Journal of Research and Agricultural Innovation Sakonnakhon Rajabhat University

วารสารวิจัยและนวัตกรรมการเกษตร 1(1): (2566) j. res. agri. inn.1(1): 2023

Effects of purple Guinea grass silage prepared with lactic acid bacteria and fibrolytic enzyme on feed intake, digestibility, and nitrogen balance in post-weaning Thai native beef cattle

Waroon Khota[1*], Suradej Pholsen[2], Sayan Subepang[3], Suwit Thip-uten[4] and Chatchai Kaewpila[1]

[1]*Faculty of Natural Resources, Rajamangala University of Technology Isan, Sakon Nakhon, Thailand, 47160*
[2]*Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand, 40002*
[3]*Faculty of Liberal Arts and Sciences, Sisaket Rajabhat University, Sisaket, Thailand, 33000*
[4]*Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon, Thailand, 47000*
**Corresponding author E-mail: waroon.kh@rmuti.ac.th*

Received: 17 January 2023
Acepted: 3 April 2023

Abstract

The objective of this study was to evaluate the effects of purple Guinea grass silage prepared with lactic acid bacteria and fibrolytic enzyme on feed intake, digestibility, and nitrogen balance in post-weaning Thai native beef cattle. A 4×4 Latin square design was conducted using 4 heads of post-weaning Thai native beef cattle (average body weight = 53.20±3 kg, and age = 6 months old), 4 treatments of purple Guinea grass silage prepared with and without (control), *Lactobacillus casei* TH14 (TH14), *Acremonium* cellulase (AC), and TH14+AC, and 4 periods (adaptation = 14 days, and collection = 7 days). In each period, cattle were fed silage *ad libitum*. The concentrate supplemented at 1.0 % body weight, and diets were fed at 09:00 and 17:00. The feaces and urine samples were obtained using a total collection technique. As a result, the silage treated with TH14 did not promote nutrient digestibility and nitrogen utilization (P>0.05). Compared with the control, in all treatments did not alter the chemical compositions of silage and feed intake (P>0.05). On the other hand, addition the TH14, AC, and TH14+AC improved the quality of purple Guinea grass silage by increasing lactic acid concentration (P<0.01) and ammonia nitrogen concentration (P<0.05). AC-treated silage showed a significant improvement in nutrient digestibility (P<0.05), while there was no significant effect on nitrogen utilization (P>0.05) in Thai native beef cattle. Therefore, the results of this study suggested that AC has greater potential as a silage additive for purple Guinea grass.

Keywords: lactic acid bacteria, cellulase, purple Guinea grass silage, Thai native beef cattle

1. Introduction

In the tropics, the major constraint for ruminant production is a shortage of roughage in terms of both quality and quantity, especially in the dry season (Alsersy et al., 2015; Khota et al., 2016).

Purple Guinea grass (*Panicum maximum* cv. 'TD 58') is widely available for planting throughout the tropical area, especially in the northeast of Thailand. This grass can adapt and grow well in a variety of soil types and tolerate continuous heavy grazing (Bureenok et al., 2016). Purple Guinea grass has a high dry matter yield in the rainy season but very low in the dry season (Yoottasanong et al., 2015). Thus, silage making is important to preserve fresh grass as a feedstock for all year-round feeding. However, purple Guinea grass is difficult to successfully ensile because it has a low water-soluble carbohydrate (WSC) and natural lactic acid bacteria (LAB) population for conducting the fermentation process (Bureenok et al., 2016). In contrast, it also has high fiber content which ruminants cannot digest easily. Therefore, the addition of LAB inoculants to forage prior to ensiling is an effective method to improve the fermentation quality by decreasing pH, butyric acid, and protein degradation as well as increasing lactic acid content (Napasirth et al., 2015; Pholsen et al., 2016).

To improve fiber degradation, the use of fibrolytic enzymes has been associated with beneficial effects on decreasing the fiber fraction and increasing digestion by ruminants (Oladosu et al., 2016) as indicated by *in vivo* study (Gado et al., 2011; Alsersy et al., 2015), due to synergistic effects between LAB inoculants and fibrolytic enzymes at ensiling. The addition of both additives is a strategy to improve both silage quality and digestibility. Nkosi et al. (2015) reported that the addition of enzymes and inoculants improved silage fermentation and increased digestion and nitrogen retention in rams. However, the information on the addition of LAB and fibrolytic enzymes, and their combination in tropical grass silage making on nutrient utilization in Thai native beef cattle is limited.

2. Objectives

The objectives of this study were to determine the effects of LAB inoculants, fibrolytic enzymes and their combinations ensiled with Guinea grass on fermentation quality, feed intake, nutrient digestion and nitrogen utilization in post-weaning Thai native beef cattle.

3. Materials and methods

3.1 Silage preparation

Purple Guinea grass (*Panicum maximum* cv. TD 58) was grown in an area of 4,800 m^2 on Korat soil series (Oxic Paleustults) at the experimental farm, Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand. Forage was cut to adjust the height level at approximately 10 cm above the ground surface. After 60 days of re-growth, forage was harvested in the early morning and immediately chopped into 2 cm lengths using a forage chopper. The chopped grass was separated into 4 equal portions and thoroughly mixed in each of 4 treatments: control (untreated), a locally selected *Lactobacillus casei* TH14 at 1.0×10^5 cfu/g of fresh matter (FM), *Acremonium* cellulase (AC) (Meiji Seika Pharma Co., Ltd, Tokyo, Japan) at 0.01% of FM, and TH14 combined with AC. For TH14-inoculant and AC application, the TH14-inoculant and/or AC were homogenized with 100 ml sterilized

distilled water and then sprayed in an aqueous solution using a sprinkler water-can into the ensiling materials which were spread equally on plastic sheets, then mixed well by hand. 100 ml of sterilized distilled water was sprayed into the control treatment. After mixing, the materials were ensiled in 200 L sealed polyethylene drums for 30 days before silage quality and chemical composition analyses and feeding to cattle.

3.2 Digestion trial

Four male calves of Thai native beef cattle, with an average body weight of 53.2 kg and 6 months of age at the start of the trial, were arranged in a 4×4 Latin square design with four experimental periods and four silage diets. Each experimental period lasted 21 days, 14 days for the preliminary adjustment period and 7 days for feces and urine collection. Before the start of each experimental period, the cattle were weighed and placed in individual pens (5×5 m) equipped with mineral blocks (FNZ Red Lick, Thai Serve Co. Ltd.; mineral composition: NaCl 930.00 g, Mg 2.00 g, Zn 0.77 g, Mn 0.50 g, Co 18.00 g, I 0.05 g, Se 0.01 g, Cu 0.22 g, another 2.50 g) and fresh water, then moved to individual metabolism cages (2×1 m) for the 7 days of each collection period. The housed animals were well ventilated and sufficiently illuminated. The chemical composition of the concentrate diet and four experimental silages are shown in Table 1. The cattle were fed *ad libitum* the assigned diet in two equal meals at 09:00 and 17:00hrs. The concentrate diet was fed at a fixed rate of 1% of animal live body weight.

Digestibility was conducted on a total collection method with 7 consecutive days in an individual metabolism cage. One kg of each silage sample was taken daily in each collection period. The orts, feces and urine of each animal were removed and weighed daily at 08.30 hrs, and were individually mixed and sampled: 1 kg for ort and feces, and 100 ml for urine, and stored frozen at –20°C. On the last day of each period, the stored orts, feces and urine were thawed and composited for each animal for chemical composition analysis. Urine was collected in a plastic box containing 100 ml of 6 N HCl to maintain a pH of 3.

3.3 Fermentation quality of silage and chemical composition analysis

Silage fermentation end products were analyzed from silage juice (Cai, 2004). For silage juice extraction, 10 g FM of silage was homogenized with 90 ml of sterilized distilled water and incubated at 4°C in a refrigerator for 12 hours. After incubation, the pH was measured immediately using a glass electrode pH meter (FiveGo; Mettler Toledo, Greifensee, Switzerland). Ammonia nitrogen content was determined using a spectrophotometer (UV/VIS Spectrometer, PG Instruments Ltd., London, UK) (Fawcett and Scott, 1960). The concentration of organic acids was measured by HPLC following the methods of Cai (2004).

The ensiled materials, concentrate, silages, orts, and feces samples were dried at 60°C for 48 h and ground to pass through a 1 mm screen, dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE) were analyzed following the methods 934.01, 942.05, 976.05 and 920.39 (AOAC,

1990), respectively. Urinary nitrogen was determined by the Kjeldahl procedure (AOAC, 1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed using a fiber analyzer (ANKOM 200, ANKOM Technology, New York, USA), based on the method of Van Soest et al. (1991).

3.4 Statistical Analysis

All data were analyzed using the SAS general linear model procedure for a Latin square design and used the following model for statistical analysis:

$$Yijk = \mu + \alpha i + \rho j + \tau k + \varepsilon ijk$$

where Yijk is the observation; μ is the overall mean, αi is the effect of animal i (i=1 to 4), ρj is the effect of the experimental period (p=1 to 4), τk is the effect of silages treatment (k=1 to 4), and εijk is the error. Duncan's test was used to determine the differences among treatmentmeans, and the significance level was considered to be $P \leq 0.05$ (Steel and Torrie, 1980).

4. Results

4.1 Chemical composition and gross energy of experimental diets

The chemical composition of the concentrate diet and purple Guinea grass silages are presented in Table 1. The CP, NDF, ADF and GE of the concentrate diet were 22.79, 16.21, 6.31 % DM, and 18.85 MJ/kg DM, respectively. All treated grass silages showed no significant differences ($P>0.05$) in chemical composition when compared with the control, except for ADF ($P<0.05$). The CP content of silages ranged from 8.06 to 8.76 % DM, NDF ranged from 70.74 to 74.84 % DM, and GE ranged from 17.70 to 17.85 MJ/kg DM. The NDF tended to be lower for AC and AC+TH14 treated silages.

Table 1 Chemical composition of concentrate diet and purple Guinea grass silages fed to Thai native beef cattle

Item	purple Guinea grass silages					1/SEM	p-value
	Concentrate	Control	TH14	AC	TH14+AC		
DM, %	88.99	23.82	22.58	23.29	23.56	1.359	0.925
OM, % DM	94.14	91.67	90.77	91.12	90.93	1.421	0.972
CP, % DM	22.79	8.53	8.06	8.76	8.64	1.321	0.983
EE, % DM	5.21	2.29	2.27	2.36	2.32	0.097	0.907
NDF, % DM	16.21	74.34	74.84	70.74	71.15	0.831	0.108
ADF, % DM	6.31	47.92^{ab}	49.12^{a}	46.28^{b}	46.70^{b}	0.629	0.038
ADL, %DM	1.44	6.43	6.67	6.42	6.90	0.285	0.607
GE, MJ/kg DM	18.85	17.70	17.69	17.85	17.76	0.068	0.978

1/SEM, standard error of the mean; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; GE, gross energy; TH14, a locally selected lactic acid bacteria *Lactobacillus casei* strain TH14; AC, a commercial cellulase enzyme *Acremonium* cellulase; a to b Means within rows with difference superscript letters differ at $P < 0.05$.

4.2 Fermentation quality of purple Guinea grass silages

The pH and fermentation products of purple Guinea grass silages are shown in Table 2. Purple Guinea grass silages treated with additives showed good quality. The TH14, AC, and TH14+AC treatments showed significantly lower pH values and more lactic acid concentration than the control. The butyric acid and ammonia nitrogen contents of the control were significantly higher than other treatments (P<0.01 and P<0.05, respectively). The lowest pH, butyric acid, and ammonia nitrogen concentration and highest lactic acid content were obtained in AC and TH14+AC treatments.

Table 2 Fermentation quality of purple Guinea grass silages

Item	Treatments				1/SEM	p-value
	Control	TH14	AC	TH14+AC		
pH	4.83[a]	4.41[b]	4.29[b]	4.31[b]	0.086	0.003
Lactic acid, g/kg DM	0.36[d]	1.23[c]	4.35[a]	3.37[b]	0.183	<0.001
Acetic acid, g/kg DM	1.02	0.74	0.83	1.12	0.151	0.353
Propionic acid, g/kg DM	0.05	0.03	0.01	0.01	0.016	0.282
Butyric acid, g/kg DM	1.25[a]	0.28[b]	0.01[b]	0.06[b]	0.088	0.001
Ammonia-N, g/kg DM	2.69[a]	2.10[ab]	1.59[b]	1.56[b]	0.099	0.020

1/SEM, standard error of the mean; DM, dry matter; TH14, a locally selected lactic acid bacteria *L. casei* strain TH14; AC, a commercial cellulase enzyme *Acremonium* cellulase; [a to c] Means within rows with difference superscript letters differ at P < 0.05.

4.3 Feed intakes and energy intakes of Thai native beef cattle fed purple Guinea grass silages

The feed intakes and energy intakes of Thai native beef cattle fed purple Guinea grass silages are shown in Table 3. The feed and energy intakes were not significantly different (P>0.05) among treatments. Dry matter intake (DMI) ranged from 1.63 to 1.78 kg/d, and from 2.61 to 2.78 % BW. The CP, NDF, and ADF intakes were 0.20, 0.91, and 0.57 kg/d, respectively. DE intake ranged from 15.70 to 18.51 MJ/d.

4.4 Apparent nutrient digestibility of Thai native beef cattle fed purple Guinea grass silages

The apparent nutrient digestibility of Thai native beef cattle fed purple Guinea grass silages are shown in Table 4. Digestibility of DM, OM, EE, NDF, ADF, and digestible energy significantly (P<0.05) increased in the AC treatment. The digestibility of CP and digestible crude protein were not significantly different (P>0.05) among treatments. Apart from ADF digestibility, there was no significant improvement in nutrient digestibility observed in the TH14 treated silage compared to the control treatment.

Table 3 Feed intakes and energy intakes of Thai native beef cattle fed purple Guinea grass silages

Item	Treatments				1/SEM	p-value
	Control	TH14	AC	TH14+AC		
Feed intakes						
DM, kg/d	1.74	1.63	1.71	1.78	0.082	0.674
DM, % BW	2.75	2.61	2.79	2.78	0.109	0.635
OM, kg/d	1.61	1.50	1.57	1.62	0.078	0.715
CP, kg/d	0.23	0.20	0.22	0.23	0.016	0.531
EE, kg/d	0.06	0.05	0.06	0.06	0.003	0.372
NDF, kg/d	0.94	0.91	0.91	0.96	0.047	0.861
ADF, kg/d	0.59	0.58	0.57	0.60	0.034	0.911
ADL, kg/d	0.08	0.08	0.08	0.09	0.005	0.242
Energy intakes						
GE, MJ/d	31.41	29.60	31.00	31.97	1.491	0.720
DE, MJ/d	17.47	15.70	18.51	18.24	1.502	0.582
ME, MJ/d	14.32	12.88	15.18	14.96	1.233	0.582

1/SEM, standard error of the mean; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; GE, gross energy; DE, digestible energy; BW, body weight; TH14, a locally selected lactic acid bacteria *Lactobacillus casei* strain TH14; AC, a commercial cellulase enzyme *Acremonium* cellulase. Metabolizable energy (ME) was calculated following the equation of NRC (2000) (ME = 0.82 × DE).

Table 4 Apparent nutrient digestibility of Thai native beef cattle fed purple Guinea grass silages

Item	Treatments				1/SEM	p-value
	Control	TH14	AC	TH14+AC		
Digestibility, %						
DM	55.69^{bc}	51.87^{c}	62.25^{a}	57.21^{b}	0.713	0.031
OM	56.57^{b}	52.08^{b}	69.25^{a}	58.20^{b}	0.833	0.026
CP	64.28	61.41	63.44	64.16	0.306	0.094
EE	71.27^{bc}	68.98^{c}	83.94^{a}	74.39^{b}	0.589	0.018
NDF	36.24^{b}	44.66^{b}	57.71^{a}	45.49^{b}	1.983	0.019
ADF	30.26^{c}	39.09^{b}	53.16^{a}	38.09^{b}	0.736	0.002
Digestible crude protein, % DM	9.31	8.23	8.99	8.79	0.182	0.137
Digestible energy, MJ/kg DM	10.92^{ab}	9.23^{b}	12.71^{a}	10.50^{b}	0.208	0.046

1/SEM, standard error of the mean; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; TH14, a locally selected lactic acid bacteria *Lactobacillus casei* strain TH14; AC, a commercial cellulase enzyme *Acremonium* cellulase; $^{a\ to\ c}$ Means within rows with difference superscript letters differ at $P < 0.05$.

4.5 Nitrogen retention of Thai native beef cattle fed purple Guinea grass silages

The nitrogen intake and nitrogen retention are presented in Table 5. There were no significant ($P>0.05$) differences among treatments. Nitrogen intake, fecal nitrogen, urinary nitrogen, digestible nitrogen, and retained nitrogen ranged from 32.04 to 37.33, 11.84 to 13.50, 13.38 to 16.16, 18.63 to 23.88, and 5.06 to 10.50 g/d, respectively.

Table 5 Nitrogen utilization of Thai native beef cattle fed purple Guinea grass silages

Item	Treatments				[1/]SEM	p-value
	Control	TH14	AC	TH14+AC		
Nitrogen intake, g/d	37.33	32.04	35.72	36.92	2.603	0.512
Fecal nitrogen, g/d	13.50	13.41	11.84	13.04	0.572	0.250
Urinary nitrogen, g/d	14.01	13.57	13.38	16.16	0.923	0.228
Digestible nitrogen, g/d	23.83	18.63	23.88	23.88	2.070	0.286
Retained nitrogen, g/d	9.82	5.06	10.50	7.73	1.782	0.233
Digestible nitrogen, % NI	63.81	57.82	67.87	65.58	2.130	0.068
Retained nitrogen, % NI	26.54	14.52	30.73	20.92	4.183	0.129

[1/]SEM, standard error of the mean; NI, nitrogen intake; TH14, a locally selected lactic acid bacteria *Lactobacillus casei* strain TH14; AC, a commercial cellulase enzyme *Acremonium* cellulase; [a to b] Means within rows with difference superscript letters differ at $P < 0.05$.

5. Discussion

Many researchers have reported the positive benefit of LAB-inoculant and fibrolytic enzyme addition to fermentation quality and fiber content of silages (Kaewpila et al., 2021a; Kaewpila et al., 2021b). In the present study, applying TH14-inoculants and cellulase enzyme alone or in mixtures improved the silage quality of purple Guinea grass with a low pH, butyric acid, and ammonia nitrogen content and high concentration of lactic acid compared with the control treatment (Table 2). Our findings agree with Cao et al. (2011); Napasirth et al. (2015); Pholsen et al. (2016) who reported that the addition of LAB could improve the fermentation quality of vegetable residues, cassava residues, purple Guinea grass and sorghum. They are also consistent with Dean et al. (2005); Khota et al. (2016) who found that enzyme addition could improve silage quality. This is because the enzyme breaks down the structural carbohydrate of plant cell walls (i.e. cellulose or fiber) to release water-soluble carbohydrates (WSC) that can be utilized by LAB to produce more lactic acid (Oladosu et al., 2016). Previous works have reported that enzyme addition can affect the degradation of forage cell walls thus lowering the total fiber of the silage materials (Dean et al., 2005; Khota et al., 2016; Oladosu et al., 2016). In the present study, both the AC and TH14+AC treatments led to a significantly ($P<0.05$) reduced the ADF contents compared to TH14 treatment alone. The NDF also tended to be lower ($p=0.108$) when compared to other treatments (Table 1), this is consistent with Sun et al. (2012); Paya et al. (2015) who reported that the addition of cellulase resulted in decreased NDF and ADF content of shrub and orange pulp silage respectively. This is also in agreement with Kung et al. (1990); Zahiroddini et al. (2004) who reported that cellulase addition did not decrease cell wall contents of silages.

WTSR (2010) reported that Thai native beef cattle, with an average body weight of 50 kg had a DMI of 2.3 % BW; this is similar to our findings (2.6 to 2.8 % BW). Phromloungsri et al. (2012) reported that Thai native beef cattle fed rice straw and concentrate feed had a voluntary DMI of 1.7% BW. TH14, AC and TH14+AC treated silages did not significantly ($P>0.05$) influence feed and energy intakes (Table 3). This study is consistent with Arriola et al. (2011) who found that applying LAB-inoculants to

corn silage did not affect intakes when fed to dairy cattle. Our findings are not in agreement with Nadeau et al. (2000) who reported that lambs fed orchard grass and alfalfa silage treated with cellulase + LAB inoculants increased total DMI by 8% when compared with control.

The addition of fibrolytic enzyme significantly increased nutrient digestibility ($P<0.05$) by 6% compared with the control treatment. This indicates that the fibrolytic enzymes break down complex plant cell wall materials into smaller sugar molecules such as glucose, which can be digested by ruminant animals (Oladosu et al., 2016; Salem et al., 2012). In addition, fibrolytic enzyme addition enhances the attachment and colonization of rumen microorganisms to the forage cell wall (Chung et al., 2012; Alsersy et al., 2015). Our findings agree with Gado et al. (2011); Alsersy et al. (2015) who reported that the addition of exogenous fibrolytic enzymes resulted in increased nutrient digestion of orange pulp and *Atriplex halimus* silages fed to lambs and sheep, respectively. In contrast, Nadeau et al. (2000) reported that lambs fed orchardgrass and alfalfa cellulase-treated silages decreased NDF digestibility. The TH14 addition did not affect ($P>0.05$) nutrient digestibility of purple Guinea grass silage when fed to Thai native beef cattle. This is in agreement with Aksu et al. (2006); Arriola et al. (2011); Fang et al. (2012) who reported that the addition of LAB-inoculants in corn silage and rice straw silage did not affect nutrient digestibility when fed to sheep, dairy cattle and wethers, respectively.

The application of fibrolytic enzymes can enhance fiber digestion through multiple mechanisms, leading to an increase in microbial biomass and polysaccharides activity, which can aid in the digestion of various feed types and the degradation of secondary metabolites. Our findings suggest that supplementing enzymes can enhance fiber digestibility, while having no significant ($P>0.05$) impact on N intake and N utilization. This is inconsistent with Alsersy et al. (2015) who reported that the addition of exogenous fibrolytic enzymes resulted in increased nitrogen retention of *Atriplex halimus* silage when fed to sheep.

6. Conclusions

The present study indicates that purple Guinea grass treated with TH14, AC, and their combination prior to ensiling improved fermentation quality by increasing lactic acid production. Using TH14 did not promote nutrient digestibility and nitrogen utilization. Feeding Thai native beef cattle with AC-treated silage resulted in improved nutrient digestibility, but did not have any effect on nitrogen utilization. Therefore, the results of this study confirm that *Acremonium* cellulase has greater potential as a silage additive for purple Guinea grass.

7. Acknowledgements

The authors would like to thank the Rajamangala University of Technology Isan, Animal Nutrition Laboratory (Khon Kaen University), and Japan International Research Center for Agricultural Sciences for the infrastructure and laboratory facilities.

8. References

AOAC. (1990). Official Methods of Analysis. (15th ed.), Association of Official Analytical Chemists. Arlington, VA, USA.

Aksu, T., Baytok, E., Karsli, M. A., and Muruz, H. (2006). Effects of formic acid, molasses and inoculant additives on corn silage composition, organic matter digestibility and microbial protein synthesis in sheep. *Small Ruminant Research*, *61*, 29–33.

Alsersy, H., Salem, A. Z. M., Borhami, B. E., Olivares, J., Gado, H. M., Mariezcurrena, M. D., Yacuot, M. H., Kholif, A. E., El-Adawy, M., and Hernandez, S. R. (2015). Effect of Mediterranean saltbush (Atriplex halimus) ensilaging with two developed enzyme cocktails on feed intake, nutrient digestibility and ruminal fermentation in sheep. *Animal Science Journal*, *86*, 51–58.

Arriola, K. G., Kim, S. C., Staples, C. R., and Adesogan, A. T. (2011). Effect of applying bacterial inoculants containing different types of bacteria to corn silage on the performance of dairy cattle. *Journal of Dairy Science*, *94*, 3973–3979.

Bureenok, S., K. Sisaath, C. Yuangklang, K. Vasupen, and J. T. Schonewille. (2016). Ensiling characteristics of silages of Stylo legume (Stylosanthes guianensis), Guinea grass (Panicum maximum) and their mixture, treated with fermented juice of lactic bacteria, and feed intake and digestibility in goats of rations based on these silages. *Small Ruminant Research, 134*, 84–89.

Cai, Y. (2004). Analysis method for silage. In: Japanese Society of Grassland Science (ed.), Field and Laboratory Methods for Grassland Science. Tosho Printing Co. Ltd., Tokyo, Japan. p. 279–282.

Cao, Y., Cai, Y., Takahashi, T., Yoshida, N., Tohno, M., Uegaki, R., Nonaka, K., and Terada, F. (2011). Effect of lactic acid bacteria inoculant and beet pulp addition on fermentation characteristics and in vitro ruminal digestion of vegetable residue silage. *Journal of Dairy Science*, *94*, 3902–3912.

Chung, Y. H., Zhou, M., Holtshausen, L., Alexander, T. W., McAllister, T. A., Guan, L. L., Oba, M., and Beauchemin, K. A. (2012). A fibrolytic enzyme additive for lactating Holstein cow diets: ruminal fermentation, rumen microbial populations, and enteric methane emissions. *Journal of Dairy Science*, *95*, 1419–1427.

Dean, D., Adesogan, A., Krueger, N., and Littell, R. (2005). Effect of Fibrolytic Enzymes on the Fermentation Characteristics, Aerobic Stability, and Digestibility of Bermudagrass Silage. *Journal of Dairy Science*, *88*, 994–1003.

Fang, J., Matsuzaki, M., Suzuki, H., Cai, Y., Horiguchi, K. I., and Takahashi, T. (2012). Effects of lactic

acid bacteria and urea treatment on fermentation quality, digestibility and ruminal fermentation of roll bale rice straw silage in wethers. *Grassland Science*, *58*, 73–78.

Fawcett, J. K., and J. E. Scott. (1960). A rapid and precise method for the determination of urea. *Journal of Clinical Pathology,* 13, 156–159.

Gado, H. M., Salem, A. Z. M., Odongo, N. E., and Borhami, B. E. (2011). Influence of exogenous enzymes ensiled with orange pulp on digestion and growth performance in lambs. *Animal Feed Science and Technology*, *165*, 131–136.

Kaewpila, C., Gunun, P., Kesorn, P., Subepang, S., Thip-uten, S., Cai, Y., Pholsen, S., Cherdthong, A., and Khota, W. (2021a). Improving ensiling characteristics by adding lactic acid bacteria modifies in vitro digestibility and methane production of forage-sorghum mixture silage. *Scientific Reports*, *11*, 1–9.

Kaewpila, C., Thip-Uten, S., Cherdthong, A., and Khota, W. (2021b). Impact of cellulase and lactic acid bacteria inoculant to modify ensiling characteristics and in vitro digestibility of sweet corn stover and cassava pulp silage. *Agriculture*, *11*, 1-12.

Khota, W., Pholsen, S., Higgs, D., and Cai, Y. (2016). Natural lactic acid bacteria population of tropical grasses and their fermentation factor analysis of silage prepared with cellulase and inoculant. *Journal of Dairy Science*, *99*, 9768–9781.

Kung, L., Carmean, B., and Tung, R. (1990). Microbial Inoculation or Cellulase Enzyme Treatment of Barley and Vetch Silage Harvested at Three Maturities. *Journal of Dairy Science*, *73*, 1304–1311.

Nadeau, E. M. G., Russellt, J. R., and Buxton, D. R. (2000). Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs. *Journal of Animal Science*, *78*, 2980–2989.

Napasirth, V., Napasirth, P., Sulinthone, T., Phommachanh, K., and Cai, Y. (2015). Microbial population, chemical composition and silage fermentation of cassava residues. *Animal Science Journal*, *86*, 842–848.

Nkosi, B. D., R. Meeske, T. Langa, M. D. Motiang, and T. F. Mutavhatsindi. (2015). The influence of ensiling potato hash waste with enzyme / bacterial inoculant mixtures on the fermentation characteristics, aerobic stability and nutrient digestion of the resultant silages by rams. *Small Ruminant Research,* 127, 28–35.

NRC. (2000). Nutrient Requirements of Beef Cattle. (7th ed.). National Academy Press. Oklahoma, USA.

Oladosu, Y., Rafii, M. Y., Abdullah, N., Magaji, U., Hussin, G., Ramli, A., and Miah, G. (2016). Fermentation Quality and Additives: A Case of Rice Straw Silage. *BioMed Research International*, 1-15.

Paya, H., Taghizadeh, A., and Lashkari, S. (2015). Effects of Lactobacillus plantarum and hydrolytic enzymes on fermentation and ruminal degradability of orange pulp silage. *Journal of BioScience and Biotechnology*, *4*, 349–357.

Pholsen, S., Khota, W., Pang, H., Higgs, D., and Cai, Y. (2016). Characterization and application of

lactic acid bacteria for tropical silage preparation. *Animal Science Journal*, *87*, 1202–1211.

Phromloungsri, A., Hayashi, K., Otsuka, M., Udchachon, S., and Sommart, K. (2012). Effects of energy intake level on methane production of Thai Native and Brahman crossbred cattle. *Khon Kaen Agriculture Journal*, *40*, 5–11.

Salem, A. Z. M., Hassan, A. A., Khalil, M. S., Gado, H. M., Alsersy, H., and Simbaya, J. (2012). Effects of sun-drying and exogenous enzymes on nutrients intake, digestibility and nitrogen utilization in sheep fed Atriplex halimus foliages. *Animal Feed Science and Technology*, *171*, 128–135.

Steel, R. G. D., and J. H. Torrie. (1980). Principles and Procedures of Statistics: A Biometrical Approach. (2nd ed.) McGraw–Hill Book Co. Inc., New York, NY, USA.

Sun, Q., Gao, F., Yu, Z., Tao, Y., Zhao, S., and Cai, Y. (2012). Fermentation quality and chemical composition of shrub silage treated with lactic acid bacteria inoculants and cellulase additives. *Animal Science Journal*, *83*, 305–309.

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. *Journal of Dairy Science*, 74, 3583–3597.

WTSR. (2010). *Nutrient Requirement of Beef Cattle in Indochinese Penin-sula. The Working Committee of Thai Feeding Standard for Ruminant.* Klungnanavitthaya Press, Khon Kaen, Thailand.

Yoottasanong, C., S. Pholsen, and D. E. B. Higgs. (2015). Dry Matter Yields and Forage Quality of Grass Alone and Grass Plus Legume Mixture in Relation to Cattle Manure Rates and Production Methods. *Pakistan Journal of Biological Sciences,* 18, 324–332.

Zahiroddini, H., Baah, J., Absalom, W., and McAllister, T. A. (2004). Effect of an inoculant and hydrolytic enzymes on fermentation and nutritive value of whole crop barley silage. *Animal Feed Science and Technology*, *117*, 317–330.