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Improvement on the Nutritive Quality of Napier Grass Silage through Inoculation of *Lactobacillus plantarum* and Formic Acid

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ABSTRACT

The potential availability of forage feed is high, but in reality this potential has not been able to meet the requirement of feed both in sustainable quantity and quality. Silage made with the use of liquid fermentation additive (FA) can be a solution for those problems. The use of different levels of FA and addition of *Lactobacillus plantarum* bacteria as well as formic acid were expected to improve the nutritive quality of napier grass silage. The first experiment was designed to measure the fermentative quality of napier grass silage. The treatments used were the levels of FA, *L. plantarum*, and formic acid supplementations. The experiment used a completely randomized design with a 3x2x2 factorial arrangement with 3 replications. The first factor was the level of liquid FA (5%, 7.5%, and 10%), and the second factor was the inoculation of *L. plantarum* (without and with inoculation of the *L. plantarum*), and the third factor was the addition of formic acid (without and with the addition of 0.15% formic acid). The second experiment was aimed to evaluate chemical and microbiological characteristics, and *in vitro* digestibility of selected napier grass silage. The results showed that napier grass silage from all treatments showed good qualities. There were interactions between FA, *L. plantarum*, and formic acid on DM content (P<0.05) and ammonia production (P<0.01). The use of FA showed an interaction (P<0.01) with the addition of *L. plantarum* and formic acid in Fleigh point. Ammonia production in rumen (P<0.01), total VFA (P<0.05), and *in vitro* digestibility (P<0.01) were significantly affected by the treatments. The optimal level of liquid FA was 7.5%. Based on the nutritive quality of silage, *L. plantarum* addition was as effective as control treatment to improve nutritive quality of napier grass silage through the increased of fermentation characteristics i.e., low pH, high DM product, high fermentation product (VFA), and digestible on rumen. Formic acid reduced ammonia production during ensiling and fermentation in rumen, but it was less effective in inhibiting the fermentation process when it was combined with *L. plantarum*.

Key words: napier grass silage, fermentation additive, Lactobacillus plantarum, formic acid

ABSTRAK

Potensi ketersediaan hijauan pakan yang tinggi pada kenyataannya belum dapat memenuhi penyediaan pakan yang cukup secara berkelanjutan dengan mutu yang baik. Silase dibuat dengan cairan aditif fermentasi (AF) sebagai solusi permasalahan di atas. Melalui evaluasi level penggunaan AF dan penambahan bakteri *Lactobacillus plantarum* serta asam formiat diharapkan memberikan pengaruh sinergi dalam meningkatkan kualitas nutrisi silase rumput gajah. Percobaan pertama menguji kualitas fisik dan fermentatif hasil akhir silase rumput gajah. Kualitas fermentatif menggunakan rancangan acak faktorial 3 x 2 x 2 dengan 3 ulangan. Faktor pertama adalah level cairan aditif fermentasi (5%, 7,5%, dan 10%), faktor kedua adalah inokulasi *L. plantarum* (tanpa dan dengan inokulasi *L. plantarum*), dan faktor ketiga adalah penambahan asam formiat (tanpa dan dengan penambahan 0,15%). Percobaan kedua menguji kualitas kimia, mikrobiologi, dan kecernaan *in vitro* silase rumput gajah terpilih. Semua silase yang dihasilkan menunjukkan karakteristik fisik yang berkualitas baik. Terdapat interaksi dari ketiga faktor pada kandungan bahan kering (P<0,05), NH_3 (P<0,01) dan nilai fleigh (P<0,01). Produksi amonia rumen (P<0,01), total VFA (P<0,05), dan kecernaan *in vitro* (P<0,01) signifikan dipengaruhi oleh perlakuan. Penggunaan AF yang optimal adalah pada level 7,5%. Berdasarkan kualitas nutrisi silase, perlakuan penambahan *L. plantarum* sama efektif dengan perlakuan kontrol

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dalam meningkatkan kualitas nutrisi silase rumput gajah melalui peningkatan karakteristik fermentasi, seperti penurunan pH, peningkatan bahan kering produk akhir, peningkatan produk fermentasi (VFA), dan mudah dicerna di dalam rumen. Asam formiat mampu menurunkan produksi amonia selama ensilase dan fermentasi di dalam rumen, namun kurang efektif ketika dikombinasikan dengan *L. plantarum***.**

Kata kunci: silase rumput gajah, aditif fermentasi, Lactobacillus plantarum, asam formiat

INTRODUCTION

The latent potential availability of feed in Indonesia was estimated very high, both derived from forage and agricultural waste. In fact, this potential has not been able to meet the national requirement of sustainable quality feed. Some of the constrain factors are: (1) poor quality of the feed nutrients, (2) seasonal production, with uneven distribution of rainfall throughout the year, and (3) the distance between the production site of the feed and the livestock production. To solve this problem, the development of an appropriate technology that includes preservation, conservation, distribution, and transportation technologies becomes of importance. The preservation method that has been widely used is silage. However, low quality and unpalatable silage, caused by poor fermentation, is often found in practice.

Some restricting factors in silage production in Indonesia are high humidity and the low nutrient content. It is difficult to ensure a good quality silage from tropical pasture crops because the materials have low water soluble carbohydrate (WSC) content, high buffering capacity, and low lactic acid bacteria (LAB) numbers (McDonald *et al*., 1991). Good quality silage is produced by converting WSC by LAB to organic acid efficiently resulting in a high nutrient content (McDonald *et al*., 1991). Low-quality tropical forages normally have crude protein (CP) contents that are lower than 70 g/kg of dry matter (DM), which is considered to be a critical factor for supporting adequate microbial growth on fibrous carbohydrates of basal forage (Lazzarini *et al*., 2009). Therefore, it is necessary to add an additive that is capable of supporting the ensilage process. Administration of liquid fermentation additive (FA) is expected to stimulate the growth of LAB that finally accelerates the decline of pH and the ensilage fermentation process. FA liquid contains a mixture of molasses, organic acids, and salts such as calcium propionate and lactic acid. This content allows the Napier grass silage to be preserved for a long period. However, the use of improper level of FA liquid causes more extensive fermentation process. This resulted in a decreased nutritional quality of the silage and would impact on the palatability of the silage, especially for small ruminants (Centras, 2013).

The critical point in fermentation process is the rate of acid production. The faster the acid production process the lower the nutrient losses of the forage because this acid production phase caused DM losses of forage. Consequently, supplementation of additives is required to improve the fermentation process efficiently and nutritive quality of Napier grass silage. A variety of additives, both biological and chemical additives, have been developed primarily to be used to improve the fermentation quality. Inoculants containing selected strains of LAB have been developed and used to stimulate fermentation (McDonald *et al*., 1991). The addition of LAB inoculant resulted in a better fermentation quality of tropical and temperate forage silage compared to commercial inoculant (Bureenok *et al*., 2006; Santoso *et al*., 2011). Homofermentative LAB strains such as *Lactobacillus plantarum*, *Enterococcus faecium*, and *Pediococcus* spp. produced the largest reduction in pH and higher lactate:acetate ratios. The function of those inoculants is to ensure a rapid and efficient fermentation of WSCs into lactic acid by homolactic fermentation and improve silage preservation with minimal losses. LAB improves the nutritive quality by stimulating lactic acid production, decreasing the pH rapidly, and extending the preservation time. *L. plantarum* is known as homolactic LAB that is able to result in rapid decrease of the pH of the silage, rapid increase in LAB, and inhibition of spoilage microorganisms (Adesoji *et al*., 2010). *L. plantarum* improves the silage fermentation by inducing more extensive homolactic fermentation producing silage with the highest level of lactic acid (Filya & Sucu, 2007).

Chemical additives are also an alternative to improve fermentation quality of the silage by acting as a fermentation inhibitor (Mc Donald *et al*., 1991). The intensive fermentation process results in a high sugar residue and organic matter in the silage. Additional additives are primarily targeted to limit the fermentation process, reduce the nutrient losses, and therefore retain the nutritive value of the ensiled herbage as much as possible. Direct addition of organic acids such as formic acid has been widely used in the silage production. The appropriate dose of acid supplementation will rapidly lower the pH of silage and limit the loss of protein and carbohydrates during fermentation (Luckstadt, 2009). Formic acid additive was effective in improving silage fermentation and reducing *silo* losses with difficult-to-ensile/DIFF (DM 163 g/kg) herbages (Lorenzo & O'Kiley, 2008) that was better than LAB. Formic acid as a fermentation inhibitor can restrict the fermentation on the ensilage process by its ability to decrease the pH and antibacterial effect that results in a limited fermentation and reduction in organic acid (Kennedy, 1990). The restriction of silage fermentation by formic acid is positively related to the synthesis of microbial protein in the rumen and induces a rumen VFA pattern with a relatively high proportion of lipogens VFAs as a milk fat precursor (Jaakkola *et al*., 2006).

The objective of this study was to evaluate the optimum level of liquid fermentation additive and the effects of inoculant *L. plantarum* and formic acid on the

fermentation characteristics, nutritive value, and *in vitro* digestibility of Napier grass silage.

MATERIALS AND METHODS

Inoculum Preparation (Cappucino & Sherman, 2001)

The isolate *L. plantarum* 1A-2 was obtained from the Biotechnology Laboratory of Indonesian Institute of Sciences (LIPI), Cibinong, Bogor. The isolate was inoculated into 10 mL sterilized deMan Rogosa and Sharpe (MRS) agar medium in petri dish (Cappuccino & Sherman, 2001). The isolate was anaerobically incubated for 18 h at temperature of 28-30°C. After the culture grew and showed no contamination, the culture was stored in the refrigerator and used as stock inoculum. Inoculum was made by inoculating 3 oses of stock *L. plantarum* into 10 mL of MRS broth media as a preculture and the 1% (v/v) preculture was transferred into another liquid MRS broth medium. LAB colony was counted using total plate count (TPC) method using MRS broth medium. LAB suspension contained 1.9 x 10^9 CFU/mL.

Silage Processing

The napier grass (*P. purpureum*) used in the experiment was harvested at 30 days of age. Furthermore, it wilted for two nights in a shaded place to lower the dry matter content. The silage production was prepared using industrial system of silage fermentation with some modifications (Adesoji *et al*., 2010). The grass was chopped to 3-5 cm long. Samples of the wilted grass were taken for dry matter analysis. The chopped grass was then divided into equal portions of 15 kg. The liquid of fermentation additive (FA) was prepared by mixing molasses (40%), organic acids, and salts such as calcium propionate and lactic acid. As a treatment, FA and the additives (0.1 mL/kg of *L. plantarum* and 0.15% formic acid) were added by spraying them on the top of grass until blended. After beeing mixed, the mixture was packed in two layer of Liner Low Density Polyethylene (LLDPE) plastic bag with 0.7 thickness and size of 50 x 85 cm^2. The grass was compressed and properly sealed to ensure the anaerobic condition. The silage was opened after 21 days of ensilage process and the samples were taken for microbiological and chemical analyses.

Experiment 1: Fermentative Quality of Napier Grass Silage

The first experiment was designed to evaluate the combination effects of additive fermentation liquid, inoculant *L. plantarum*, and formic acid on fermentative quality of napier grass silage. Fermentative qualities observed were physical quality and fermentative characteristics. After being stored for 3 wk, the silage was observed for its colour, smell, texture, and the presence of fungi. Physical characteristics as a result of fermentation products were generally able to describe the quality of the silage (Ferreira & Merten, 2005). Organoleptic observations were made by observing the top, middle, and bottom part of each bag. Observations were assisted with visual media (photos) and scored to facilitate the analysis. Colour assessment was assisted by using visual observation with colour charts, while the smell of the silage was relatively assessed as pleasant or fruity alcoholic aroma. About 10 g sample of the silage was mixed with 100 mL sterile distilled water and shaked using shaker model VRN-360 at 600 rpm for 10 min. The filtrate (napier grass silage juice) was used for determination of the pH with pH meter (HACH Spain) and N-NH3 was analyzed according to Conway method (General Laboratory Procedure, 1966). The dry matter content of silage was determined by oven dried at 60°C. The dried silage was then milled to pass through 0.5-1.5 mm aperture sieve for proximate, fiber fraction, and *in vitro* digestibility analyses. Fleigh point of the silage was used as a parameter of fermentation quality (Kilic, 1986).

Experiment 2: Chemical and Microbiological Qualities, and *in Vitro* Digestibility of Napier Grass Silage

Selected treatment from the first experiment was further analyzed for parameters of chemical and microbiological qualities and *in vitro* digestibility. Oven dried sample from Experiment 1 was also used for analyses of chemical quality. Parameters of chemical qualities analyzed were ash, crude fiber, and ether extract content using standard procedures of proximate according to AOAC (2005). Crude protein content of the Napier grass silage was also determined by the Kjeldahl method. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined according to Van Soest method (Van Soest *et al.* 1991).

Microbiological quality was determined by LAB population in the napier grass silage. Fresh silage was diluted 10x in sterile distilled water and plated on the MRS agar media to count the LAB population (duplicate). The plate was incubated at 37°C for 3 d according to TPC method (Cappuccino & Sherman, 2001). Lactic acid content of the silage was determined using spectrophotometer (Baker & Summerson, 1941).

The dry sample was also analyzed for *in vitro* digestibility by using Tilley & Terry (1963) methods. Ruminal fermentability including total VFA (steam distillation), VFA, and molar portion was measured by using gas chromatography Shimadzu GC-8A, coloum no 80/100 Chromosorb W (General Laboratory Procedure, 1966), and N-NH_3 (micro diffusion Conway). Rumen fluids were obtained from fistulated Ongole Grade (PO) cattle in Indonesian Institute of Science, Cibinong, Bogor. The cattle was fed fresh napier grass as a diet.

Experimental Design and Statistical Analysis

Experiment 1: Fermentative quality of napier grass silage. Fermentative qualities observed were physical quality and fermentative characteristics. The physical quality (colour, smell, texture and the presence of the fungi) was determined by organoleptic observations. Fermentative characteristic study used a completely ran-

domized design with a 3 x 2 x 2 factorial arrangement with 3 replication bags. The first factor was the dose of fermentation additive liquid with 3 levels i.e., 5%, 7.5%, and 10% of fresh forage. The second factor was inoculation of *L. plantarum* with 2 levels i.e., 0 and 10^8 cfu/kg fresh forage. The third factor was application of formic acid with 2 levels i.e., 0 and 0.15% of fresh forage. Variation among treatments and their interactions were analyzed using ANOVA followed by Duncan's test (Steel & Torrie, 1993).

Experiment 2: Chemical and microbiological qualities, and *in vitro* digestibility of napier grass silage. Chemical and microbiological qualities were analyzed by using completely randomized design of 4 treatments with 3 replications. The treatment was consisted of P0= napier grass silage as a control; P1= P0 + formic acid (0.15%); P2= P0 + *L. plantarum* (1.9 x 10^8 CFU/kg), and P3= P0 + formic acid (0.15%) + *L. plantarum* (1.9 x 10^8 CFU/kg). Variations among treatments were analyzed by using ANOVA followed by Duncan's test (Steel & Torrie, 1993). The *in vitro* test used randomized blocked design, consisted of 4 treatments and 3 rumen fluids as block, and 3 replications bags as subreplication. Variations among treatments were analyzed using ANOVA followed by Duncan's test (Steel & Torrie, 1993).

RESULTS

Experiment 1: Fermentative Quality of Napier Grass Silage

The napier grass silage produced in all treatments showed light green to brown colour. The smell was pleasantly acidic and in some treatments, especially in 10% AF level, it was fruity alcoholic. The physical appearance and texture of the fermented forage was dry, but the leaves contained more moisture. The mold is absence in all napier grass silage treatments.

Some chemical fermentation characteristics measured were pH, N-NH_3, and Fleigh point of napier grass silage (Table 1). There were interactions between those three factors (P<0.01). Addition of *L. plantarum* bacteria produced the lowest pH value of all treatments, including formic acid (P<0.01), especially at the level of FA 7.5% and 10%. There was an interaction between *L. plantarum* bacteria and formic acid (P<0.01) on the pH value.

The inefficient fermentation process resulted in a higher nutrient breakdown in the forage, which was seen in the low content of DM in the silage (Table 1). There were interactions between FA, *L. plantarum*, and formic acid on DM content (P<0.05). Both types of additives used showed the same effectivity in decreasing ammonia production in napier grass silage when combined with FA (P<0.01). The use of FA showed an interaction with the addition of *L. plantarum* and formic acid (P<0.01) in Fleigh point. The higher the level of FA used the higher the fleigh point of napier grass silage.

Experiment 2: Chemical and Microbiological Qualities, and *in Vitro* Digestibility of Napier Grass Silage

Nutrients analysis of napier grass silage was shown in Table 2. In general, there was no difference between treatments and control in terms of nutrient contents of napier grass silage.

Microbiological characteristics were measured by the population of LAB and lactic acid produced (Figure 1). LAB population during the 21 days of ensiling was similar in all treatments. The highest LAB population was found in control treatment. Although the addition of *L. plantarum* did not show any significant differences

Table 1. Fermentative characteristics of Napier grass silage

Variables	Inoculation *L. plantarum*	Formic acid	Level of fermentation additive liquid			Significant interaction			
			5%	7.50%	10%	FA x LP	FA x Formic	LP x formic	FA x LP x formic
pH	-	0	3.58± 0.22^{abc}	3.68±0.23^{ab}	3.26± 0.04^{bcd}	ns	*	ns	**
		0.15%	4.00± 0.25^{a}	3.32±0.09^{bcd}	3.73± 0.35^{ab}				
	+	0	3.40± 0.12^{bcd}	3.08±0.10^{de}	3.02± 0.11^{e}				
		0.15%	3.71± 0.44^{ab}	3.35±0.05^{bcd}	3.20± 0.07^{bcd}				
Dry matter (%)	-	0	24.80± 1.03^{abc}	22.75±0.36^{c}	22.88± 1.43^{c}	ns	ns	ns	*
		0.15%	23.08± 1.67^{c}	25.10±0.29^{abc}	23.78± 0.36^{bc}				
	+	0	24.80± 0.15^{abc}	26.66±2.71^{a}	26.93± 1.00^{a}				
		0.15%	26.24± 0.94^{ab}	25.58±1.33^{ab}	25.83± 1.74^{ab}				
NH_3 (g $100g^{-1}$)	-	0	0.79± 0.14^{ab}	1.02±0.17^{a}	0.49± 0.12^{bcd}	*	ns	**	**
		0.15%	0.59± 0.07^{bcd}	0.35±0.15^{d}	0.46± 0.06^{cd}				
	+	0	0.66± 0.02^{bc}	0.46±0.08^{cd}	0.71± 0.14^{bc}				
		0.15%	0.55± 0.13^{bcd}	0.71±0.10^{bc}	0.51± 0.12^{bcd}				
Fleigh pPoint	-	0	111.26± 6.58^{abc}	103.43±9.64^{ab}	120.23± 1.73^{bcd}	ns	**	ns	**
		0.15%	91.01± 6.83^{a}	122.53±2.90^{bcd}	103.49±14.74^{ab}				
	+	0	118.47± 5.05^{bcd}	134.99±3.90^{d}	138.19± 3.90^{a}				
		0.15%	108.95±16.99^{abc}	122.29±4.46^{bcd}	128.80± 6.18^{cd}				

Note: Means in the same variables with different superscripts differ significantly (* P<0.05, ** P<0.01). FA= fermentation additive liquid, LP= inoculant *L. plantarum*.

in LAB population, it did increase the lactic acid content in the silage.

Formic acid did not affect the population of LAB in napier grass silage. The ability of a feed product to support the process of fermentation in the rumen was shown through its fermentation products (Table 3). Although the addition of either formic acid or *L. plantarum* bacteria were able to reduce proteolysis in the silage, both showed different effects on rumen fluid. The average range of NH_3 concentration was 4-6.3 mM. The range of ammonia concentration had reached the ideal range according to Boucher *et al.* (2007) to support the growth of rumen bacteria i.e., 5-13 mg/dL or 2.94-7.65 mM. Napier grass silage supplemented with formic acid showed the lowest ammonia concentration in rumen compared to that supplemented with *L. plantarum*. On the other hand, the addition of *L. plantarum* bacteria increased ammonia concentration in the rumen compared to control and the silage supplemented with formic acid.

Total VFA was significantly affected by the treatment (P<0.05). The range of total mean of VFA was 115-134 mM. Control silage contained the higest total VFA in the rumen, followed by that supplemented with *L. plantarum* and formic acid either single or in combination. The addition of formic acid or *L. plantarum* could not increase the concentration of VFA in the rumen as compared to control. Isovaleric acid concentration was affected significantly by the treatments. The highest concentration was found in control silage and the one supplemented with *L. plantarum* inoculant.

In vitro dry matter and organic matter digestibility coefficients were in line and showed significant differences among treatments (P<0.01). The highest digestibility was found in the control silage and the one supplemented with *L. plantarum* bacteria. The addition of formic acid and its combinations with *L. plantarum* resulted in lower digestibility of napier grass silage.

Table 2. Nutrient composition of napier grass silage after ensiling 21 d (100% DM)

Nutrient composition	Napier grass silage			
	P0	P1	P2	P3
Ash	12.08	10.71	11.52	10.96
Crude protein (CP)	9.36	9.14	10.14	9.70
Crude fiber (CF)	32.60	35.25	30.80	36.00
Ether extract (EE)	2.31	2.42	3.41	4.40
Nitrogen free extract (NFE)	43.66	42.48	44.13	38.94
Total digestible nutrient (TDN)*	50.99	49.01	53.67	50.55
Cellulose	56.81	47.52	45.37	47.01
Hemicellulose	10.22	17.26	19.26	14.14
Neutral detergent fiber	69.18	72.59	76.15	71.92
Acid detergent fiber	62.03	59.84	62.19	59.80
Lignin	12.86	7.90	12.44	12.07

Note: P0= Napier grass silage control; P1= P0 + formic acid; P2= P0 + *L. plantarum*; P3 = P0 + formic acid + *L. plantarum*; *) TDN estimated by Sutardi formula (2001): TDN = 2.79 + 1.17 CP + 1.74 EE – 0.295 CF + 0.810 NFE.

DISCUSSION

Experiment 1: Fermentative Quality of Napier Grass Silage

Molasses content in the FA caused the color of forage to change to light brownish. This color change was also affected by the wilting process. The highest level of FA (10%) supplementation did not reveal any effluent dripping or wet texture of napier grass silage. The presence of mold in the ensilage product was a problem that often occured. The presence of the mold was associated with a high humidity, especially in tropical countries such as Indonesia. The humid condition is very suitable for the growth of molds. Although the well-preserved silage could be considered as an anaerobic mass of plant material, many species of fungi were isolated from different kinds of silages, depending on crop or silage additives used (Li & Nishino, 2011). The final results showed the absence of mold in the napier grass silage indicating that the quality of silage produced was good and safe to be fed to the animal, with or without the addition of LP and formic acid.

FA contains acids, organic salts, and molasses as an energy source for bacteria. The sugar content in molasses during the fermentation process will be used by bacteria to produce organic acids such as lactic acid and a small portion of acetic and butyric acid. Molasses supplementation to the silage would increase the available sugar that would decrease the pH faster and inhibit deamination and decarboxylation of amino acid (Abarghoei *et al.*, 2011). *L. plantarum* bacteria as homofermentative bacteria produced lactic acid as a primary product. The lactic acid produced by *L. plantarum* bacteria led to a faster decrease in pH, through the restriction of plant respiration and enzyme activity, thereby inhibiting the growth of other bacteria (Adesoji *et al.*, 2012). In limiting fermentation, formic acid lowered the pH immediately after its supplementation and worked by reducing the activity of saccharolytic enterobacteri and clostridia bacteria (Lorenzo & O'Kiely, 2008). The pH value was greater in combination treatment than in single formic acid treatment. This indicated the dominance

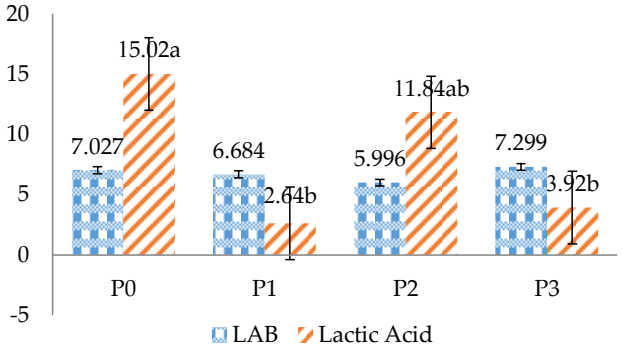

Figure 1. Population of lactic acid bacteria (log cfu g^{-1}) and lactic acid (g kg^{-1} DM) napier grass silage. P0= napier grass silage control, P1= P0 + formic acid, P2 = P0 + *L. plantarum*, P3 = P0 + formic acid + *L. plantarum*.

Table 3. *In vitro* fermentability and digestibility of napier grass silage

Variables	Treatment				SEM	Significant
	P0	P1	P2	P3		
Dry matter digestibility (%)	56.09[a]	51.41[b]	55.37[ab]	51.15[b]	0.52	**
Organic matter digestibility (%)	52.21[ab]	48.06[b]	52.65[a]	48.36[ab]	0.54	**
NH_3 (mM)	4.37[bc]	4.05[d]	5.28[ab]	6.30[a]	0.21	**
Total vollatile fatty acid (mM)	134.09[a]	119.89[b]	123.86[ab]	115.76[b]	2.43	*
Acetic (mM %VFA)	65.33	57.86	58.50	55.24	1.15	ns
Propionic (mM %VFA)	43.25	39.81	40.46	38.99	0.84	ns
Isobutyric (mM % VFA)	8.29	7.59	7.54	6.86	0.15	ns
Butyric (mM % VFA)	11.29	11.33	11.43	10.61	0.27	ns
Isovaleric (mM % VFA)	2.90[a]	2.57[ab]	2.85[a]	2.09[b]	0.08	*
Valeric (mM % VFA)	0.67	0.73	0.79	0.80	0.05	ns
Acetic-Propionic ratio	1.51	1.45	1.45	1.42	0.01	ns

Note: P0= Napier grass silage control; P1= P0 + formic acid; P2= P0 + *L. plantarum*; P3 = P0 + formic acid + *L. plantarum*; means in the same row with different superscripts differ significantly * P<0.05, ** P<0.01.

effect of the *L. plantarum* bacteria against formic acid on pH. The lactic acid produced by *L. plantarum* bacteria had a stronger role in lowering the pH as compared to formic acid.

The increased levels of AF up to 7.5% increased the final DM that was similar to the effect of AF supplementation up to 10%. There was an interaction between FA, *L. plantarum*, and formic acid on DM content. The result showed that *L. plantarum* bacteria supplementation produced the best results on the highest levels of DM (24.80%-26.93%). High value of DM was caused by bacteria that were capable of converting WSC to lactic acid more efficiently. This result was in line with that reported by Filya (2003) on the use of LP and *L. buchneri* to decrease the pH and suppress the DM losses. The effect of formic acid supplementation on limiting fermentation was not found on the dry matter content of napier grass silage, although it was better as compared to control treatment. Formic acid limits the fermentation by lowering pH and the antimicrobial effect of formic acid will interfere with cell function and inhibit the growth of both mold and bacteria (Luckstadt, 2009).

The two types of additives showed the same effectivity in decreasing ammonia production in napier grass silage when they were combined with FA. This result is in agreement with that of Saarisalo *et al.* (2006). Formic acid is effective in inhibiting amino acid breakdown during ensilage process as reflected by the lowest level of N-NH_3 in the silage supplemented with formic acid especially at the level of 7.5% FA. Similar finding was also reported by Guo *et al.* (2008) that the higher value of amino acid was found in silage supplemented with formic acid. The increased level of AF also contributed to the decresed N-NH_3 on Napier grass silage.

Fleigh point was one of the parameters indicating the quality of silage fermentation. The used of FA showed an interaction effect with the supplementation of *L. plantarum* and formic acid on fleigh point. The higher the level of FA supplementation the higher the fleigh point of napier grass silage. By grouping the fleigh point (Idikut *et al*., 2009), the three levels of FA produced very good quality of napier grass silage. Generally, the use of FA at the level of 7.5% could produce the best quality of napier grass silage, both physically and fermentatively.

Experiment 2. Chemical and Microbiological Qualities, and *in Vitro* Digestibility of Napier Grass Silage

The result of nutrient analysis of napier grass silage was shown in Table 2. In general, there was no difference between treatments and control in terms of nutrient contents of the silage. Addition of *L. plantarum* bacteria showed greater effects on the content of crude protein, crude fat, TDN, and nitrogen free extract of silage. *L. plantarum* bacteria improved the fermentation during ensilage process, by lowering the pH, reducing the formation of N-NH3, and increasing dry matter (%) content in the final product, resulted in a more soluble protein remaining in silage. This results were similar to that reported by Lee *et al*. (2008), that *L. plantarum* bacteria supplementation improved fermentation process and decreased proteolysis. The proportion of fibrous components (ADF) were lower in the silage supplemented with LAB inoculant and formic acid as compared to control, meanwhile the hemicellulose content was higher. Similar result was also reported by Santoso *et al.* (2014) with addition of LAB on silage. The explanation was that the enzymatic actions (hemicellulase and cellulase) present in the original forage degraded the cell wall during ensiling (de Oliveira *et al*., 2009).

The highest LAB population was found in control treatment. The growth of LAB was supported by the availability of WSC in napier grass silage which was provided by FA. Although the addition of the *L. plantarum* did not show significant differences in LAB population, it increased the production of lactic acid. As observed by Ratnakomala *et al.* (2006), *L. plantarum* bacteria 1A2 produced a higher lactid acid as compared to the other inoculant (1BL2) and resulted in the largest decline in LAB population. The lactic acid produced in the fermentation would decrease the final pH and therefore restricted the growth of LAB on the napier grass silage. As observed in the previous study (Saarisalo *et*

al., 2007), the total number of LAB in the final silage was smaller in the inoculated than in the limiting fermentation or in untreated silage, probably due to the low pH and autolysis.

Formic acid was able to restrict fermentation indirectly by limiting the activity of LAB to produce lactic acid. The result was consistent with that reported by Saarisalo *et al.* (2006), but different from that reported by Aksu *et al*. (2006) that formic acid supplementation did not alter lactid acid concentration. These results suggest that the effects of formic acid on lactid acid concentration are inconsistent. Formic acid did not affect the population of LAB in napier grass silage. This result indicated that the formic acid supplementation at the level of 0.15% only restricted the LAB's fermentation process, without interfering the growth of LAB in the silage. Rowghani & Zamiri (2009) reported that formic acid had an antibacterial effect on some bacterial species, included the LAB.

The two types of additives (formic acid and inoculant *L. plantarum* bacteria) did not show significant differences in the fermentation type. Formic acid supplementation restricted the production of lactic acid resulting in the higher average pH (by 0.51) as compared to that in inoculated treatment. The inoculant of *L. plantarum* bacteria enhanced lactid acid production and resulted in the lowest pH. The ability of a feed product to support the process of fermentation in the rumen was showed through its fermentation products (Table 3). Although the addition of either formic acid or *L. plantarum* bacteria were able to reduce proteolysis in silage, both treatments showed different effects on the rumen fluid. The range of ammonia concentration reached the ideal range (5-13 mg/dL or 2.94-7.65 mM) according to Boucher *et al.* (2007) that supported the growth of rumen bacteria. Formic acid treatment on napier grass silage showed the lowest ammonia concentration in the rumen as compared with *L. plantarum* treatment. Formic acid as a fermentation inhibitor reduced the degradation of protein in the rumen. This result is supported by the data reported by Aksu *et al.* (2006) that acid treatment reduced the apparent digestibility of crude protein in the rumen through its effect on preventing the conversion of protein to ammonia that eventually increased the proportion of by-pass protein entering the duodenum. This result indicated that the application of formic acid was an important factor in determining the effect of formic acid on the fermentation,the synthesis of microbial protein in the rumen, and the supply of nutrients to the ruminants (Jaakola *et al.,* 2006). On the other hand, the addition of *L. plantarum* bacteria increased ammonia concentration in the rumen compared to control and acid treatments. This result indicated that the higher rate of protein degradation into ammonia by fermentation are during ensilage process and in the rumen.

Microbial protein synthesis requires a balance availability of nitrogen and energy source (Pathak, 2008). VFA is a product of carbohydrate by microbial fermentation in the rumen. The higest total VFA concentration in the rumen fluid was found in the control treatment, followed by the one supplemented with *L. plantarum* and formic acid either single or in combination with *L. plantarum*. The supplementation of formic acid or *L. plantarum* affected the concentration of VFA in the rumen as compared to control. The use of formic acid or *L. plantarum* inhibited the fermentation, resulting in lower VFA concentration compared to that in control. Jaakola *et al*. (1991) reported that formic acid acts as a fermentation inhibitor, resulting in higher residual sugar that improved protein microbial synthesis and lower rumen VFA than compared to control. Isovaleric acid concentration was significantly affected by the treatments. The highest concentration was found in control silage and the one supplemented with *L. plantarum* inoculant. In addition to ammonia, branched chain fatty acids (isobutyrate and isovaleric) are also the result of protein degradation. Isovaleric acids affect fiber digestibility in the rumen of cattle, through an increase in the population of cellulolytic bacteria (Liu *et al*., 2014). The use of *L. plantarum* 1A-2 on silage increased the population of rumen bacteria and celulolytic bacteria in cattle (Widyastuti, 2008).

The highest digestibility was found in the control silage and the one supplemented with *L. plantarum* bacteria. The use of LAB in silage production was largely due to its ability to survive in the digestive tract of cattle. *L. plantarum* is potential as probiotics because of its tolerance to low pH and bile salts, its sensitivity to some antibiotics and its ability to produce extracellular enzymes (Arasu *et al*., 2014).

The addition of formic acid and its combinations with *L. plantarum* showed a lower digestibility of napier grass silage. This was evident from the low content of ADF in the treatment of acid based (Table 2). It appears that during the ensilage process, the most easily degradable fiber fractions are hydrolysed, whereas the remaining fractions are less digestible in the rumen (Jaakola *et al*., 2006).

CONCLUSION

Napier grass silage showed a good quality, especially with the use of liquid fermentation additive at the level of 7.5%. Based on the nutritive quality of silage, *L. plantarum* addition was as effective as control treatment to improve nutritive quality of napier grass silage through the increased of fermentation characteristics i.e., low pH, high DM product, high fermentation product (VFA), and digestible on rumen. Formic acid reduced ammonia production during ensiling and fermentation in rumen, but it was less effective in inhibiting the fermentation process when it was combined with *L. plantarum.*

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