86 to 6.96.

At 6 and 24 h, LPT, LA, and LPA showed higher ($P<0.05$) ammonia-N concentrations. In the analysis of the chemical composition of IRG silage on the 42nd day, the higher the crude protein content, the more proportional to the *in vitro* ammonia-N content were noted, which could be compared to the earlier ensiled silages. The ammonia-N concentration was different ($P<0.05$) at 6 and 24 h incubation period. The LPA, LA, and LPT produced higher ($P<0.05$) ammonia-N compared to the other treatments. In Table 2, it showed that these three treatments had higher CP concentration, which explains the higher *in vitro* ammonia-N concentration. Wanapat and Pimpa (1999) mentioned that ammonia-N is a critical nutrient to support rumen fermentation.

The VFA concentration during *in vitro* rumen fermentation is presented in Table 4. Total VFA did not differ among the experimental groups at 6 h of incubation. However, at 24 h,

Table 2. Effect of different lactic acid bacteria on chemical composition (%) of Italian ryegrass silage (dry matter basis unless otherwise stated)

Days of ensiling	Treatment	Dry matter	Organic matter	Crude protein	NDF	ADF
Original		43.69	93.50	8.28	60.06	34.33
3	NC	44.90^{bc}	93.08	8.81	59.11^{b}	34.40
	PC	45.30^{abc}	93.81	8.42	61.39^{a}	36.21
	LPL	45.92^{ab}	92.99	8.85	59.68^{b}	34.97
	LPA	45.36^{abc}	93.44	8.73	59.95^{ab}	34.82
	LA	44.28^{c}	93.21	9.09	60.35^{ab}	35.39
	LPT	46.27^{a}	92.89	8.73	59.21^{b}	34.45
	SEM	0.342	0.229	0.133	0.509	0.322
	Sig.	*	NS	NS	*	NS
7	NC	46.53^{a}	93.37	9.04^{ab}	59.15	34.72^{a}
	PC	46.25^{ab}	93.00	8.89^{abc}	58.81	34.51^{a}
	LPL	46.21^{ab}	93.17	8.50^{c}	59.35	35.08^{a}
	LPA	46.08^{ab}	93.08	8.47^{c}	59.45	34.69^{a}
	LA	45.48^{c}	93.00	8.77^{bc}	59.68	34.71^{a}
	LPT	45.85^{bc}	93.24	9.29^{a}	58.38	33.71^{b}
	SEM	0.179	0.125	0.113	0.407	0.268
	Sig.	*	NS	*	NS	*
21	NC	46.03^{a}	92.56^{c}	8.88	59.35	35.40
	PC	44.92^{bc}	93.02^{bc}	8.81	60.03	35.90
	LPL	44.90^{bc}	93.01^{bc}	8.81	60.04	36.08
	LPA	45.04^{b}	93.89^{a}	8.76	59.29	34.91
	LA	44.39^{c}	93.46^{ab}	9.01	59.86	35.01
	LPT	44.95^{bc}	92.81^{bc}	8.88	58.90	35.05
	SEM	0.179	0.199	0.141	1.715	1.080
	Sig.	*	*	NS	NS	NS
42	NC	45.25^{a}	92.79	8.80^{b}	60.38^{abc}	36.09^{a}
	PC	44.88^{ab}	93.37	8.85^{b}	61.26^{a}	36.07^{a}
	LPL	45.45^{a}	92.99	8.74^{b}	60.67^{ab}	36.00^{a}
	LPA	45.52^{a}	93.00	9.04^{a}	58.45^{c}	34.65^{b}
	LA	44.35^{ab}	92.91	9.24^{a}	59.02^{bc}	34.81^{b}
	LPT	43.76^{b}	92.54	9.22^{a}	59.22^{bc}	35.16^{ab}
	SEM	0.349	0.290	0.108	0.619	0.333
	Sig.	*	NS	*	*	*

Original = Italian ryegrass used for ensiling (only Italian ryegrass); NC = Negative control (without any additives); PC = Positive control (with a commercial silage additive); LPL, LPA, LA and LPT=Italian ryegrass silage treated with *L. plantarum, L. paracasei, L. acidophilus and L. pentosus*, respectively.

SEM = Standard error of the mean; NS = not significant; *= $P<0.05$

NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber.

^{a,b,c} Superscripts within columns significantly differ at $P<0.05$

the LPA treatment has shown the highest value ($P<0.05$). At 6 h incubation period, all treatments showed lower ($P<0.05$) acetic acid compared to NC. However, there was no difference between NC and treatment groups at 24 h for acetic acid concentration. In the case of propionic acid, PC, LPA, LA, and LPT at 6 h incubation period showed higher ($P<0.05$) value. Our results were somewhat different from the study conducted by Liu et al. (2016), which reported no significant differences in total VFA, acetate and propionate proportion when inoculated various LAB to alfalfa silage. However, previous studies have

Table 3. Effect of Italian ryegrass silage prepared with several lactic acid bacterial strains on *in vitro* rumen fermentation characteristics

Incubation time, h	Treatments						SEM	Sig.
	NC	PC	LPL	LPA	LA	LPT		
				Dry matter degradability, %				
6 h	33.62	35.06	33.86	34.12	30.66	31.57	1.035	NS
24 h	53.21	53.62	51.40	53.22	51.88	51.77	1.223	NS
				pH				
6 h	6.94^{a}	6.89^{ab}	6.90^{ab}	6.89^{b}	6.94^{a}	6.94^{a}	0.015	*
24 h	6.96^{a}	6.95^{a}	6.96^{a}	6.90^{b}	6.86^{c}	6.96^{a}	0.011	*
				Ammonia-N, mg/100 mL				
6 h	5.28^{c}	5.65^{bc}	5.91^{bc}	6.18^{ab}	6.29^{ab}	6.57^{a}	0.367	*
24 h	3.85^{e}	4.58^{d}	4.06^{e}	5.07^{c}	6.47^{a}	5.52^{b}	0.232	*

NC = Negative control (without any additives); PC = Positive control (with a commercial silage additive); LPL, LPA, LA and LPT = Italian ryegrass silage treated with *L. plantarum, L. paracasei, L. acidophilus and L. pentosus*, respectively.
SEM = Standard error of the mean; NS = not significant; *= $P<0.05$
^{a,b,c,d} Superscripts within rows significantly differ at $P<0.05$

Table 4. Effect of Italian ryegrass silage prepared with several lactic acid bacterial strains on VFA concentration during *in vitro* rumen fermentation

Incubation time	Treatment	Total VFA (mM)	Acetic acid, % of total VFA	Propionic acid, % of total VFA	Butyric acid, % of total VFA	A/P ratio
6 h	NC	26.37	63.50^{a}	23.86^{c}	9.10^{b}	2.66^{a}
	PC	22.48	62.69^{bc}	24.53^{ab}	9.04^{b}	2.56^{bc}
	LPL	28.48	63.13^{ab}	24.16^{bc}	9.14^{b}	2.61^{ab}
	LPA	28.17	62.30^{c}	24.74^{a}	9.16^{b}	2.52^{c}
	LA	24.72	62.17^{c}	24.49^{ab}	9.49^{a}	2.54^{c}
	LPT	22.78	62.36^{c}	24.40^{ab}	9.40^{a}	2.56^{bc}
	SEM	0.901	0.129	0.080	0.042	0.013
	Sig.	NS	*	*	*	*
24 h	NC	44.92^{bc}	57.32	30.76^{a}	8.01^{b}	1.86
	PC	48.91^{bc}	57.79	30.41^{ab}	8.02^{b}	1.90
	LPL	36.06^{c}	57.68	30.64^{a}	7.90^{b}	1.88
	LPA	70.85^{a}	55.81	30.72^{a}	9.30^{a}	1.82
	LA	57.02^{ab}	56.78	29.47^{b}	9.46^{a}	1.93
	LPT	41.00^{bc}	58.54	29.46^{b}	8.25^{b}	1.99
	SEM	3.283	0.307	0.181	0.173	0.020
	Sig.	*	NS	*	*	NS

NC = Negative control (without any additives); PC = Positive control (with a commercial silage additive); LPL, LPA, LA and LPT = Italian ryegrass silage treated with *L. plantarum, L. paracasei, L. acidophilus and L. pentosus*, respectively.
SEM = Standard error of the mean; NS = not significant; *= $P<0.05$
^{a,b,c} Superscripts within columns significantly differ at $P<0.05$

also mentioned that ensiling not only able to decrease the ADF contents but also able to increase the hemicellulose content, which responsible in enhancing the molar propionate proportion in the rumen (Contreras-Govea et al., 2009; Lima et al., 2010; Contreras-Govea et al., 2011). The shift of this rumen fermentation product is likely to be beneficial especially on animal production (Rigout et al., 2003).

Ⅳ. CONCLUSION

In this study, the addition of LAB to IRG significantly improved the fermentation characteristics and *in vitro* rumen degradability. A single strain of LAB enhanced the lactic acid production, leading to the pH drop, which restrained the proteolysis in silage and resulted in low ammonia-N concentration in the silage compared to the negative control. Such effects were comparable with a commercial silage additive, which was the mixture of several micro-organisms. Interestingly, *L. paracasei*, which belongs to heterofermentative LAB, seemed to produce a better quality of silage among the LAB. Significantly higher CP and lower NDF, ADF contents were also observed from the silage fermented with *L. paracasei*. During the *in vitro* rumen fermentation, IRG inoculated with *L. paracasei* exhibited higher ammonia-N and VFA production than the other treatments. It is unclear if *L. paracasei* is specifically favorable for the fermentation of IRG or not in the rumen. Further studies with IRG grown and/or harvested via a different cropping system may be necessary.

Ⅴ. ACKNOWLEDGMENTS

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