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FERMENTATION QUALITY OF KING GRASS SILAGE TREATED WITH LIQUID OR DRIED INOCULANT OF LACTIC ACID BACTERIA

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ABSTRAK

Tujuan penelitian ini adalah untuk mengevaluasi nilai nutrisi dan karakteristik fermentasi silase rumput raja yang diberi perlakuan penambahan inokulan cair atau kering bakteri asam laktat (BAL). Penelitian ini disusun dengan rancangan acak lengkap dengan 4 perlakuan dan 3 ulangan. Empat perlakuan yaitu (A) rumput raja tanpa inokulan BAL sebagai kontrol; (B) rumput raja + 30 mL inokulan cair BAL/kg hijauan segar; (C) rumput raja + 30 g inokulan kering beku BAL/kg hijauan segar; (D) rumput raja + 30 g inokulan kering sentrifugasi BAL/kg hijauan segar. Inokulan cair BAL disemprot pada permukaan rumput dan selanjutnya dicampur hingga rata. Konsentrasi awal BAL pada semua perlakuan adalah $1,0 \times 10^6$ cfu/g. Sebanyak 500 g bahan silase diensilase dalam silo plastik dan disimpan pada temperatur ruang (28^{o}C) selama 30 hari. Hasil penelitian menunjukkan bahwa nilai pH, konsentrasi asam laktat, N-amonia (N-NH_3), asam butirat, total *volatile fatty acids* (VFA) serta Nilai Fleigh dipengaruhi oleh perlahuan inokulan BAL. Silase yang diberi perlakuan inokulan cair atau kering BAL memiliki nilai pH lebih rendah (P<0,01) dibandingkan silase kontrol. Konsentrasi N-NH_3 dan asam butirat signifikan lebih rendah (P<0,01) pada silase yang diberi perlakuan inokulan cair atau kering BAL dibandingkan silase kontrol. Silase yang diberi perlakuan inokulan BAL kering sentrifugasi memiliki Nilai Fleigh tertinggi (P<0,05) dibanding silase lain. Kecernaan *neutral detergent fiber* (NDF) *in vitro* lebih tinggi (P<0,05) pada silase yang diberi perlakuan inokulan BAL dibandingkan silase kontrol.

Kata kunci:bakteri asam laktat, fermentasi, inokulan, rumput, silase

ABSTRACT

The aim of this study was to evaluate the nutritive value and fermentation characteristic of king grass silage treated with addition of liquid or dried lactic acid bacteria (LAB) inoculant. Experiment was arranged to a completely randomized design with four treatments and three replications. Four treatments as follows (A) king grass without LAB inoculant as the control; (B) king grass + 30 ml of liquid of epiphytic LAB inoculant/kg of fresh forage; (C) king grass + 30 g of freeze-dried powder of LAB inoculant/kg of fresh forage; (D) king grass + 30 g of centrifuged powder of LAB inoculant/kg of fresh forage. The liquid LAB inoculant was sprayed on the top of grass and then mannualy mixed by hand. The intial LAB concentration in all inoculants were 1.0×10^6 cfu/g. About 500 g of silage materials were ensiled into plastic silos and stored at room temperatures (approximately 28^{o}C) for 30 days. Results showed that pH value, concentrations of lactic acid, N-amonia (NH_3-N), butyric acid and total volatile fatty acids (VFA) as well as Fleigh Point were affected by treatment of LAB inoculant. Silage treated with liquid or dried of LAB inoculant had lower (P<0.01) pH value compared to the control silage. Concentrations of NH_3-N and butyric acid significantly decreased (P<0.01) in silage treated with LAB

inoculants. Silage treated with centrifuged powder of LAB inoculant had the highest (P<0.05) Fleigh Point than other silage. In vitro neutral detergent fiber (NDF) digestibility was significantly higher (P<0.05) in silage treated with LAB inoculant compared to the control silage.

Keywords: fermentation, inoculant, grass, lactic acid bacteria, silage

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*., 2004). 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).

The LAB plays an important role in silage fermentation and influence silage quality. Under natural circumstances, LAB grows as epiphytic bacteria, however, the population of LAB is usually low and variable with standing crops (Muck, 1990). Thus, addition of LAB inoculant is needed to improve silage quality (Bureenok *et al*., 2006). In the previous studies, Yahaya *et al*. (2004); Bureenok *et al.* (2006) stated that tropical and temperate forages ensiled with addition of epiphytic lactic acid bacteria inoculant resulting good fermentation quality compared to commercial inoculant. Santoso *et al.* (2009) concluded that fermentative quality of grass silage treated with epiphytic LAB prepared from king grass was better than those prepared from elephant grass. Similar result was reported in other experiment of Santoso *et al*. (2011); Santoso *et al*. (2012) that king grass and rice crop residue silages with addition of epiphytic LAB had good fermentation quality compared to control silage, as indicated by high lactic acid content and *in vitro* nutrient digestibility, and low ammonia-N (NH_3-N) concentration. Wang *et al*. (2009) revealed that the effect of LAB from forage crop may be comparable or even better than commercial bacterial culture, because the commercial bacterial does not grow well on the target crop. Furthermore, addition of LAB inoculant in silage materials could improve fermentation quality of silage and nutrient digestibility. Whiter and Kung (2001) reported that microbial inoculant in a liquid-form was more effective than in a dry-form. Meanwhile, Kizilsimsek *et al*. (2007) stated that application of inoculant as a fresh culture in alfalfa silage resulted good fermentation profile than freeze-dried culture. Jeni *et al*. (2010) concluded method of drying by centrifugation produced higher bacteria viability than the method freezedrying. This experiment was carried out to evaluate the nutritive value and fermentation characteristic of king grass silage treated with addition of dried inoculant or liquid epiphytic LAB.

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, University of Papua in Manokwari. Grass was harvested with a hand clipper 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.

Preparation of Liquid and Dry Inoculants

Preparation of liquid LAB inoculant according to modified of Bureenok *et al*. (2006) procedure as previously described by Santoso *et al.* (2009); Santoso *et al*. (2011); Santoso *et al.* (2012). The liquid inoculant 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 cheese cloths, 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 30ºC. The number of LAB in the liquid or dried inoculants 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).

Preparation of both freeze-dried powder and centrifuged powder of LAB inoculants according to modified method of Jeni *et al*. (2010). Briefly,

1 liter of LAB culture was added to sterilized cassava powder and throughly mixed by hand. The mixed material was put on glass bottle and then dried by using freeze-dryer (Martin Christ 18436) for 36 hours. Furthermore, the freeze-dried powder of LAB was used as additive of king grass silage.

One liter of LAB culture was put in 4 glass bottle with capacity of 250 ml and centrifuged at 10.000 rpm for 5 min. The supernantant was removed and remained about 10-15 ml for each bottle. The remained supernatant was mixed with 250 g of sterilized cassava powder. Futhermore, the mixture LAB culture was used as additive of king grass silage.

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 4 treatments were as follows (A) king grass without LAB as the control; (B) king grass + 30 ml of liquid of epiphytic LAB inoculant/kg of fresh forage; (C) king grass + 30 g of freeze-dried powder of LAB inoculant/kg of fresh forage; (D) king grass + 30 g of centrifuged powder of LAB inoculant/kg of fresh forage. The intial LAB concentration in all inoculants were 1.0×10^6 cfu/g. About 500 g of silage materials were packed into plastic silos and stored in the room temperature for 30 days. Each treatment was prepared in triplicate.

Chemical Analyses

Dried samples were used to determine dry matter (DM), ash and crude protein (CP) according to procedure of AOAC (2005). Procedure of Van Soest *et al.* (1991) was used to determined concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). NDF was determined without the use of α-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 then homogenized for 15 min by using a shaker and filtered through a Whatman No. 1542 filter paper. The filtrate was used for determine of 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 method of Barker and Summerson (1941); Chaney and Marbach (1962), respectively. Fleigh Point of the silage were calculated according to formulae as follows : Fleigh Point = 220 + (2 × DM% - 15) - (40 × pH), where Fleigh Point denote values between 85 and 100, very good quality; 60 and 80, good quality; 55 and 60, moderate quality; 25 and 40, satisfying quality; < 20 worthless (Ozturk *et al*., 2006).

Statistical Analysis

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 DICUSSION

Chemical Composition of Silage

The nutrients content of king grass silage treated with dry or liquid LAB inoculants are presented in Table 1. The DM content of silages was lower than the value of 30% for ideal silage as suggested by Chamberlain and Wilkinson (1996). Organic matter and crude protein contents in all silages were similar with the average values of 93.1% and 7.7%, respectively. Organic matter content in this silage was slightly lower than value of 95.3% as reported by of Santoso *et al*. (2011). A slightly lower ADF content was observed in silage treated with LAB inoculants (B, C and D) than control silage. It has been reported that activity of cellulase and hemicellulase enzymes was high during ensilage (Yahaya *et al*., 2004). Similar results were also reported in previous findings using guinea grass and king grass silages (Ando *et al*., 2006; Santoso *et al*., 2009; Santoso *et al*., 2011).

Characteristics Fermentation of Silage

The final pH of silage treated with liquid and dry LAB inoculant was lower ($P<0.01$) than untreated silage (A) after 30 days of ensilaging (Table 2). Addition of lactic acid bacteria inoculants at ensiling ensured rapid and vigorous fermentation which resulted in faster accumulation of lactic acid. Furthermore, a high lactic acid concentration in those silages resulted in lower pH value. This result indicates that LAB

Table 1. Chemical Composition (%) of King Grass Silage Treated with Liquid or Dried Lactic Acid Bacteria Inoculants

	Silages			
	A	B	C	D
Dry matter	21.3	20.8	19.4	21.1
Organic matter	93.3	93.5	92.4	93.3
Crude protein	7.7	7.7	7.7	7.6
NDF	79.4	76.5	77.2	78.6
ADF	52.9	49.7	48.8	47.8
Hemicelullose	26.5	26.8	28.4	30.8

A: Silage without LAB inoculant; B: silage treated with liquid of epiphytic LAB inoculant; C: silage treated with freeze-dried powder of LAB inoculant; D: silage treated with centrifuged powder of LAB inoculant.

Table 2. Fermentation Characteristics of King Grass Silage Treated with Liquid or Dried Lactic Acid Bacteria Inoculants After 30 Days of Ensilage

	Silages				SEM	*P*
	A	B	C	D		
pH	5.55^{A}	5.16^{B}	4.93^{B}	4.92^{B}	0.05	0.01
Lactic acid (g/kg DM)	54.6^{B}	67.3^{A}	72.7^{A}	65.7^{AB}	2.51	0.01
NH_3-N (g/kg total N)	89.7^{A}	46.6^{B}	45.0^{B}	39.4^{C}	4.40	0.01
Acetic acid (g/kg DM)	11.3	10.0	7.8	10.0	0.75	0.06
Propionic acid (g/kg DM)	3.7	3.0	2.2	2.1	0.78	0.45
Butyric acid (g/kg DM)	8.0^{A}	4.9^{B}	7.5^{A}	1.8^{C}	0.43	0.01
Total VFA (g/kg DM)	23.0^{a}	17.9^{b}	17.5^{b}	13.8^{b}	1.47	0.05
Fleigh Point	18.6^{b}	37.5^{ab}	47.1^{ab}	50.2^{a}	5.59	0.02

Means in the same row followed by different superscript are different ($^{a-b}P<0.05$; $^{A-C}P<0.01$).

produced high lactic acid concentration during incubation which resulted in low pH value. This result is consistent with previous studies by Santoso *et al*. (2009); Santoso *et al.* (2011) that pH value in king grass extract declined from average of 6.62 to 3.40 after 48 h of incubation at 30°C. Trend declined pH value in extracts of grass and legume after 48 h of incubation have been also reported by Bureenok *et al*. (2006) and Wang *et al*. (2009). Similar to this findings, Kizilsimsek *et al*. (2007) reported that the addition of an inoculant in water was more effective than in a dry application of the same inoculant in grass silage. In the previous study, Whiter and Kung (2001) revealed that alfalfa silage with 30% DM treated with both dry and liquid of LAB inoculant had lower ($P<0.05$) pH value than untreated silage at 2 days after ensiling.

Concentrations of lactic acid, NH_3-N, butyric acid, total VFA and Fleigh Point were affected ($P<0.01$) by LAB inoculants. Concentration of lactic acid in silages of B and C 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*. (2004); Bureenok *et al*. (2006), and lucerne silage by 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. However, concentration of lactic acid in king grass silages treated LAB were slightly lower than the ideal range of lactic acid concentration from 80 to 120 g/kg DM as recommended by Chamberlain and Wilkinson (1996).

Silages treated with LAB inoculants (B, C and D) had lower (P<0.01) concentration of NH_3-N than control silage (A). This result was consistent with previous study of Santoso *et al*. (2011) who found that use of fermented forage extract increased lactic acid and greatly inhibited the clostridial activity to protect proteins from extensive degradation. Owens *et al.* (2002) revealed 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 and 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 and D compared to silage A. This condition could depress the growth of proteolytic clostridia. The target value for NH_3 -N in silage is less than 50 g/kg total N (Chamberlain and Wilkinson, 1996). Based on NH_3 -N concentration in the Table 3, silage B, C and D could be classified in normal range of NH_3-N concentration.

Concentration of butyric acid was lower (P<0.01) in silage B and D compared to control silage (A). The result indicates that clostridia bacteria was more active in control silage (A) than silage treated with liquid epiphytic LAB or centrifuged powder of LAB inoculant. According to Chamberlain and Wilkinson (1996), secondary fermentation occurs insufficient acid is produced by the primary fermentation to reduce the pH to below a critical level of about 4.5. The bacteria responsible for secondary fermentations are mainly the clostridia. These bacteria may convert lactic acid to butyric, or may degrade proteins, peptides and amino acids to amines and ammonia. McDonald *et al.* (1987) also reported that butyric acid is produced by saccharolytic clostridia *i.e*. *Clostridium butyricum*. The total VFA concentration was slightly lower in silages treated with LAB inoculant than control silage. The result indicates that addition of LAB inoculant to king grass could improve fermentative quality of silage. Chamberlain and Wilkinson (1996) stated that the VFA comprise acetic acid, propionic acid, butyric acid and other acids. The production of these acids is 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 amino acid from skeleton of the amino acid. In the present study, the proportion of VFA to total acid

Table 3. *In vitro* Nutrient Digestibilities of King Grass Silage Treated with Liquid or Dried LAB Inoculants

	Silage				SEM	*P*
	A	B	C	D		
Dry matter	47.9	52.7	49.1	49.0	1.91	0.43
Organic matter	48.0	54.3	51.4	51.1	1.22	0.29
NDF	19.0^{b}	28.2^{a}	27.0^{a}	27.2^{a}	1.37	0.03

Means in the same row followed by different superscripts are significantly different ($^{a-b}$P<0.05).

was 29.6, 21.0, 19.4 and 17.4%, respectively for silage A, B, C and D. The result indicates that fermentation of silage D was more efficient than silage A, B and C. However, the values found in silage A and B are still above than ideal value of 20% as recommended by Chamberlain and Wilkinson (1996).

Silage D had the highest ($P<0.05$) Fleigh Point than other silages, suggesting that silage treated with centrifuged powder of LAB inoculant at level of 3% (v/w) had better fermentative quality as compared to other silages. In addition, Fleigh point found in the present study was lower than the value of 72.83 in alfalfa-maize silage mixture and 62.3 in rice crop residue-based silage as reported by Ozturk *et al*. (2006) and Santoso *et al*. (2012), respectively.

***In vitro* Nutrient Digestibilities**

Table 3 depicts the result of *in vitro* DM, OM and NDF digestibilities of king grass silage treated with liquid and dried LAB inoculants. Treatments of LAB inoculants had no effect on IVDMD and IVOMD values ($P>0.05$). This result was inconsistant with previous finding of Ando *et al*. (2006) that addition of LAB increased the digestibility of DM, OM, and CP of guinea grass silage. On the other hand, IVNDFD of king grass silage was affected by addition of LAB inoculant ($P<0.05$). The IVNDFD increased by average of 44.6% when liquid or dried LAB inoculants was added. Increasing IVNDFD in silages with addition of LAB inoculant (B, C and D) in the present study could be due to lower NDF and ADF contents. It has been reported that activity of cellulase and hemicellulase enzymes was high during ensilage (Yahaya *et al*., 2004).). In other study, Nsereko *et al*. (2008) concluded that some LAB produced ferulate esterase that can increase susceptibility of plant cell walls to enzymatic hydrolysis. Similar results were also reported in other experiments using guinea grass and king grass silages (Ando *et al*., 2006; Santoso *et al*., 2009; Santoso *et al*., 2011).

CONCLUSION

Application of the LAB inoculant as a dry formulation with centrifugation method resulted in faster decline silage pH, decreased NH_3-N and butyric acid concentration and increased Fleigh Point than when the inoculant was applied as a liquid formulation.

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