Using fermented juice of epiphytic lactic acid bacteria (FJLB) and molasses to improve digestibility and rumen fermentation characteristics of ruzigrass silage fed to dairy cows

Smerjai Bureenok, Chalermpon Yuangklang and Kraisit Vasupen

Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima, 30000 Thailand

Contact email: asmerjai@hotmail.com

Abstract. The effects of fermented juice of epiphytic lactic acid bacteria (FJLB) and molasses (MO) on ruzigrass silage digestibility and rumen fermentation characteristics in dairy cows were studied. All treated silages were well-preserved as indicated by the pH value and NH_3-N content. Silage treated with MO or MO-FJLB had lower pH and higher lactic acid contents than untreated silages and FJLB silages. Butyric acid was not detected in the FJLB silage. Water soluble carbohydrate (WSC) was higher in the MO silages; neutral detergent fiber (NDF) and acid detergent fiber (ADF) did not differ significantly. Dry matter intake in cows fed with all silages did not differ among diets. The CP digestibility of the FJLB silage was higher than the other silages. For rumen characteristics, pH value was lower in cows fed with MO silages. However, volatile fatty acid content in rumen fluid and blood urea nitrogen (BUN) contents did not differ significantly among silages. The cellulolytic bacteria populations were significantly higher in cows fed FJLB and MO-FJLB silages than in cows fed untreated silages. In conclusion, adding FJLB to ruzigrass silage improved fermentative quality, digestibility of crude protein and increased cellulolytic bacteria counts in cows.

Keywords: Lactic acid bacteria, molasses, silage, dairy cow, rumen.

Introduction

Profitable livestock production from forages in tropical regions depends largely on the quantity and quality of forage produced, the continuity of forage supply throughout the year, the animal's capacity to utilize forages efficiently and the ability of livestock producers to manage forage feeding systems. In seasonally-dry tropical areas, dry season feed shortages are often the major limitation to animal productivity from forage feeding systems. A typical example is Ruzigrass (*Brachiaria ruziziensis*); a tropical forage crop grown widely in many areas of Asia, especially in Thailand, which is highly productive during the wet season but is very susceptible to seasonal drought. Preservation of forages such as Ruzigrass grown during the rainy season is a practical tool to improve continuity of forage supply for animals throughout the year.

Forage crops can be conserved through the fermentation process of silage making, but tropical forages are known to be difficult to ensile, and the resulting fermentation quality, intake, and digestibility are frequently low (McDonald *et al.* 1991). Successful silage production requires epiphytic lactic acid bacteria (LAB) and water soluble carbohydrate (WSC) to produce sufficient lactic acid for rapid pH reduction (Rooke 1990). The numbers of epiphytic lactic acid bacteria (LAB) in tropical forages may be too low (Ohmomo *et al.* 2002) to ensure an uncomplicated process of fermentation. Bureenok *et al.* (2005; 2006) reported that the addition of fermented juice of epiphytic lactic acid bacteria (FJLB) to tropical forages improved the quality of the silage. Low levels of WSC in silage are commonly addressed through the use of , molasses as an additive (Yokota *et al.* 1991; Van Niekerk *et al.* 2007). The aim of this research was to investigate the effects of both hand-made bacteria inoculums (FJLB) and molasses as silage additives on Ruzigrass (*Brachiaria ruziziensis*) silage quality and nutritive values, including voluntary feed intake, rumen ecology and digestibility in dairy cows.

Methods

FJLB Preparation

A 200 g fresh sample of Ruzigrass was chopped and macerated in 1,000 mL of sterilized distilled water with a home blender. The juice was filtered through a double layer of cheesecloth. The filtrate was transferred to a bottle and 2% glucose added. The bottle was then anaerobically incubated at 30°C for 2 d before the contents were used as a silage additive. The LAB from grass and FJLB were plated out onto MRS agar and incubated at 35°C for 3 d, after which viable colony-forming unit (cfu) was confirmed (Bureenok *et al.* 2006).

Silage Preparation

Ruzigrass was harvested and chopped with a forage cutter into 2- to 3-cm lengths and mixed with the silage additives to create the following treatments: (1) no additive (Untreated); (2) 5% molasses (MO); (3) 1% FJLB (FJLB); and (4) 5% molasses plus 1% FJLB (MO-FJLB) based on fresh weight. Distilled water (1% of fresh weight) was added to the Untreated and MO treatments to adjust the moisture content to be equivalent to the treatments that had

added FJLB. The mixtures were then packed tightly in 100-kg plastic drums and stored at room temperature (27–30°C). At 45 d of ensiling, three samples per treatment were randomly collected and evaluated for fermentative quality, and the remainder was used for the feeding trial.

Animals, Feeding

Four fistulated Holstein Friesian x Red Sindi crossbred cows with average body weight, 481 ± 48 kg were individually housed in metabolic cages. The cows were randomly assigned to receive dietary treatments in a 4x 4 Latin square design. The dietary treatments were: (1) untreated silage; (2) MO silage; (3) FJLB silage; and (4) MO-FJLB silage. All cows were fed 1.5% body weight (BW) of a concentrate containing 16% CP. The 28-d experimental period consisted of a 21 d feed intake trial and 7 d of sampling. Feed was offered twice daily at 08:00 and 15:00 h, and the refused portions were weighed daily before the morning feeding. During the 7 d sampling, all faeces were collected from each cow at morning and afternoon, according to the total collection method.

Silage quality

Subsamples of each silage treatment (50 g) were macerated with 150 mL of distilled water and stored in a refrigerator at 4°C for 12 h. The extract was filtered with No. 5 filter paper. The silage pH was determined with a pH meter (Lab 860, Schott). Lactic acid and volatile fatty acids were determined by high performance liquid chromatography (HPLC). The NH_3-N content was determined by steam distillation (Cai, 2004).

Sampling Procedures

At the end of each experimental period, rumen fluid and jugular blood samples were collected at 0, 2, and 4 h after the morning feeding; the rumen fluid was then filtered through 2 layers of cheesecloth. The pH of the filtrates was immediately measured with a glass electrode pH meter (Lab 860, Schott). The filtrates were then used to determine the NH_3-N, volatile fatty acids (VFA) and rumen microbial counts. For NH_3-N and VFA , the 90-mL filtrate samples were acidified with 10 mL of 1 M H_2SO_4, centrifuged at 16,000 × *g* for 15 min, and the supernatant was stored at -20°C before analysis. The filtrate was fixed with 10% formalin solution in sterilized 0.9% saline solution and used to determine the total direct counts of bacteria, protozoa, and fungal zoospores (Galyean 1989). The filtrate was diluted for identification of cellulolytic, proteolytic and amylolytic bacteria as total viable count bacteria with the roll-tube technique (Hungate, 1969).Blood sample from a jugular vein was collected into EDTA tubes at the same time as rumen fluid sampling for blood urea nitrogen (Crocker, 1967).

Chemical composition.

The DM content of roughages, concentrates, and faeces were determined by oven drying at 60°C for 48 h. The WSC content was determined by the method of Dubois *et al.* (1956) after extraction with 80% ethanol. The total nitrogen (N) content was determined by the Kjeldahl procedure, and CP was calculated by multiplying N by the 6.25 conversion factor (AOAC, 1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined by the methods of Van Soest *et al.* (1991) on an ash-free basis.

Statistical analyses

Statistical analyses were performed using the general linear models (GLM) procedure of SAS (SAS Institute Inc., Cary, NC). Data of silage fermentation (n=3) were compared by Duncan's multiple range test (DMRT). In the feeding trial, data were analyzed using the procedures of SAS for a 4×4 Latin square design. Rumen fermentation parameters and plasma metabolites were analyzed as repeated measures (n=4) at 0, 2, and 4 h after the morning feeding.

Results

Ruzigrass leaf material was characterized by 23.2 %DM, 5.4% CP, 64.8% NDF, 45.8% ADF and 56.5% WSC on a dry matter basis. The LAB counts on ruzigrass extract were about 4 log cfu/ml and increased to7.64 log cfu/ml after incubation. The dry matter content of silages treated with MO and MO-FJLB were higher (P<0.05) than in the FJLB and untreated silages. WSC residues were higher (P<0.05) in MO and MO-FJLB silages than in FJLB and untreated silages. Addition of MO and their combined additives increased the WSC concentration of the Ruzigrass silage.

Silage Quality

At 45 days of ensiling, silage treated with MO and MO-FJLB resulted in lower pH and higher lactic acid content (P<0.05) compared with untreated and FJLB (Table 1). At

Table 1. Effect of the fermented juice of epiphytic lactic acid bacteria (FJLB) and molasses (MO) on the fermentative quality and nutrient composition of ruzigrass silages.

Items	Treatments				SEM	*P* -value
	Untreated	MO	FJLB	MO-FJLB		
pH	4.08 a	3.81 b	4.03 a	3.84 b	0.02	<0.001
Lactic acid, g/kg DM	53.1 d	127.9 a	73.3 c	108.2 b	6.19	<0.001
Acetic acid, g/kg DM	21.1	23.7	19.8	19.1	4.49	0.729
Propionic acid, g/kg DM	6.4 b	20.7 a	3.7 b	0 b	2.47	<0.001
Butyric acid, g/kg DM	9.8 a	9.9 a	0 b	0 b	0.94	<0.001
Lactic acid:acetic acid ratio	2.9 c	5.4 a	3.7 b	5.5 a	0.47	0.004
NH_3-N, g/ kg total N	121.7 a	103.9 b	109.5 b	91.5 b	4.53	0.004
Chemical composition						
DM , g/kg	240.0 b	275.0 a	255.0 b	274.0 a	12.49	<0.001
CP, g/kg DM	49.4	48.1	47.6	50.2	0.87	0.179
WSC, g/kg DM	14.7 b	29.8 a	15.5 b	25.1 a	2.06	<0.001
NDF, g/kg DM	718.9	715.2	749.6	697.7	23.05	0.480
ADF, g/kg DM	377.3	370.2	418.6	402.6	16.91	0.276

Mean values in a row with different letters were significantly different (P < 0.05); SEM = standard error of the mean. Adapted from Bureenok *et al.* (2011).

Table 2. Effect of the fermented juice of epiphytic lactic acid bacteria (FJLB) and molasses (MO) on voluntary feed intake and nutrient digestibility in cows fed ruzigrass silages.

Items	Treatments				SEM	*P*-value
	Untreated	MO	FJLB	MO-FJLB		
Silage DM intake						
% BW	0.66	0.78	0.64	0.63	0.07	0.535
g/kg $BW^{0.75}$	30.96	36.65	29.84	29.24	3.44	0.464
Total intake						
% BW	2.13	2.2	2.01	1.98	0.07	0.535
g/kg $BW^{0.75}$	98.81	102.17	92.52	91.7	2.89	0.122
Apparent digestibility, g/kg						
DM	768.9	774.9	783.6	744.9	12.49	0.291
OM	790.4	795.9	803.7	767.3	11.49	0.273
CP	764.7 b	783.2 ab	803.3 a	782.0 ab	10.24	0.002
NDF	520.0	549.3	539.1	451.5	44.17	0.519
ADF	522.4	547.5	547.0	459.7	39.26	0.477

Mean values in a row with different letters were significantly different ($P < 0.05$); SEM = standard error of the mean. Adapted from Bureenok *et al.* (2011).

Table 3. Effects of the fermented juice of epiphytic lactic acid bacteria (FJLB) and molasses (MO) on rumen fermentation characteristics and microbial counts in cows fed ruzigrass silages.

Items	Treatments				SEM	*P*-value
	Untreated	MO	FJLB	MO-FJLB		
pH	6.47 bc	6.27 c	6.71 ab	6.8 4a	0.10	0.023
Rumen VFA, mol/100 mol						
Acetic acid (C2)	69.59	69.43	69.95	68.29	3.90	0.992
Propionic acid (C3)	20.29	20.89	21.45	22.81	3.24	0.951
Butyric acid (C4)	10.80	9.09	8.59	8.89	1.29	0.642
C2:C3	3.43	3.37	3.26	2.99	0.74	0.679
NH_3-N, mg/dL	15.0	14.5	15.0	15.3	0.24	0.876
BUN, mg/dL	9.75	9.92	10.75	8.75	0.99	0.590
Viable bacteria, log cfu/mL						
Amylolytic	5.78	5.8	5.79	5.88	0.07	0.816
Proteolytic	5.71	5.76	5.77	5.79	0.02	0.178
Cellulolytic	7.05 b	7.23 ab	7.3 a	7.27 a	0.06	0.025
Rumen microbes, log cells/mL						
Bacteria	13.33	13.38	12.37	13.32	0.03	0.627
Protozoa	6.36	6.41	6.47	6.45	0.04	0.260

Mean values in a row with different letters were significantly different ($P < 0.05$); SEM = standard error of the mean. Adapted from Bureenok *et al.* (2011).

the same WSC content in the grass, the addition of FJLB produced more lactic acid than the Untreated silage. Lactic to acetic acid ratio was lower in untreated silages than in all treated silages. Propionic acid content was higher ($P<0.05$) in MO silages than the others. The butyric acid content was higher in the untreated and MO silages. NH_3-N content of untreated silages was higher ($P<0.05$) than the others. The NH_3-N content of treated silages was low but the level was not significantly different among the treated silages.

Feed Intake and Digestibility

The effect of silage additives on feed intake of dairy cows is presented in Table 2. No significant differences were detected between treatments on Silage DMI or total DMI. The digestibility of CP was higher ($P<0.05$) in cows fed FJLB than those fed untreated silage.

Rumen Characteristics

The pH value was lowest ($P<0.05$) in cows fed with MO silages (Table 3). There were no significant differences between treatments for acetic, propionic and butyric acid contents in rumen fluid, the ruminal NH_3-N content, BUN content or ruminal microbial (bacteria and protozoa). Cellulolytic bacteria counts were higher ($P<0.05$) in cows fed with FJLB-silages than the untreated silage.

Conclusions

The results of this study indicate that FJLB prepared from Ruzigrass could feasibly be used as a starter LAB culture for ensiling. Addition of FJLB or FJLB combined with MO to Ruzigrass silage significantly increased lactic acid content and decreased NH_3-N and butyric acid contents. In the feeding trial, Ruzigrass silage treated with FJLB demonstrated improved digestibility of CP and increased cellulolytic bacteria population in cows. The use of FJLB as a silage additive could improve the fermentative quality of Ruzigrass silage and its feeding value in dairy cows. Furthermore, the preparation of fermented juice requires little cost and simple techniques which both small and scale large farmers could apply at any scale.

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