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Strategic Addition of Different Additives to Improve Silage Fermentation, Aerobic Stability and In Vitro Digestibility of Napier Grasses at Late Maturity Stage

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Abstract: Napier cultivars are widespread in the tropics. To effectively prepare two Napier grasses at the late maturity stageas silage for ruminant feedstock, the silage fermentation characteristics, aerobic stability, in vitro digestibility, and gas kinetics were studied. Napier Pakchong grass (NP) and sweet grass (SG) were harvested at 120 dof regrowth and untreated (control) or treated with normal or a double dose of cellulase, urea, and formic acid. After 30 d of ensiling, the pH values of silages (ranging from 3.91 to 7.79) were affected ($p < 0.05$) by additives and lower in control and cellulase-treated silages than in urea- and formic acid-treated silages. Adding cellulase resulted in greater lactic acid concentrations in SG silage. Adding urea boosted acetic acid, propionic acid, butyric acid, and ammonia nitrogen levels of silages. Adding formic acid spoiled silages and shortened the aerobic stability of NP silage. Compared with the control, the addition of cellulase at a double dose enhanced in vitro dry matter digestibility, and the gas production kinetics parameters of silages. Taking silage fermentation quality and in vitro dry matter digestibility into consideration, adding cellulase (0.2 g/kg fresh matter) to Napier grasses at the late maturity stage could be a better option compared with the other additives.

Keywords: additives; in vitro digestibility; Napier grass; silage fermentation

1. Introduction

Napier grass (*Pennisetum purpureum*) is widely used in ruminant feed in the tropics, and "silage-making practice" is an optional method for year-round feeding. In Thailand, cultivations of Napier Pakchong grass (NP) (*P. purpureum* × *P. americanum* cv. Pakchong 1) and sweet grass (SG) (*P. purpureum* cv. Mahasarakham) are well established. The NP option has a high dry matter (DM) yield from 63 to 87 t/ha/year, is seasonal over the short rainy season and has an optimum stage of maturity of about 60 d of regrowth [1]. The high biomass production makes the Napier grass an interesting crop for biogas, electricity, bio-ethanol, and animal feedstock production [1,2]. For silage purposes, Khota et al. [3,4] reported that NP's high water soluble carbohydrate (WSC) content enhanced its quality and was, sufficient for a rapid pH decrease. SG is a dwarf Napier grass and has an excellent crude protein (CP) content of 151 g/kg DM when harvested at 42 d of regrowth [5]. Chaikong et al. [6] reported that the CP contents of SG harvested at 28, 42, 56, and 70 d of regrowth were 238, 184, 159, and 156 g/kg DM, respectively. Unfortunately, the DM contents of fresh NP and SG are low, ranging from 104 to 179 g/kg [3–5], and Napier grasses at both optimum and late maturity stages are commonly used in this area [1]. The lignin fractions in grasses have also increased due to cutting at a later stage [7].

The nature of lignification bound to the structural carbohydrate can bea major barrier to nutrient digestibility [7], which is very important in ruminant production [8].

To improve digestibility for ruminants, numerous publications have evidenced the effects of cell-wall-degrading enzymes, urea, or formic acid on the fermentation of grass silage. Cellulase can degrade fiber fractions into fermentable WSC content important for producing lactic acid to rapidly decrease the pH [9]. The urea approach is based on ureolysis—converting urea into ammonia—which has an alkalizing corrosive effect on the forage cell walls [10]. The formic acid additive associates with an acidification around the forage, thus rapidly decreasing the pH and degrading the forage content [11–13].

Limited information is available on the effect of cellulase enzyme, urea, or formic acid on the nutrient preservation and silage quality for ruminants of NP and SG silages at the late maturity stage. In addition, the most important variable in additives for silage is the amount added. The choices of normal dose and dose increments for subsequent doses are ordinary objectives that need to be evaluated. Therefore, the objective of this experiment was to study silage fermentation quality, aerobic stability, in vitro digestibility, and gas kinetics of NP and SG harvested at the late stage of maturity and prepared with cellulase enzyme, urea, or formic acid at different levels.

2. Materials and Methods

2.1. Ensiling Material Preparation

The NP and SG were grown in May 2019 at the experimental farm of Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand, in an area of 800 m^2 for each plant in Korat soil series (Oxic Paleustults). Before planting, both plots were ploughed and harrowed once. The stem cuttings of both grasses were planted into rows by hand at distances between and within rows of 120 by 80 cm and 65 by 40 cm for NP and SG, respectively. Basal dressing N:P:K fertilizers (15:15:15) and cattle manure were applied at 300 and 12,500 kg/ha, and urea fertilizer (46:0:0) at a rate of 60 kg/ha was split applied. Both Napier cultivars were harvested after 120 d of regrowth, cut close to the soil surface. Three sub-plots ofeach cultivar were established using quadrate samplers (2 by 2 m). Then, these six samples (two grasses by three sub-plots) were chopped individually with a theoretical length of 1 cm via a chopper (chopper model 8-inch knives, Supachai Factory, Kanchanaburi, Thailand). Each grass sample was then divided into 22 portions of 300 g of fresh matter (FM). The first portion was used immediately to analyze the microbial counts and chemical composition of the fresh materials, and the other portion was used to prepare silages.

2.2. Experimental Design and Silage Preparation

This experiment was conducted as a completely randomized design for studying individual grass with different additives at different levels. The NP and SG were untreated (control) or treated with three additives on an FM basis *viz.* cellulase enzyme 0.1 or 0.2 g/kg (CE-N or CE-D, respectively), urea 5.5 or 11.0 g/kg (U-N or U-D, respectively), and formic acid 6.0 or 12.0 g/kg (FA-N or FA-D, respectively). These two concentrations were estimated to represent the normal and double dose rates of each additive. The normal dose rate for cellulase enzyme, urea, and formic acid in this study was a modest dosage for preparing forage silages suggested by Khota et al. [4], Wanapat et al. [10], and Waldo et al. [11], respectively. A cellulase enzyme (*Acremonium cellulolyticus*, glucanase and pectinase 7350 U/g) [4], a reagent-grade urea (NH_2CONH_2, 99.5% purity), and a reagent-grade formic acid (HCOOH, 98% purity) were used. For every 300 g of FM grass, the individual cellulase and urea additives were mixed with 3 mL of sterilized distilled water, and formic acid additives were used undiluted. The additives were added by spraying onto grass and were mixed homogeneously. The forage mixtures were packed into laboratory-scale silos laminated with nylon and polyethylene (Hiryu KN type, Asahi Kasei Pax Corp., Tokyo, Japan) and sealed via a vacuum sealer (SQ-303, Asahi Kasei Pax Corp.). Three bags per treatment were prepared, which was regarded as an adequate sample size for the silage evaluations based on a small-scale silo technique according to previous studies [3,14].

Silages were preserved at room temperature (25 to 37 °C) and opened after 30 d of fermentation to entera stable phase [15].

2.3. Microbial Analysis

Following Kozaki et al.'s [16] method, samples of fresh materials and silages were analyzed for microbiological counts. Each 10-g FM sample was homogenized in 90 mL of sterilized water and serially diluted from 10^{-1} to 10^{-5} with a 0.85% NaCl solution. Each 20-µL dilution was spread on prepared agar plates. The lactic acid bacteria colonies were counted on a Lactobacilli de Man, Rogosa, Sharpe (MRS) agar (Difco Laboratories Inc., Detroit, MI, USA) after incubating at 30 °C for 48 h in an anaerobic chamber (Sugiyamagen Ltd., Tokyo, Japan). The coliform bacteria were counted on a blue light broth agar (Nissui Ltd., Tokyo, Japan) after incubating at 30 °C for 48 h. Aerobic bacteria were counted on a nutrient agar (Difco Laboratories Inc.) after incubating at 30 °C for 24 h. Yeast and mold were counted on a potato dextrose agar (Nissui Ltd.) after incubating at 30 °C for 24 h. Yeasts were distinguished from molds or bacteria by observing colony appearance and cell morphology. The microbial counts were reported in colony forming units (cfu) on the FM basis.

2.4. Fermentation End Products Analysis of Silages

A silage sample (10 g of FM) was homogenized in 90 mL of sterilized distilled water and incubated at 4 °C in a refrigerator overnight [9]. Then, the pH was measured using a glass electrode pH meter (FiveGo; Mettler-Toledo GmbH, Greifensee, Switzerland). The ammonia nitrogen and lactic acid concentrations were analyzed using a spectrophotometer (UV/VIS Spectrometer, PG Instruments Ltd., London, UK) via the methods of Fawcett and Scott [17] and Borshchevskaya et al. [18], respectively. Based on the procedure Cai [19] described, the acetic acid, propionic acid, and butyric acid contents were measured using a gas chromatograph (GC 8890; Agilent technologies Ltd., Santa Clara County, CA, USA) equipped with a capillary column (molecular sieve 13×, 30/60 mesh, Alltech Associates Inc., Deerfield, IL, USA).

2.5. Chemical Composition, Lactate Buffering Capacity, and Aerobic Stability Analyses

Aliquots of fresh materials and silages were dried in a forced air oven at 60 °C for 48 h, then grinded to pass through a 1-mm mesh screen using a Retsch mill (Retsch SK-1, Retsch GmbH, Haan, Germany). The chemical composition of the ground samples was analyzed following the standard Association of Official Analytical Chemists (AOAC) [20] method, including DM (method 930.15), organic matter (OM; method 942.05 for determination of total ash), CP (method 954.01), and ether extract (EE; method 920.39). The neutral detergent fiber (NDF, assayed with a heat stable amylase and expressed inclusive of residual ash) and acid detergent fiber (ADF, expressed inclusive of residual ash) were analyzed via a fiber analyzer (ANKOM 200, ANKOM Technology, New York, NY, USA) using the method described by Van Soest et al. [21]. The acid detergent lignin (ADL) was assessed by solubilization with sulfuric acid following the method of Faichney and White [22].

The lactic acid buffering capacity (LBC) of the fresh materials was determined by titrating the samples (a mixture of 10 g of FM sample and 90 mL of distilled water) with 0.1 M of HCl (to reduce the initial pH to three) and then with 0.1 M of NaOH (changing the pH from three to six) [23].

Aerobic stability of silage samples was determined according to the method Moselhy et al. [24] described; it was defined as how many hours it took to increase the silage temperature to more than 2 °C above room temperature. Briefly, a sample (150 g of FM) was incubated in a 500-mL plastic beaker covered by two layers of cheesecloth at room temperature (26 to 35 °C). The silage temperature was measured every hour via a digital thermometer probe.

2.6. In Vitro Gas Production Technique

All animal-related procedures used in this study were reviewed and approved by the Animal Ethics Committee of Rajamangala University of Technology Isan (Project ID: 6/2563). An in vitro gas

production technique was used, following the procedure described by Makkar et al. [25]. The rumen fluid was collected before morning feeding from two dairy steers by a stomach-tube sucker. The daily cattle's diet was a concentrate diet (0.5% of their body weight, including cassava chips, rice bran, coconut kernels cake, palm kernels cake, urea, and a vitamin-mineral mixture at 500, 300, 110, 60, 10, and 20 g/kg, respectively, on a DM basis), and rice straw was fed ad libitum. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed (39 °C) thermo bottles, and transported immediately to the laboratory. The rumen fluid was mixed with a buffer solution [25]. Ground samples (0.5 g) were pre-weighed into 50-mL serum bottles (six bottles per experimental unit: three bottles to determine in vitro digestibility at 48 h and another to determine gas production at 96 h of incubation). The bottles were closed by rubber stoppers with aluminum seal caps. The rumen fluid-buffer mixture (40 mL) was injected into each sample bottle using a 60-mL syringes with a 1.5-inch 18-gauge needle and anaerobically flushed via CO_2. One run was used, and all sample bottles (two grass silages by seven treatments by three replications by six bottles) were incubated in a water-bath shaker (WNB22, Memmert GmbH + Co. KG, Schwabach, Germany) at 39 °C. Six bottles containing only rumen inoculum were incubated as blanks. Gas production was measured after incubation at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 14, 18, 24, 30, 36, 42, 48, 60, 72, and 96 h using a pressure transducer and a calibrated glass syringe following Cherdthong and Wanapat [26]. Cumulative gas production data were fitted to the model of Ørskov and McDonald [27], as follows:

$$y = a + b\ (1 - e^{-ct}), \quad (1)$$

where a is the gas production from the immediately soluble fraction, b is the gas production from the insoluble fraction, c is the gas production rate constant for the insoluble fraction (b), t is incubation time, a + b is the potential extent of gas production, and y is gas produced at time "t" [26]. At 48 h after incubation, three bottles per treatment were opened and their pH was measured immediately. The undigested sample was filtered through a glass filter crucible (ROBU 50 mL-Por.1, ROBU Glasfilter-Geräte GmbH, Hattert, Germany), dried at 100 °C in a forced air oven for 24 h, and weighed for in vitro DM digestibility (IVDMD) determination according to a procedure as used previously by Khota et al. [4] and Yuan et al. [28]. The dried residues were burned in the muffle furnace at 550 °C for 3 h to measure the in vitro OM digestibility (IVOMD).

2.7. Statistical Analysis

The data obtained from each type of grass silage were analyzed for variance using an ANOVA procedure in SAS version 6.12 (SAS Institute Inc., Cary, NC, USA). Data were analyzed using the following model:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}, \quad (2)$$

where Y_{ij} is observation, μ is overall mean, α_i is additive effect (i = 1 to 7), and ε_{ij} is error. The significant differences among treatment means were assessed by Duncan's new multiple range test (DMRT) and the significance level was set at $p < 0.05$ [29].

3. Results

3.1. Microorganism Counts and Chemical Composition of Napier grasses at the Late Maturity Stage

Microbial populations of the fresh NP and SG used in this study are presented in Table 1. Total lactic acid bacteria counts ranged from 10^3 to 10^4 cfu/g FM. The coliform bacteria and aerobic bacteria counts ranged from 10^6 to 10^7cfu/g FM. The yeast and mold counts of NP and SG were 10^5 and 10^7 cfu/g FM, respectively.

The DM contents (Table 2) of NP and SG were 157 and 131 g/kg, respectively. Compared to other chemical compositions, the CP and NDF contents varied between NP and SG. The LBC contents of NP and SG ranged from 560 to 757 mEq/kg DM.

Table 1. Microbial counts of fresh Napier grasses at the late maturity stage used in this study.

Item [1]	Lactic Acid Bacteria	Coliform Bacteria	Aerobic Bacteria	Yeasts	Molds
	(cfu/g FM)				
NP	2.2×10^3	3.2×10^6	8.0×10^7	2.9×10^5	3.6×10^7
SG	2.7×10^4	2.3×10^7	4.7×10^6	2.5×10^5	2.8×10^7

[1] NP, Napier Pakchong grass; SG, sweet grass; cfu, colony forming unit; and FM, fresh matter.

Table 2. Chemical composition and lactate buffer capacity of fresh Napier grasses at the late maturity stage used in this study.

Item [1]	DM	OM	CP	EE	NDF	ADF	ADL	LBC
	(g/kg)	(g/kg DM)						(mEq/kg DM)
NP	157	937	71.2	26.5	755	486	55.6	757
SG	131	924	122	31.4	699	424	42.1	560

[1] NP, Napier Pakchong grass; SG, sweet grass; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; and LBC, lactate buffer capacity.

3.2. Fermentation Characteristics of Silages

The fermentation endproducts of silages prepared from NP and SG at the late maturity stage are shown in Table 3. In NP silage, the pH values were lower ($p < 0.05$) for the control, CE-N, or CE-D treatment compared with FA-N, U-N, FA-D, and U-D treatments. The lactic acid concentrations of NP silage differed ($p < 0.05$) among treatments; they were greater for control, CE-N, and CE-D than for other treatments. Compared with U-N, the addition of U-D to NP silage had consistently greater ($p < 0.05$) acetic acid, propionic acid, butyric acid, and ammonia nitrogen concentrations. In SG silage, control, CE-N, or CE-D resulted in lower ($p < 0.05$) pH values compared with other treatments. The lactic acid concentrations from CE-N or CE-D treatment were greater ($p < 0.05$) than that of control and other additive treatments. Adding FA-D or U-D increased ($p < 0.05$) acetic acid, propionic acid, butyric acid, and ammonia nitrogen concentrations of SG silage.

Table 3. Fermentation characteristics of silage safter 30 d of fermentation.

Item [1]	Additive at Ensiling	pH	Lactic Acid	Acetic Acid	Propionic Acid	Butyric Acid	NH_3-N (g/kg TN)
			(g/kg DM)				
NP silage	Control	4.05 ^{e}	18.7 ^{a}	1.27 ^{b}	<0.001 ^{c}	0.408 ^{b}	8.55 ^{c}
	CE-N	4.01 ^{e}	22.0 ^{a}	1.93 ^{b}	<0.001 ^{c}	<0.001 ^{b}	9.57 ^{c}
	CE-D	3.91 ^{e}	23.2 ^{a}	1.81 ^{b}	<0.001 ^{c}	<0.001 ^{b}	8.28 ^{c}
	U-N	5.54 ^{c}	6.41 ^{c}	10.6 ^{a}	0.283 ^{b}	4.61 ^{a}	149 ^{b}
	U-D	7.79 ^{a}	3.05 ^{c}	11.9 ^{a}	1.45 ^{a}	3.94 ^{a}	177 ^{a}
	FA-N	5.01 ^{d}	4.23 ^{c}	1.42 ^{b}	0.146 ^{bc}	0.895 ^{b}	6.93 ^{c}
	FA-D	7.34 ^{b}	1.33 ^{b}	2.25 ^{b}	<.001 ^{c}	1.08 ^{b}	17.5 ^{c}
	SEM	0.093	1.571	1.004	0.0558	0.4292	6.869
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SG silage	Control	4.06 ^{d}	19.9 ^{b}	1.53 ^{c}	<0.001 ^{c}	<0.001 ^{b}	23.8 ^{d}
	CE-N	3.98 ^{d}	25.5 ^{a}	2.11 ^{c}	<0.001 ^{c}	<0.001 ^{b}	22.0 ^{d}
	CE-D	3.97 ^{d}	27.1 ^{a}	2.26 ^{c}	<0.001 ^{c}	<0.001 ^{b}	23.3 ^{d}
	U-N	6.17 ^{b}	6.29 ^{c}	10.2 ^{b}	0.360 ^{bc}	3.81 ^{a}	203 ^{b}
	U-D	6.79 ^{b}	3.27 ^{cd}	19.1 ^{a}	1.30 ^{a}	4.81 ^{a}	243 ^{a}
	FA-N	5.31 ^{c}	1.60 ^{d}	<0.001 ^{c}	<0.001 ^{c}	<0.001 ^{b}	80.3 ^{c}
	FA-D	7.56 ^{a}	0.380 ^{d}	9.94 ^{b}	0.966 ^{ab}	4.87 ^{a}	111 ^{c}
	SEM	0.252	1.4208	1.0952	0.2769	0.8150	11.45
	p	<0.001	<0.001	<0.001	0.018	<0.001	<0.001

[1] NP, Napier Pakchong grass; SG, sweet grass; CE-N, cellulase enzyme normal dose (0.1 g/kg FM); CE-D, cellulase enzyme double dose (0.2 g/kg FM); U-N, urea normal dose (5.5 g/kg FM); U-D, urea double dose (11.0 g/kg FM); FA-N, formic acid normal dose (6.0 g/kg FM); FA-D, formic acid double dose (12.0 g/kg FM); SEM, standard error of the means; DM, dry matter; NH_3-N, ammonia nitrogen; and TN, total nitrogen. $^{a–e}$ Means within columns with difference superscript letters differ at $p < 0.05$.

3.3. Chemical Compositions of Silages

The chemical compositions of silages prepared from NP and SG at the late stage of maturity are presented in Table 4. In NP silage, DM content was higher ($p < 0.05$) for the control compared with adding U-N, FA-N, or FA-D. Adding CE-D, U-D, and FA-D to NP resulted in the highest ($p < 0.05$) OM, CP, and ADL contents, respectively. The EE ($p = 0.52$) and NDF ($p = 0.14$) contents of NP silage were not different among treatments. The ADF contents of NP silage trended to be higher ($p = 0.09$) in the U-N, U-D, FA-N, or FA-D treatment, followed by the control or CE-N and then CE-D. The highest ($p < 0.05$) DM content in SG silage was in the control treatment. The OM contents of SG silage prepared from control, CE-N, and CE-D were greater ($p < 0.05$) than from U-N, U-D, FA-N, and FA-D. Regarding the EE contents of SG silage, adding U-D or FA-N resulted in greater ($p < 0.05$) values than the control treatment. The ADF contents of SG silage were lower ($p < 0.05$) for CE-D and higher for FA-N compared with other treatments. However, the NDF ($p = 0.14$) and ADL ($p = 0.57$) contents of SG silage were not affected by treatments.

Table 4. Chemical composition of silages after 30 d of fermentation.

Item [1]	Additive at Ensiling	DM	OM	CP	EE	NDF	ADF	ADL
		(g/kg)	(g/kg DM)					
NP silage	Control	163 ^{a}	899 ^{bc}	69.3 ^{b}	11.5	761	486 ^{yz}	57.3 ^{ab}
	CE-N	144 ^{ab}	908 ^{b}	71.0 ^{b}	14.0	741	468 ^{yz}	52.5 ^{bc}
	CE-D	143 ^{ab}	927 ^{a}	71.5 ^{b}	16.1	736	445 ^{z}	47.6 ^{c}
	U-N	135 ^{b}	913 ^{ab}	79.3 ^{b}	13.3	763	498 ^{y}	56.9 ^{ab}
	U-D	145 ^{ab}	889 ^{c}	104 ^{a}	15.5	774	509 ^{y}	55.0 ^{abc}
	FA-N	116 ^{c}	886 ^{c}	70.1 ^{b}	15.5	745	493 ^{y}	59.0 ^{ab}
	FA-D	113 ^{c}	895 ^{bc}	69.5 ^{b}	14.1	736	501 ^{y}	60.6 ^{a}
	SEM	6.0	5.6	4.64	1.67	10.9	14.5	2.36
	p	<0.001	<0.001	<0.001	0.525	0.139	0.090	0.026
SG silage	Control	123 ^{a}	894 ^{a}	131 ^{b}	24.4 ^{c}	642	377 ^{cd}	41.7
	CE-N	110 ^{b}	902 ^{a}	131 ^{b}	25.4 ^{bc}	634	370 ^{d}	41.4
	CE-D	111 ^{b}	894 ^{a}	136 ^{b}	26.7 ^{abc}	626	336 ^{e}	41.9
	U-N	113 ^{b}	869 ^{bc}	134 ^{b}	24.6 ^{c}	649	390 ^{bcd}	42.1
	U-D	103 ^{bc}	863 ^{c}	157 ^{a}	27.8 ^{ab}	645	406 ^{ab}	41.0
	FA-N	95.9 ^{c}	878 ^{b}	126 ^{b}	28.3 ^{a}	668	418 ^{a}	43.4
	FA-D	95.6 ^{c}	843 ^{d}	137 ^{b}	26.2 ^{abc}	657	394 ^{bc}	46.3
	SEM	3.12	3.3	3.7	0.86	9.8	7.2	1.99
	p	<0.001	<0.001	0.002	0.038	0.136	<0.001	0.573

[1] NP, Napier Pakchong grass; SG, sweet grass; CE-N, cellulase enzyme normal dose (0.1 g/kg FM); CE-D, cellulase enzyme double dose (0.2 g/kg FM); U-N, urea normal dose (5.5 g/kg FM); U-D, urea double dose (11.0 g/kg FM); FA-N, formic acid normal dose (6.0 g/kg FM); FA-D, formic acid double dose (12.0 g/kg FM); SEM, standard error of the means; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; and ADL, acid detergent lignin. $^{a–e}$ Means within columns with difference superscript letters differ at $p < 0.05$. $^{y–z}$ Mean separations, refer to the differences as tendencies ($p < 0.10$).

3.4. Aerobic Stability and Microbial Counts of Silages

The aerobic stability and microbial population of the tested silages are presented in Table 5. In NP silage, aerobic stability (h) was longer ($p < 0.05$) in the U-N or U-D treatment, followed by CE-N or CE-D, and control, FA-N, or FA-D, respectively. The lactic acid bacteria ($p = 0.08$) and aerobic bacteria ($p = 0.051$) counts of NP silage trended to be different among treatments. The yeast counts of NP silage were greater ($p < 0.05$) when FA-N was added, followed by FA-D and then the other treatments. In SG silage, adding CE-D, FA-N, and FA-D resulted in the shortest ($p < 0.05$) aerobic stability. The lactic acid bacteria ($p = 0.09$) and aerobic bacteria ($p = 0.24$) counts of SG silage were not different among treatments. The yeast counts of SG silage were higher ($p < 0.05$) when FA-N was added compared with other treatments. Molds were detected (10^7 cfu/g FM) in NP and SG silages treated only with

FA-N and FA-D. In all silages, the coliform bacteria counts decreased to below the detectable level (<10^2 cfu/g FM).

Table 5. Aerobic stability and microbial populationof silages after 30 d of fermentation.

Item [1]	Additive at Ensiling	Aerobic Stability (h)	Lactic Acid Bacteria	Coliform Bacteria	Aerobic Bacteria	Yeast	Mold
			(cfu/g FM)				
NP silage	Control	26.0 ^{c}	3.4×10^{5} ^{yz}	ND	7.5×10^{4} ^{z}	9.5×10^{4} ^{b}	ND
	CE-N	63.5 ^{b}	3.4×10^{5} ^{yz}	ND	4.1×10^{5} ^{yz}	5.3×10^{4b}	ND
	CE-D	75.0 ^{b}	3.8×10^{5} ^{y}	ND	3.7×10^{5} ^{yz}	1.2×10^{5} ^{b}	ND
	U-N	96.0 ^{a}	1.3×10^{5} ^{z}	ND	6.3×10^{5} ^{y}	4.6×10^{4} ^{b}	ND
	U-D	96.0 ^{a}	1.1×10^{5} ^{z}	ND	1.2×10^{5} ^{z}	5.2×10^{4} ^{b}	ND
	FA-N	22.0 ^{c}	1.5×10^{5} ^{yz}	ND	9.9×10^{4} ^{z}	1.0×10^{7} ^{a}	2.0×10^{7}
	FA-D	22.0 ^{c}	1.7×10^{5} ^{yz}	ND	1.2×10^{5} ^{z}	4.4×10^{5} ^{ab}	2.1×10^{7}
	SEM	3.73	74.71	-	126.02	222.85	-
	p	<0.001	0.082	-	0.051	0.047	-
SG silage	Control	75.0 ^{ab}	1.2×10^{5} ^{z}	ND	1.1×10^{5}	9.7×10^{4} ^{c}	ND
	CE-N	63.5 ^{b}	4.5×10^{5} ^{yz}	ND	1.1×10^{5}	4.1×10^{4} ^{d}	ND
	CE-D	39.0 ^{c}	6.1×10^{5} ^{yz}	ND	2.9×10^{5}	2.8×10^{4} ^{e}	ND
	U-N	75.0 ^{ab}	1.2×10^{5} ^{z}	ND	1.0×10^{7}	1.2×10^{5} ^{b}	ND
	U-D	96.0 ^{a}	8.1×10^{5} ^{y}	ND	9.8×10^{5}	1.6×10^{5} ^{b}	ND
	FA-N	26.0 ^{c}	4.7×10^{5} ^{yz}	ND	1.5×10^{7}	2.1×10^{5} ^{a}	2.2×10^{7}
	FA-D	39.0 ^{c}	1.3×10^{5} ^{z}	ND	1.4×10^{7}	2.8×10^{4} ^{e}	2.4×10^{7}
	SEM	6.19	178.02	-	501.11	22.37	-
	p	0.001	0.085	-	0.240	<0.001	-

[1] NP, Napier Pakchong grass; SG, sweet grass; CE-N, cellulase enzyme normal dose (0.1 g/kg FM); CE-D, cellulase enzyme double dose (0.2 g/kg FM); U-N, urea normal dose (5.5 g/kg FM); U-D, urea double dose (11.0 g/kg FM); FA-N, formic acid normal dose (6.0 g/kg FM); FA-D, formic acid double dose (12.0 g/kg FM); SEM, standard error of the means; cfu, colony forming unit; FM, fresh matter; and ND, not detected (<10^2 cfu/g FM). $^{a–e}$ Means within columns with difference superscript letters differ at $p < 0.05$. $^{y–z}$ Mean separations, refer to the differences as tendencies ($p < 0.10$).

3.5. In Vitro Digestibility of Silages

The in vitro digestibility, pH and ammonia nitrogen ofgrass silages after 48 h incubation are shown in Table 6. In NP silage, the IVDMD and IVOMD consistently improved ($p < 0.05$) when CE-D or FA-N was added compared with the control and other additives. The pH values of NP silage ranged from 7.11 to 7.39 ($p < 0.05$), and the ammonia nitrogen concentrations were greater ($p < 0.05$) following FA-N or FA-D treatment than other treatments. In SG silage, CE-D resulted in consistently greater ($p < 0.05$) IVDMD and IVOMD compared with other treatments. The pH values of SG silage were not affected ($p = 0.20$) by treatments, and the ammonia nitrogen concentrations were higher ($p < 0.05$) for CE-D, FA-N, and FA-D.

Table 6. In vitro digestibility, pH and ammonia nitrogen concentration after 48 h incubation of silages after 30 d of fermentation.

Item [1]	Additive at Ensiling	IVDMD (g/kg)	IVOMD (g/kg)	pH	NH_3-N (mg/L)
NP silage	Control	444 ^{c}	457 ^{b}	7.11 ^{b}	130 ^{b}
	CE-N	457 ^{bc}	464 ^{b}	7.11 ^{b}	143 ^{b}
	CE-D	531 ^{a}	532 ^{a}	7.26 ^{ab}	145 ^{b}
	U-N	450 ^{c}	452 ^{b}	7.39 ^{a}	137 ^{b}
	U-D	461 ^{bc}	454 ^{b}	7.25 ^{ab}	142 ^{b}
	FA-N	523 ^{a}	508 ^{a}	7.23 ^{ab}	182 ^{a}
	FA-D	486 ^{b}	499 ^{a}	7.13 ^{b}	186 ^{a}
	SEM	10.2	11.6	0.059	10.1
	p	<0.001	<0.001	0.041	0.007

Table 6. *Cont.*

Item [1]	Additive at Ensiling	IVDMD (g/kg)	IVOMD (g/kg)	pH	NH_3-N (mg/L)
SG silage	Control	454 [c]	465 [b]	6.85	170 [d]
	CE-N	473 [bc]	491 [b]	6.88	193 [bc]
	CE-D	556 [a]	567 [a]	7.03	190 [bc]
	U-N	520 [ab]	516 [b]	6.88	188 [c]
	U-D	515 [ab]	509 [b]	6.87	207 [ab]
	FA-N	495 [bc]	489 [b]	6.90	222 [a]
	FA-D	506 [abc]	498 [b]	6.90	219 [a]
	SEM	15.9	15.6	0.045	5.8
	p	0.012	0.013	0.204	<0.001

[1] NP, Napier Pakchong grass; SG, sweet grass; CE-N, cellulase enzyme normal dose (0.1 g/kg FM); CE-D, cellulase enzyme double dose (0.2 g/kg FM); U-N, urea normal dose (5.5 g/kg FM); U-D, urea double dose (11.0 g/kg FM); FA-N, formic acid normal dose (6.0 g/kg FM); FA-D, formic acid double dose (12.0 g/kg FM); SEM, standard error of the means; IVDMD, in vitro dry matter digestibility; IVOMD, in vitro organic matter digestibility; and NH_3-N, ammonia nitrogen. [a–d] Means within columns with difference superscript letters differ at $p < 0.05$.

3.6. Gas Kinetics of Silages

The values for the estimated parameters obtained from the kinetics of gas production models for the silages studied are given in Table 7. In NP silage, the gas production from soluble fractions (a) ranged from −2.95 to 1.63 and was greater ($p < 0.05$) for control than additive treatments. The gas production from insoluble fractions (b) and the potential extent of gas production (a + b) of NP silage were greater ($p < 0.05$) when CE-D or FA-N were added, followed by CE-N, U-D, or FA-D, then U-N and control, respectively. The NP silage prepared with CE-D had a greater ($p < 0.05$) gas production rate (c) compared with the other treatments. In addition, the total gas production obtained from CE-D, U-D, FA-N, or FA-D treatment was greater ($p < 0.05$) than that of the control. In SG silage, the gas production from soluble fractions (a) ($p = 0.09$) and total gas production ($p = 0.07$) trended to differ among treatments, while the gas production rate (c) was not different ($p = 0.76$). However, adding CE-D, U-N, or U-D to SG resulted in higher ($p < 0.05$) gas production from insoluble fractions (b) and potential extent of gas production (a + b) compared with control.

Table 7. Gas kinetics and total gas production after 96 h of incubation of silages after 30 d of fermentation.

Item [1]	Additive at Ensiling	Gas Kinetics (mL)				TGP
		a	b	c	a + b	(mL)
NP silage	Control	1.63 [a]	101 [c]	0.028 [b]	103 [c]	95.4 [c]
	CE-N	−2.75 [b]	116 [ab]	0.028 [b]	118 [ab]	105 [bc]
	CE-D	−2.08 [b]	125 [a]	0.040 [a]	127 [a]	120 [a]
	U-N	−1.47 [b]	113 [b]	0.031 [b]	114 [b]	105 [bc]
	U-D	−1.54 [b]	122 [ab]	0.027 [b]	124 [ab]	111 [ab]
	FA-N	−2.95 [b]	125 [a]	0.029 [b]	128 [a]	114 [ab]
	FA-D	−2.56 [b]	119 [ab]	0.031 [b]	122 [ab]	110 [ab]
	SEM	0.618	3.2	0.0023	3.4	4.21
	p	0.002	0.001	0.011	0.002	0.026

Table 7. *Cont.*

Item [1]	Additive at Ensiling	Gas Kinetics (mL)				TGP
		a	b	c	a + b	(mL)
SG silage	Control	−0.93 ^{yz}	105 ^{d}	0.029	107 ^{c}	97.4 ^{z}
	CE-N	−1.47 ^{yz}	110 ^{cd}	0.034	112 ^{bc}	104 ^{z}
	CE-D	−1.54 ^{yz}	130 ^{a}	0.035	132 ^{a}	124 ^{y}
	U-N	−3.55 ^{z}	124 ^{ab}	0.031	127 ^{a}	114 ^{yz}
	U-D	−3.85 ^{z}	121 ^{abc}	0.032	125 ^{ab}	112 ^{yz}
	FA-N	−0.27 ^{y}	113 ^{bcd}	0.030	113 ^{bc}	106 ^{z}
	FA-D	−3.26 ^{yz}	117 ^{abcd}	0.031	120 ^{abc}	107 ^{z}
	SEM	0.930	4.1	0.0028	4.3	5.30
	p	0.094	0.011	0.762	0.009	0.068

[1] NP, Napier Pakchong grass; SG, sweet grass; CE-N, cellulase enzyme normal dose (0.1 g/kg FM); CE-D, cellulase enzyme double dose (0.2 g/kg FM); U-N, urea normal dose (5.5 g/kg FM); U-D, urea double dose (11.0 g/kg FM); FA-N, formic acid normal dose (6.0 g/kg FM); FA-D, formic acid double dose (12.0 g/kg FM); SEM, standard error of the means; a, the gas production from soluble fractions; b, the gas production from insoluble fractions; c, the gas production rate, a + b, the potential extent of gas production; and TGP, total gas production. $^{a–d}$ Means within columns with difference superscript letters differ at $p < 0.05$. $^{y–z}$ Mean separations, refer to the differences as tendencies ($p < 0.10$).

4. Discussion

4.1. Microorganism Counts and Chemical Composition of Napier Grasses at the Late MaturityStage

Lactic acid bacteria are a potential strain for fermenting good quality silage. In this study (Table 1), NP and SG at the late stage of maturity contained low lactic acid bacterial counts ranging from 10^3 to 10^4 cfu/g FM. This result agrees with previous reports on the 60-d cutting age NP [3,4].

Our data (Table 2) implied that SG's CP content is greater than that of NP (122 vs. 71 g/kg DM). The ADL content of NP obtained after 120 d of regrowth in this study was greater than that after 60 to 70 d of regrowth (55.6 vs. 31.5 g/kg DM) reported by Khota et al. [3,4]. For SG, Mapato and Wanapat [5] reported a greater CP level (122 vs. 151 g/kg DM) and a lower NDF level (699 vs. 617 g/kg DM) than in the present findings. These differences are associated with the fact that SG was cut at a later stage in this study. Mapato and Wanapat [5] stated that the high nutritive characteristics of SG occur when it is harvested at 42 d of regrowth, when it has a high leaf-to-stem ratio.

4.2. Effects on Fermentation Characteristics of Silages

The results revealed that the pH of control silages prepared from NP and SG at the late maturity stage decreased below 4.2 (Table 3). This result agreed with previous studies [3–5]. Thus, the NP and SG used in this study can be well preserved as silage with good quality which probably compares to those at the optimum stage of maturity [3–5]. This pH level was recommended to inhibit the growth of harmful bacteria, especially clostridia [23]. In addition to that of control, the pH of cellulase treatments (CE-N and CE-D) was low, with a concurrently improved lactic acid production in SG silage ($p < 0.05$). This finding agrees with Li et al. [30] and Khota et al. [3,4] that the enzyme increased lactic acid levels in forage silages. According to the report of Wu et al. [31], adding 3-phenyllactic acid decreased the pH of alfalfa silage. Thus, the present finding could be attributed to the cellulase enzyme degrading plant fiber and increasing the fermentable sugars, an essential substrate for lactic acid producing bacteria growth [23].

In contrast, our results show that the quality of both NP and SG silages, as indicated by pH, lactic acid, butyric acid, and ammonia nitrogen levels ($p < 0.05$), was very poor when urea or formic acid was added. However, silage quality is unreliable for evaluating the effects of urea addition because the urea approach promotes an alkaline fermentation. The urea results support the work of Heinrichs and Conrad [32], who found that anhydrous ammonia increased the pH of maize silage, consistently indicating a different fermentation type in the alkaline-treated silages. For formic

acid, our results indicated a spoilage silage effect because of high pH levels (ranging from 5.01 to 7.56). Acid production shifted to less lactic acid and more acetic and butyric acids with greater ammonia nitrogen levels. This effect was surprising because formic acid has been used worldwide for acidification. Adding formic acid rapidly reduces the pH of fresh materials, thus inhibiting plants' protease enzymes [33]. Waldo et al. [11] confirmed this earlier using alfalfa and orchardgrass. The results showed that the pH was progressively less than the control (3.98 vs. 5.07) as the formic acid additive level was only 5 g/kg of FM [11]. Adding formic acid consistently benefited silages via high moisture crops, including in lucerne and cocksfoot silages [34] and in bur clover and annual ryegrass [13]. The current study indicated that both normal (6.0 g/kg FM) and double (12.0 g/kg FM) dose rates of formic acid might perform insufficiently for NP and SG at the late maturity stage. Less desirable fermentation might also relate to the direct and indirect effects of grasses' buffering capacities. The buffering capacity might be able to inhibit acidification.

Our results (Table 4) suggested that the DM content of SG silage prepared with additives was dramatically reduced from that of the control silage ($p < 0.05$), and the relative change was up to 27% lower than in the control after adding formic acid. Regarding formic acid's effects, poor fermentation occurs due to the activities of plant enzymes, enterobacteria, and clostridia causing silage spoilage and nutrient losses [23], which would decrease the silage DM content in a closed container that prevents moisture loss.

4.3. Effects on Aerobic Stability of Silages

Our results (Table 5) imply that the effects of additives on the aerobic stability of silages could be inconsistent between Napier grasses. The results indicated thatthe CE-D and formic acid treatments reduced the aerobic stability of SG silage ($p < 0.05$), in which most microbial counts were not affected. Aerobic stability indicates a risk of aerobic deterioration during feed out, which spoils silages and reduces palatability. The specific factors for minimizing the silage's internal temperature increase after the ensiled mass's contact with air are still unknown [24]. Muck [35] suggested that the aerobic stability of typical low-pH silages ranged from 25 to 503 h. Cai et al. [9] indicated that the aerobic deterioration of silages is associated with high concentrations of residual WSC and lactic acid and with a lack of volatile fatty acids. However, air-dried rice straw silages with high acetic acid concentration did not spoil over 792 h [36]. Recently, Santos et al. [37] and Zhang et al. [38] noted that the lack of spoilage reflects the intense effects of reactions caused by filamentous fungi, yeast, and aerobic bacteria. However, the present finding for the overall effects of cellulase on the aerobic stability and microbial counts of NP silage agrees with the work of Dean et al. [39], who showed that the aerobic stability of Bermuda grass silages increased when adding cellulase enzyme, despite unchanged microbial counts. The results found that the addition of urea improved ($p < 0.05$) the aerobic stability of NP silage. These results support the work of Santos et al. [37], who suggested that the addition of 5 g/kg urea is sufficient to improve the aerobic stability of sorghum silage. This effect could relate to alkalinizing because urea causes an unfavorable environment for the development of enterobacteria and clostridia, which were in sporulated form [37]. In contrast, formic acid caused spoilage fermentation in silages in this study, which could increase harmful microbial numbers and shorten aerobic stability.

4.4. Effects on In Vitro Digestibility and Gas Kinetics of Silages

It is well established that the potential digestibility of forages for ruminants could be estimated with reasonable accuracy by using rumen fluid in vitro [40]. Therefore, in vitro experiments have become significant in determining whether to apply potential treatments to animals. The results showed that the IVDMD of NP and SG silages improved with the double dose of cellulase treatment ($p < 0.05$; Table 6). This effect was expected because cellulase should cause the plant cell wall fractions to degrade [9–12]. The superior effect of high levels of cellulase enzyme (CE-D) in our study is partly because of the increased activity of the cellulase enzyme. This result implies that the CE-D is important for increasing the digestibility of silages prepared from the NP and SG at the

late maturity stage. Fermentation in the rumen produces microbial protein, gases, and volatile fatty acids [26]. However, the slow-release of ammonia nitrogen is critical for efficient microbial protein synthesis. In this study, greater levels of ammonia nitrogen in formic acid-treated silages ($p < 0.05$) might be associated with protein deterioration reactions from spoilage. Poor quality silage generally supplies a rapid-release of nitrogen into the rumen, reducing nitrogen-use efficiency for microbial mass synthesis [7].

The in vitro gas production technique has a remarkable effect, and data on the fermentation kinetics of numerous feeds are available. Iqbal et al. [41] demonstrated that the microbial community in an in vitro rumen fermentation can remain similar among different high-fiber forages. The results (Table 7) suggest that the silage additives performed generally better than the control silages. These findings could result from positive effects among digestibility, gas kinetics, and accumulated gas production. The results confirmed the work of Sommart et al. [42] because increasing IVDMD positively related with the volume of gas released during fermentation. Similarly, Li et al. [30] reported that cellulase enzyme could improve the ruminal degradation rate of crop silage because the enzyme enhances the degradation of fiber by releasing a soluble carbohydrate. However, the results also showed a reduction in gas production from soluble fractions (a) when NP silage was prepared with the additives ($p < 0.05$). Thus, Paya et al. [43] suggested that an abundance of lactic acid that is metabolized in silage probably slows the release of gas, so gas production from soluble fractions (a) can be low.

5. Conclusions

The present results demonstrate that the addition of cellulase enzyme (0.2 g/kg FM) or urea (5.5 g/kg FM) favored silage preservation and increased the IVDMD of Napier grasses harvested at the late maturity stage. Before expanding to the farm, the cellulase and urea should be tested in vivo to evaluate their additive effects on the intake, apparent digestibility, and performance of animal-fed Napier grass silages.

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