Effects of Napiergrass silages treated with various additives on feed intake, digestibility and rumen fermentation characteristics

Smerjai Bureenok, Warangkana Homsai, Achara Lukkananukool[1], Chalermpon Yuangklang, Krisit Vasupen and Yasuhiro Kawamoto[2]

Faculty of Natural Resources, Rajamangala University of Technology-Isan, Sakon Nakhon Campus, Sakon Nakhon, Thailand
[1]School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology
[2]Faculty of Agriculture, University of the Ryukyus, Nishiahara-cho, Okinawa, Japan

Summary
The Napiergrass silage was treated with fermented juice of epiphytic lactic acid bacteria (1% FJLB), 5% molasses (w/w) and 5% cassava meal (w/w). The effect of Napiergrass silages treated with various additives on voluntary feed intake, digestibility, rumen fermentation and microbial rumen fermentation were determined in 4 fistulated cows using 4 × 4 Latin square design. Experimental period were 28 days long and consisted of 21 days of feed intake and 7 days of sampling. Cows were randomly assigned to 1 of 4 ad libitum diets: 1) untreated silage, 2) FJLB-silages, 3) molasses - silages and 4) cassava – silages.

The control silage resulted in higher pH and butyric acid content compared with the treated silages ($P< 0.05$). Neutral detergent fiber and acid detergent fiber of silage treated with cassava meal were significantly higher ($P< 0.05$) than the others. The dry matter intake ($BW^{0.75}$) increased ($P< 0.05$) in cow fed with the treated silage. The NDF digestibility was higher in cow fed with the molasses-silage compared with the other diets. However, rumen parameters: ruminal pH, NH_3-N, volatile fatty acid (VFA) and blood urea nitrogen (BUN) were not significantly different among the treatment. The cellulolytic, amylolytic, proteolytic bacteria and protozoa populations were not significantly different among the treatment.

In conclusion, these studies confirmed that the applying of FJLB improved fermentative quality of Napiergrass. The NDF digestibility in cow fed with FJLB-silage was lower than molasses-silage but was similar for the cassava-silages. However, the ruminal fermentation pattern was not affected by the additives.

Key words: Napiergrass, silage, lactic acid bacteria, molasses, cassava

Introduction
The fermented juice of epiphytic lactic acid bacteria (FJLB) additive was recommended as a silage additive for improving the fermentative quality of tropical grass silage (Bureenok et al., 2005a, b). However, this is sometimes not effective because of the low lactic acid bacteria (LAB) and low water-soluble carbohydrates (WSC) content of the grasses. Previous studies have suggested that addition of molasses as a source of readily fermentable WSC has improved the fermentation of tropical pasture silage (Catchpoole and Henzell, 1971). In the present study, we compared the effects of FJLB, molasses and cassava meal on the feeding value of silage.

Materials and methods
Silage preparation
Napiergrass was harvested at 60 d after planting; silages were untreated (CO), or prepared with 1% FJLB (FJLB), 5% molasses (MO), or 5% cassava (CA). To adjust the moisture content of the MO, CA and CO silages, distilled water (1% of fresh matter) was

added. Napiergrass was chopped into 2- to 3-cm lengths and mixed with the silage additives. These mixtures were then packed tightly in 100-kg plastic drums and stored at room temperature (27–30°C) for 45 d.

Animals, Feeding

Four fistulated Holstein Friesian crossbred cows (mean body weight, 403 kg) were individually housed in metabolic cages. The cows were randomly assigned to receive 1 of 4 ad libitum diets: 1) CO silage, 2) FJLB silage, 3) MO silage, or 4) CA silage. All cows were also fed 1.5% body weight (BW) of a concentrate containing 16% CP. The 28-d experimental period consisted of a 21 d of feed intake 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. BW was measured before the morning feeding at the beginning and end of each experimental period. The daily DM intake per unit of metabolic BW was calculated with the mean value of initial BW and final BW of each period. During the 7 d sampling, all feces were collected in the morning before feeding. All procedures were approved by the Ethical Principles for the Use of Animals for Scientific Purposes of the National Research Council of Thailand.

Chemical Analyses

Chemical composition

The dry matter (DM) content of the grass and silages were determined by oven drying at 70°C for 48 h. The dried sample was milled to pass through a 1.0 mm sieve. The samples were extracted with ethanol, and the concentration of water-soluble carbohydrates (WSC) was estimated as described by Dubois et al. (1956). The concentration of total nitrogen (N) was determined by the Kjeldahl procedure (AOAC, 1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were determined by methods described by Van Soest et al. (1991). NDF and ADF concentrations were calculated on an ash-free basis. Hemicellulose was calculated as the difference between NDF and ADF.

Silage

After 45 d of fermentation, silage samples from the center of each plastic drum in each treatment were collected. Subsamples (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 using No.5 filter paper (Whatman, England). The pH of silage was determined with a pH meter (Lab 860, Schott). Lactic acid and volatile fatty acid was determined by HPLC (Aminex® HPX-87H, 300 mm x 7.8 mm i.d; column temperature, 40°C; flow rate, 0.60ml/min, Shimzu Co., Ltd., Kyoto, Japan). The NH_3-N content was determined using a steam distillation technique (Japan Grassland Farming Forage Seed Association, 1994).

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 immediately filtered through 2 layers of cheesecloth. The pH of the filtrates was measured with a glass electrode pH meter (Lab 860, Schott). The filtrates were divided into three portions. The first portion was used to determine the NH_3-N and volatile fatty acids. 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 second portion was fixed with 10% formalin solution in sterilize 0.9% saline solution. The total direct counts of bacteria, protozoa, and fungal zoospores were determined with a hemocytometer (Boeco, Hamburg, Germany). The last portion was diluted for identification of bacterial groups (e.g., cellulolytic, proteolytic, amylolytic) and total viable count bacteria with the roll-tube technique (Hungate, 1969).

A 20-mL blood sample from a jugular vein was collected into EDTA tubes at the same time as rumen fluid sampling. The plasma was separated by centrifugation at 2,500 × *g* for 15 min and stored at -20°C until blood urea nitrogen (BUN) analysis was performed (Crocker, 1967).

Statistical analyses

Statistical analyses were performed using the general linear models (GLM) procedure of SAS (SAS Institute Inc., Cary, NC). For silage quality, group means were compared by Duncan's multiple range test (DMRT). For 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 at 0, 2, and 4 h after the morning feeding.

Results and discussion

The control silage resulted in higher pH and butyric acid content compared with the treated silages ($P< 0.05$). Neutral detergent fiber and acid detergent fiber of silage treated with cassava meal were significantly higher ($P< 0.05$) than the others (Table 1). The dry matter intake ($BW^{0.75}$) increased ($P< 0.05$) in cow fed with the treated silage. The NDF digestibility was higher in cow fed with the molasses-silage compared with the other diets (Table 2). However, rumen parameters: ruminal pH, NH_3-N, volatile fatty acid (VFA) and blood urea nitrogen (BUN) were not significantly different among the treatment. The cellulolytic, amylolytic, proteolytic bacteria and protozoa populations were not significantly different among the treatment (Table 3).

Table 1 Fermentation quality and chemical composition of Napiergrass silages

	CO	1% FJLB	5% MO	5% CA	SEM
Fermentation profile					
pH	3.96 [a]	3.72 [b]	3.75 [b]	3.73 [b]	0.04
LA (g/kg DM)	49.34 [c]	79.34 [ab]	94.42 [a]	61.93 [bc]	5.59
AA (g/kg DM)	10.9 [b]	9.16 [b]	20.82 [a]	7.2 [c]	2.03
PA (g/kg DM)	1.72 [b]	3.69 [a]	0 [c]	0 [c]	0.43
BA (g/kg DM)	16.1 [a]	5.89 [b]	7.68 [b]	2.65 [b]	3.22
NH_3-N (g/kg TN)	72.91	63.04	67.81	66.61	4.45
Nutrient composition					
DM (g/kg)	298 [a]	265 [b]	280 [ab]	293 [a]	5.84
CP (g/kg DM)	42.74	49.15	44.64	45.40	3.58
WSC (g/kg DM)	12.00 [b]	15.44 [ab]	23.52 [a]	12.48 [b]	2.82
NDF (g/kg DM)	712.1 [ab]	736.3 [a]	704.6 [b]	623.3 [c]	8.99
ADF (g/kg DM)	470.10 [ab]	487.9 [a]	461.3 [b]	392.2 [c]	5.51

Means of triplicate silages. Values in the same row followed by different letters are significantly different ($p<0.05$). SEM = standard error of means.

Table 2 Effect of the FJLB**,** molasses and cassava meal on voluntary feed intake and nutrient digestibility in cows fed Napiergrass silages

	CO	1%FJLB	5%MO	5%CA	SEM
Initial BW, kg	388.50	415.33	405.37	404.12	-
Total VFI					
%BW	2.14 [b]	2.19 [b]	2.41 [a]	2.12 [b]	0.02
$g/kgBW^{0.75}$	94.8 [d]	99.2 [b]	108 [a]	95.1 [c]	0.01

Silage Intake								
DM intake (kg/d)	3.05	d	3.80	b	4.37	a	3.11	c	0.01
%BW	0.79	b	0.84	b	1.06	a	0.77	b	0.02
g/kgBW$^{0.75}$	35.1	b	38.57	b	47.62	a	34.73	b	1.24
Digestibility, %									
DM	75.64	b	75.99	ab	81.54	a	76.18	ab	1.43
OM	77.43	b	77.82	b	83.22	a	78.06	ab	1.36
CP	76.95	b	75.27	b	81.09	a	77.50	ab	0.98
NDF	66.60	b	67.26	b	76.75	a	68.57	b	1.76
ADF	66.76		67.63		76.33		65.22		2.91

Means of triplicate silages. Values in the same row followed by different letters are significantly different ($p<0.05$). SEM = standard error of means.

Table 3 Effects of silage additives on rumen fermentation characteristics and microbial counts in cows fed Napiergrass silages

	CO	1%FJLB	5% MO	5% CA	SEM
pH	6.84	6.85	6.52	6.78	0.21
Acetic acid,C_2 (mol/100 mol)	72.58	72.16	70.64	67.32	4.08
Propionic acid,C_3(mol/100 mol)	18.21	17.89	18.51	19.47	3.14
Butyric acid,C_4(mol/100 mol)	9.19	9.94	10.72	13.21	1.60
C_2:C_3	5.34	4.18	6.13	4.92	1.08
NH_3-N, mg%	17.06	17.58	20.10	20.82	1.18
BUN, mg%	13.33	17.16	13.58	15.42	2.66
Viable bacteria, log cfu/ml					
Amylolytic bacteria	5.78	5.69	5.48	5.41	0.05
Proteolytic bacteria	5.79	5.90	6.14	5.72	0.18
Cellulolytic bacteria	7.11	7.19	7.35	7.20	0.17
Rumen microbes, log cells/ml					
Bacteria	13.29 a	13.22 b	13.23 b	13.22 b	0.02
Protozoa	6.48	6.47	6.53	6.42	0.14

Means of triplicate silages. Values in the same row followed by different letters are significantly different ($p<0.05$). SEM = standard error of means.

Conclusion

In conclusion, these studies confirmed that the applying of FJLB improved fermentative quality of Napiergrass. The NDF digestibility in cow fed with FJLB-silage was lower than molasses-silage but was similar for the cassava-silages. However, the ruminal fermentation pattern was not affected by the additives.

.

Acknowledgements

Authors are greatly indebted to the National Research Council of Thailand (NRCT) for funding this research.

References

AOAC, 1995. *Official Methods of Analysis*. 16th ed. Association of Official Analytical Chemists, Arlington. VA, USA.

Bureenok, S., T. Namihira, Y. Kawamoto and T. Nakada. 2005a. *Additive effects of fermented juice of epiphytic lactic acid bacteria on the fermentative quality of guineagrass (Panicum maximum Jacq.) silage.* Grassl. Sci. 51: 243-248.

Bureenok, S., T. Namihira, M. Tamaki, S. Mizumachi, Y. Kawamoto and T. Nakada. 2005b. *Fermentative quality of guineagrass silage by using fermented juice of the epiphytic lactic acid bacteria (FJLB) as a silage additive.* Asian-Aust. J. Anim. Sci. 18: 807-811.

Catchpoole, V.R. and E.F Henzell.1971. *Silage and silage making from tropical herbage species.* Herbage Abstracts 41:213-221.

Carpintero M. C., Holding A. J. and McDonald P. 1969. *Fermentation studies on lucerne.* J. Sci. Food Agric. 20: 677-681.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1956. *Calorimetric method for determination of sugars and related substances.* Anal. Chem. 28: 350-356.

Haigh P. M. 1990. *Effect of herbage water-soluble carbohydrate content and weather conditions at ensilage on the fermentation of grass silages made on commercial farm.* Grass and Forage Sci. 45: 263-271.

Japan Grassland Farming Forage Seed Association.1994. *Guide Book for Quality Evaluation of forage.* Tokyo, Japan. (Jpn.). pp. 82-87.

Van Soest, P.J., J.B. Robertson and B.A Lewis. 1991. *Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition.* J. Dairy Sci. 74: 3583-3597.