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Silage Quality of King Grass (*Pennisetum purpureophoides*) Treated with Epiphytic Lactic Acid Bacteria and Tannin of Acacia

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ABSTRAK

Tujuan penelitian ini adalah untuk mengevaluasi kualitas silase rumput raja (*Pennisetum purpureophoides*) dengan penambahan bakteri asam laktat (BAL) epifit yang diperoleh dari ekstrak rumput terfermentasi (ERT) atau dikombinasi dengan tanin dari akasia. Percobaan disusun menggunakan rancangan acak lengkap dengan 6 perlakuan dan 3 ulangan. Perlakuan meliputi: (A) rumput raja tanpa aditif sebagai kontrol; (B) rumput raja + 3% ERT (v/b); (C) rumput raja + 3% ERT (v/b) + 10 ml ekstrak akasia (50 g/100 ml); (D) rumput raja + 3% ERT (v/b) + 10 ml ekstrak akasia (50 g/75 ml); (E) rumput raja + 3% ERT (v/b) + 10 ml ekstrak akasia (50 g/50 ml); (F) rumput raja + 3% ERT (v/b) + 10 ml ekstrak akasia (50 g/25 ml). Sebanyak 250 g bahan silase diensilase di dalam silo botol berukuran 400 ml dan disimpan pada suhu ruang (28 ^{o}C) selama 30 hari. Variabel yang diukur adalah karakterisistik ERT, karakteristik fermentasi, dan komposisi kimia silase. Data dianalisis menggunakan analisis varians dan perbedaan antar perlakuan diuji menggunakan uji wilayah ganda Duncan. Hasil penelitian menunjukkan bahwa jumlah BAL pada ERT meningkat dari 0,8 × 10^7 cfu/ml menjadi 2,9 × 10^7 cfu/ml setelah diinkubasi selama 2 hari. Konsentrasi asam laktat pada silase dengan penambahan ERT atau dikombinasi dengan tanin akasia (B, C, D, E, dan F) lebih tinggi (P<0,01) dibanding silase A (kontrol). Silase dengan penambahan ERT atau dikombinasi dengan tanin akasia (C, D, E, dan F) mempunyai nilai pH yang lebih rendah dibandingkan silase A dan B. Konsentrasi N-NH_3 menurun sejalan dengan meningkatnya konsentrasi tanin akasia. Konsentrasi asam butirat lebih rendah pada silase B, C, D, E, dan F dibanding silase A. Penambahan ERT yang dikombinasikan dengan tanin yang berasal dari daun akasia meningkatkan kualitas silase rumput raja.

Kata kunci: silase, rumput, bakteri asam laktat, tanin

ABSTRACT

The aim of this study was to evaluate the silage quality of king grass (*Pennisetum purpureophoides*) treated with addition of epiphytic lactic acid bacteria (LAB) prepared from fermented grass extract (FGE) or combined with tannin of acacia. Experiment was arranged to a completely randomized design with six treatments and three replications. Treatments were (A) king grass without additive as a control; (B) king grass + 3% (v/w) of FGE; (C) king grass + 3% (v/w) of FGE + 10 ml of acacia extract (50 g/100 ml); (D) king grass + 3% of FGE (v/w) + 10 ml of acacia extract (50 g/75 ml); (E) king grass + 3% of FGE (v/w) + 10 ml of acacia extract (50 g/50 ml), and (F) king grass + 3% of FGE (v/w) + 10 ml of acacia extract (50 g/25 ml). About 250 g of silage materials were ensiled in 400 ml bottle silos at room temperatures (approximately 28 °C) for 30 days. Variables measured were characteristics of FGE, fermentation characteristics and chemical composition of silage. Data were analyzed by analysis of variance and the significance differences among means were tested by the Duncan's multiple range test. Results showed that the number of lactic acid bacteria in FGE increased from 0.8 × 10^7 cfu/ml to 2.9 × 10^7 cfu/ml after 2 days anaerobic incubation. Concentration of lactic acid in silages with addition of FGE or combined with tannin of acacia (B, C, D, E, and F) were higher (P<0.01) than that of silage A (control). Silages with addition of FGE combined with tannin of acacia (C, D, E, and F) had lower pH value than that of silages A and B. Concentrations of NH_3-N decreased with increasing concentration of tannin. Butyric acid concentration decreased in silages B, C, D, E, and F as compared to that in silage

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A. Addition of FGE combined with tannin prepared from acacia leaf improved fermentation quality of king grass silage.

Key words: silage, grass, lactic acid bacteria, tannin

INTRODUCTION

It is recognized that tropical grasses have low water soluble carbohydrate content, high buffering capacity and low lactic acid bacteria (LAB) number (Yahaya *et al*., 2004b). These properties result in low lactic acid production; hence it is difficult to produce good-quality silage from tropical grasses. The epiphytic microorganisms existed naturally in forage crops are responsible for silage fermentation and also influence the effectiveness of silage bacterial inoculation. However, the number of LAB is usually low and vary depended on growing crops (McDonald, 1981).

Fermented grass extract of epiphytic lactic acid bacteria (FGE) is prepared by culturing microorganism adherent to the grass materials before preparation of silage and the grown microorganism are used as a starter of silage fermentation. Masuko *et al*. (2002) revealed that FGE was prepared by macerating grass in water and incubating it anaerobically at 30 °C for 2 days. Previous studies of Masuko *et al*. (2002); Yahaya *et al*. (2004b); Bureenok *et al.* (2006); Horiguchi & Takahashi (2007) found that addition of fermented grass extract on silage materials could increase lactic acid production, decrease NH_3-N concentration, increase lactic acid bacteria population, and improve fermentation quality of grasses and green soybean stover silage. In addition, Wang *et al*. (2009) concluded that fermented grass extract can be used as a good source of LAB for direct cut alfalfa silage.

During the ensiling process of forage, extensive proteolysis occurs due to the combined action of both plant and microbial enzymes resulting in conversion of most protein to non-protein nitrogen (NPN) fractions mainly amino acid N, peptide N, and ammonia N (Owens *et al.* 2002; Givens & Rulquin, 2004). The rapid rate of degradation of silage NPN and soluble-protein N in the rumen results in a pronounced peak in rumen ammonia concentration following ingestion. Excess quantities of ammonia in the rumen is absorbed into the blood stream, converted to urea in liver and subsequently excreted in the urine, contributing to environmental pollution (Swensson, 2003).

Tannins are polyphenolic compounds of plant origin that have ability to bind protein. It has been reported that tannin-containing species such as quebracho, chestnut, residues of green tea and black tea reduced protein degradation during ensiling and protein disappearance in the rumen (Salawu *et al*., 2001; Kondo *et al*., 2004; Tabacco *et al*., 2006; Santoso *et al*. 2007) by inhibiting plant and microbial enzymes or by forming complexes with protein (McSweeney *et al*., 2001). Hariadi & Santoso (2010) reported that acacia leaf (*Acacia mangium* Willd) contains 5.4% total tannin; hence it is potentially used as silage additive to protect protein degradation during ensiling.

The objective of this experiment was to evaluate the fermentation quality of king grass (*Pennisetum purpureophoides*) silage treated with addition of epiphytic LAB prepared from fermented grass extract or combined with tannin of acacia.

MATERIALS AND METHODS

Forage Material

King grass (*Pennisetum purpureophoides*) was planted in a 9 m^2 plot without fertilizer at the experimental field of Faculty of Animal Science, Fishery and Marine Science, State University of Papua in Manokwari. Grass was harvested with a hand clipper in May 2009 after 50 days of regrowth defoliation. The experimental field is located at 134°04′ longitude and 00°48′ latitude. The area is located at an altitude of 110 m above sea level. The mean annual rainfall and temperature were 159.9 mm and 27.1 °C, respectively.

Preparations of Fermented Grass Extract and Acacia Extract

Preparation of FGE according to modified of Bureenok *et al*. (2006) procedure as previously described by Santoso *et al.* (2009). The FGE was prepared using 220 g of fresh king grass, which was macerated in 1000 ml of distilled water using a high-speed blender for 4 min. The macerated material was filtered through two layers of cheesecloths, and 600 ml of filtrate was collected in erlenmeyer glass containing 18 g of glucose. The filtrate was mixed well and incubated anaerobically for 48 h at 30 ºC. At the end of 48 h, FGE was used as source of LAB. The number of LAB in the FGE was counted before the experiments by using de Man, Rogosa, and Sharpe which were incubated for 3 days at 35 °C (Bureenok *et al*., 2006).

About 50 g of finely acacia leaf were weighed into 100 ml beaker glass and added 25 ml of distilled water. The same procedure was also performed for 50, 75, and 100 ml of distilled water. The mixtures were boiled for 10 min on a hotplate and filtered through 2 layers of cheesecloth. The filtrates were collected and stored at 4 °C for further use.

Silage Preparation and Treatments

The fresh king grass was wilted at room temperature (approximately 28 °C) for 24 h and chopped into 3-5 cm. The chopped grass was thoroughly mixed and a representative samples obtained. Total of 6 treatments were as follows (A) king grass without additive as the control; (B) king grass + 3% (v/w) of FGE; (C) king grass + 3% (v/w) of FGE + 10 ml of acacia extract (50 g/100

ml); (D) king grass + 3% of FGE (v/w) + 10 ml of acacia extract (50 g/75 ml); (E) king grass + 3% of FGE (v/w) + 10 ml of acacia extract (50 g/50 ml), and (F) king grass + 3% of FGE (v/w) + 10 ml of acacia extract (50 g/25 ml). Both FGE and acacia extract were sprayed onto silage materials using a hand sprayer and subsequently mixed by hand. Based on the concentration of LAB in FGE, the final application was 5.8×10^6 cfu/g of fresh forage. Tannin concentration calculated in silage C, D, E, and F were 2.7, 3.6, 5.2, and 10.3 g/kg, respectively. About 250 g of silage materials were packed into 400 ml laboratory glass bottle silos. Each treatment was prepared in triplicate and the silos were stored in room temperature for 30 days.

Analytical Procedure

Dried samples were used to determine DM, ash, and crude protein (CP) according to the procedure of AOAC (2005). Procedure of Van Soest *et al.* (1991) was used to determine concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). NDF was determined without the use of ∝-amylase and sodium sulfite.

A 20 g of silage was macerated with 70 ml of distilled water and stored at 4 °C for 24 h. It was than homogenized for 15 min by using a shaker and filtered through a Whatman No. 1542 filter paper. The filtrate was used to determine pH, VFAs, lactic acid and NH_3-N. The pH value was determined using a pH meter (Hanna Hi 9025). Concentrations of individual VFAs were analyzed using a gas chromatography (Varian CP-9002 GC, Shimadzu, Japan) equipped with flame ionization detector (FID) and stainless steel column (1500 mm × 3 mm i.d). The pressure of nitrogen was 1.25 kg/cm^2. The temperature of injector oven, column oven and detector were 220, 130, and 220 °C, respectively. Concentrations of lactic acid and NH_3-N were analyzed according to the method of Barker & Summerson (1941); Chaney & Marbach (1962), respectively.

Statistical Analysis

Silage fermentation data were subjected to analysis of variance for a completely randomized design. Duncan's multiple range test was used to separate treatment means, when probability was less than 0.05.

RESULTS AND DISCUSSION

Characteristics of Fermented Grass Extract

Table 1 depicts the result of pH value and LAB number in the fermented grass extract used as additive at ensiling. The epiphytic LAB number of the material grass was 0.8×10^7 cfu/ml fresh matter. After 48 h of incubation at 30 °C, the LAB number in the FGE increased to 2.9×10^7 cfu/ml. Increased LAB number resulted in high concentration of lactic acid, thereby decreasing in pH value from 6.71 to 3.51. In previous study, Santoso *et al*. (2009) also reported that pH value of fermented grass extract prepared with king grass reduced from 6.41 to 3.45. The patterns of reduced pH value in fermented extracts of grass or legume, and increased LAB number after 48 h of incubation are agree with previous reports of Nishino & Uchida (1999); Bureenok *et al*. (2006); Wang *et al*. (2009). Nishino & Uchida (1999) revealed that several strains of LAB *e.g*. *Lactobacillus plantarum*, *Lactobacillus viridescens*, *Lactobacillus fermentum*, *Pediococcus acidilactici* were isolated from Lucerne, timothy and coksfoot fermented extracts. However predominant strains were different depended on the material crop used for fermented extract preparation.

Table 1. The pH value and lactic acid bacteria (LAB) number in grass extract before and after incubation 48 h

	Before incubation	After incubation
pH	6,71	3,51
LAB (x 10^7 cfu/ml)	0,80	2,90

Fermentative Quality of the Silages

Fermentation characteristics of king grass silage treated with epiphytic lactic acid bacteria and tannin of acacia are shown in Table 2. The pH values in silages of C, D, E and F were lower ($P<0.01$) than that of silage A (control). Lower pH value in silage treated with epiphytic LAB and acacia tannin could be due to higher lactic acid concentration in those silages. Seglar (2003) stated that lactic acid is the strongest of all silage acids and its presence will drop pH more effectively than the other volatile fatty acids. Even though, lower pH value found in silage treated with epiphytic LAB, however, the final pH value except silage D are still above than ideal silage pH of 4.0 to 4.5. As reported by Chamberlain & Wilkinson (1996) that secondary fermentation occurs when insufficient acid is produced by primary fermentation to reduce the pH to below a critical level of about 4.5.

Concentration of lactic acid in silages of B, C, D, E and F were significantly ($P<0.01$) higher as compared to control silage (A). This result agrees with previous study on silages prepared with tropical grasses as reported by Yahaya *et al*. (2004a); Yahaya *et al*. (2004b); Bureenok *et al*. (2006), and lucerne silage by Nishino & Uchida (1999); Filya *et al.* (2007); Wang *et al*. (2009). Increased lactic acid concentration in silage treated with epiphytic LAB could be due to increasing fermentation process by LAB which converts monosaccharide such as glucose and fructose to lactic acid. Higher concentration of lactic acid in silage has positive effect to animal due to it can be converted to propionic acid in the rumen. In a study by Takahashi *et al*. (2005), the molar proportion of propionic acid tended to increase, and the ratio of acetic acid to propionic acid decreased, at 4 h after feeding in sheep fed whole crop rice silage with epiphytic LAB prepared from fresh rice straw. Increased propionic acid concentration might be due to addition of epiphytic LAB to silage materials

Table 2. Fermentation characteristics of king grass silage treated with epiphytic lactic acid bacteria and tannin of acacia after 30 days ensilage

	Silages						SEM	P
	A	B	C	D	E	F		
LAB (x 10^7 cfu/ml)	2.3	2.5	1.4	1.1	1.3	2.1	0.41	0.14
pH	5.28^{A}	4.67^{AB}	4.52^{B}	4.31^{B}	4.61^{B}	4.56^{B}	0.14	<0.01
Lactic acid (g/kg DM)	0.6^{b}	43.2^{A}	39.7^{A}	39.2^{A}	42.6^{A}	37.4^{A}	6.23	<0.01
NH_3-N (g/kg total N)	289.7^{a}	159.7^{b}	132.2^{b}	124.0^{b}	120.1^{b}	105.8^{b}	39.3	0.05
Acetic acid (g/kg DM)	73.0	95.2	110.0	44.1	75.8	69.2	29.51	0.71
Propionic acid (g/kg DM)	11.6	3.9	9.6	6.2	6.3	0	3.50	0.30
Butyric acid (g/kg DM)	25.6^{A}	4.7^{B}	4.0^{B}	6.3^{B}	6.7^{B}	5.6^{B}	3.06	0.01
Total VFA (g/kg DM)	110.2	103.8	123.6	56.5	88.7	74.8	27.15	0.56

Note: Means in the same row followed by different letters are different ($^{a-b}$ P<0.05; $^{A-B}$ P<0.01). A= king grass without additive; B= king grass + 3% (v/w) of fermented grass extract (FGE); C= king grass + 3% (v/w) of FGE + 10 ml of acacia extract (50 g/100 ml); D= king grass + 3% of FGE (v/w) + 10 ml of acacia extract (50 g/75 ml); E= king grass + 3% of FGE (v/w) + 10 ml of acacia extract (50 g/50 ml); F= king grass + 3% of FGE (v/w) + 10 ml of acacia extract (50 g/25 ml).

changed the microbial populations in the rumen causing higher propionic acid fermentation. Alternatively, the higher concentration of lactic acid in the silage may have been metabolized into propionic acid by the rumen microorganisms. Propionic acid is then absorbed into blood stream via rumen wall and converted to glucose in the liver. Glucose formed is used by animal as energy source for maintenance, production and reproduction activities. In addition, Weinberg *et al*. (2004) concluded that LAB from silage has potential role as probiotic which beneficially affects the host animal by improving its intestinal microbial balance.

Concentration of lactic acid in silage treated with combination of LAB and acacia tannin (C, D, E and F) tended to be lower in comparison with silage treated with LAB alone (B). McSweeney *et al*. (2001) revealed that tannin has ability to bind to macromolecules such as structural carbohydrate and starch, thereby impairing their degradation. However, concentration of lactic acid in king grass silages treated LAB and acacia tannin were slightly lower than the ideal range of lactic acid concentration from 80 to 120 g/kg DM.

Concentration of NH_3-N in silages treated with LAB or combined with acacia tannin (B, C, D, E, and F) were lower (P<0.05) than that of control silage. The finding was consistent with previous work of Nishino & Uchida (1999) who found that use of fermented Lucerne extract increased lactic acid and greatly inhibited the clostridial activity to protect proteins from extensive degradation. As stated by Owens *et al.* (2002) that during ensiling protein is degraded to peptides and free amino acids by plant proteases. In addition, degradation of amino acids to ammonia and non-protein nitrogenous fraction is predominantly due to proteolytic clostridia. Chamberlain & Wilkinson (1996) concluded that ammonia-N is as an indicator of the proportion of the total N which has been completely degraded during ensiling. Hence, concentration of ammonia-N is the best indicator of secondary fermentation. The growth of proteolytic clostridia, which degrade protein and amino acids to NH_3 is inhibited by low pH (McDonald, 1981; Winters *et al*., 2000). This result was supported by the low pH values in silage B, C, D, E, and F compared to silage A. This condition could depress the growth of proteolytic clostridia. Concentration of NH_3-N reduced with increasing tannin addition in silage materials. This could be due to tannin can bind to protein and protect them from microbial degradation. Tabacco *et al*. (2006) reported that tannin were able to protect herbage proteins from plant/microbial enzyme hydrolysis during ensiling. Concentration of NH_3-N in silage C, D, E, and F decreased with increasing tannin concentration added in silage. This result is in agreement with previous study by Tabacco *et al*. (2006) that concentrations of NH_3-N and NPN were reduced with increasing tannin concentration in silage. The normal range of NH_3-N concentration in silage is 50 to 150 g NH_3-N/kg DM. However, the target value for NH_3-N is less than 50 g/kg total N (Chamberlain & Wilkinson, 1996). Based on NH_3-N concentration, silage C, D, E and F could be classified in normal range of NH_3-N concentration.

The VFAs comprise of acetic acid, propionic acid, butyric acid and other acids. The production of these acids is a reflection of an inefficient fermentation or of secondary fermentation of lactic acid to butyric acid and degradation of amino acids to ammonia with the production of acetic acid from the carbon skeleton of the amino acid (Chamberlain & Wilkinson, 1996). Concentrations of total VFA, acetic acid and propionic acid were not significantly different (P>0.05) among treatments silage. However, silage treated with epiphytic LAB and tannin had lower (P<0.01) butyric acid concentration compared to control silage. The reduced butyric acid in those silages could be due to lower pH silage which may inhibit the activity of clostridia. McDonald (1981) stated that reducing pH silage prevented the growth of undesirable microbes *e.g*. listeria, clostridia, enterobacteriaceae, and moulds. Another possible explanation for the reduction of butyric acid in silage treated with acacia tannin (silage

Table 3. Chemical composition (%) of silages after 30 days ensilage

	Silages						SEM	P
	A	B	C	D	E	F		
Dry matter (DM)	17.5^{ab}	17.5^{ab}	18.2^{a}	16.2^{d}	16.8bc	17.9^{a}	0.32	0.02
	----------% of DM----------							
Organic matter (OM)	94.6	95.3	95.5	96.2	95.2	95.4	0.45	0.27
Crude protein (CP)	12.8^{B}	16.2^{A}	15.7^{A}	15.3^{A}	15.7^{A}	15.7^{A}	0.19	<0.01
Neutral detergent fiber (NDF)	71.0^{A}	65.3^{B}	68.0^{AB}	67.3^{B}	67.9^{AB}	66.9^{B}	0.96	<0.01
Acid detergent fiber (ADF)	43.4^{a}	41.7^{abc}	40.2^{c}	40.7^{bc}	42.5^{ab}	41.8^{abc}	0.55	0.02
Hemicellulose	28.6^{A}	23.5^{B}	27.8^{A}	26.6^{AB}	25.4^{AB}	25.1^{AB}	0.79	<0.01

Note: Means in the same row followed by different letters are different ($^{a-b}$ P<0.05; $^{A-B}$ P<0.01). A= king grass without additive; B= king grass + 3% (v/w) of fermented grass extract (FGE); C= king grass + 3% (v/w) of FGE + 10 ml of acacia extract (50 g/100 ml); D= king grass + 3% of FGE (v/w) + 10 ml of acacia extract (50 g/75 ml); E= king grass + 3% of FGE (v/w) + 10 ml of acacia extract (50 g/50 ml); F= king grass + 3% of FGE (v/w) + 10 ml of acacia extract (50 g/25 ml).

C, D, E and F) might be due to inhibited the activity of clostridia. It is well known that clostridia are responsible for most of the butyric acid in silage (McDonald, 1981).

Visually, there were no moulds on the top of silages treated with acacia tannin. This was probably due to toxic effect of tannin to silage moulds. This result is supported with previous study of McDonald (1981) that higher tannin in the silage reduced the activity of moulds.

Chemical Composition of Silages

Chemical composition of silages treated with epiphytic LAB and tannin of acacia is given at Table 3. Dry matter content in six silages was still lower than the target value for DM in ideal silage as recommended by Chamberlain & Wilkinson (1996). The lower DM content in all silages could be attributed to DM content of king grass used as silage material was less than 20%. The OM content in silage A was similar to silages treated with epiphytic LAB and tannin of acacia. Concentration of CP in silages treated with epiphytic LAB and tannin of acacia was higher (P<0.01) than that of control silage. The higher CP content in those silages could be due to lower activity proteolytic as resulted by low in pH value and protein protection by tannin acacia. Other explanation for higher CP content in silage treated with epiphytic LAB and tannin of acacia is addition of protein obtained from grass and acacia extracts.

The NDF content in silages B, D, and F was lower (P<0.01) than that of control silage. Silages C and D had lower (P<0.05) ADF content compared with control silage. This results, however is in agreement with previous studies of Yahaya *et al.* (2004b); de Oliveira *et al*. (2007). One of the explanations for the lower NDF and ADF in those silage is that enzymatic action *e.g*. hemicellulases, cellulase present in the original forage on cell wall during ensiling. Decreased NDF and ADF concentrations in silage treated with epiphytic LAB or combined with tannin of acacia had beneficial effect of silage nutritive value and leading to an increase in silage digestibility in the rumen.

CONCLUSION

Fermented grass extract prepared from king grass could be used as a good source of lactic acid bacteria pre-ensiling. Addition of epiphytic LAB or combined with acacia tannin have beneficial effect on fermentation and nutritive qualities of king grass silage indicated by a high lactic acid production, low pH value, as well as concentrations of NH_3-N and butyric acid, high crude protein content, and low cell wall component contents *i.e*. NDF, ADF, and hemicellulose compared with control silage.

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