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# Influence of Starch Type as Substrate Material in Dry Lactic Acid Bacteria Inoculant Preparation on Fermentation Quality and Nutrient Digestibility of King Grass Silage

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**ABSTRACT:** The lactic acid bacteria (LAB) play an important role in silage fermentation and influence silage quality. The population of LAB is usually low and varies with standing crops. Thus, addition of LAB inoculant is needed to improve silage quality. Use of liquid LAB has limited time during storage, thus development of dry LAB inoculant is needed. The aim of this study was to evaluate fermentation quality and nutrient digestibility of king grass silage treated with dry L. plantarum inoculant prepared with different type of starch as substrate material. Five treatments used in this study were A. King grass without LAB inoculant as control; B. King grass + 3% liquid LAB; C. King grass + 3% dry LAB with cassava starch as substrate material; D. King grass + 3% dry LAB with sago starch as susbtrate material; E. King grass + 3% dry LAB with combination of cassava and sago starches as susbtrate material. About 1.5 kg of silage materials were packed into plastic silos and tied with a string. Three replicate silos were prepared for each treatment and stored in room temperature for 30 d. The results showed that the number of LAB in dry inoculants varied from $2.6 \times 10^7$ to $4.0 \times 10^7$ cfu/g. Addition of dry LAB inoculant containing cassava starch (C) significantly enhanced ($P<0.01$) lactic acid concentration. Otherwise, this treatment had the lowest N-NH3 concentration compared to other treatments. In vitro dry matter and organic matter were higher ($P<0.01$) in silages treated with liquid and dry LAB inoculant (B, C, D, E) compared to control silage (A). However, silage treated with dry LAB inoculant containing cassava and sago starches (C, D) had higher ($P<0.01$) in vitro organic matter than liquid LAB inoculant (B). It was concluded that dry LAB inoculant prepared with cassava starch as substrate material had the best quality fermentation of king grass silage than other LAB inoculants.

**Keywords:** Silage, Lactic acid bacteria, King grass, Starch, In vitro

## 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 LAB play an important role in silage fermentation and influence silage quality. Under natural circumtances 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). Inoculating silages with lactic acid bacteria (LAB) has improved silage fermentation (Bureenok *et al*., 2006; Santoso *et al*., 2011; Santoso *et al*., 2012). Inoculation with these microbes has increased the rate and extent of lactic acid production in silages, decreased proteolysis, and decreased the production of volatile organic acids (Santoso *et al*., 2011; Santoso *et al*., 2012).

In the previous studies, most researchers used liquid BAL in silage preparation. However,

the use of liquid inoculant has a limited in the time of storage so that it becomes a problem when applied to farmers. Hariadi *et al*. (2013) concluded that addition of dry LAB inoculant prepared by centrifugation method in king grass silage resulted a good fermentation as compared to silage added with dry LAB inoculant prepared by freeze dried method. The aim of this study was to evaluate fermentation quality and nutrient digestibility of king grass silage treated with dry *L. plantarum* inoculant prepared with different type of starch as substrate material.

## MATERIAL 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.

### Preparation of Liquid LAB Inoculant

Preparation of liquid LAB inoculant according to modified of Bureenok *et al.* (2006) procedure as previously described by Santoso *et al.* (2009) and Santoso *et al.* (2012). The 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 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, extract was used as source of LAB. The number of LAB in the extract 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 Dry LAB Inoculant

Preparation of dry LAB inoculant based on modified of Jeni *et al.* (2010) procedure. Briefly, dry cassava and sago starches were strilized using autoclace at pressure of 1 atm, temperature of 121 C for 2 hours. One litre of fresh LAB inoculant was centrifugated at 1000 rpm for 90 minutes. About 50 ml of supernatant was mixed with 1 kg of sterilized cassava or sago starches.

### Silage Preparation and Treaments

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 5 treatments were as follows (A) King grass without LAB inoculant as control; (B) King grass + 3% liquid LAB; (C) King grass + 3% dry LAB with cassava starch as substrate material; (D) King grass + 3% dry LAB with sago starch as susbtrate material; (E) King grass + 3% dry LAB with combination of cassava and sago starches as susbtrate material. The liquid LAB was 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 × 106 cfu/g of fresh forage. About 1.5 kg of silage materials were packed into plastic silos and tied with a string. Each treatment was prepared in triplicate and the silos were stored in room temperature for 30 days.

### Chemical Analyses

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 NH3-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. 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 and Summerson (1941); Chaney and Marbach (1962), respectively.

**Statistical Analyses**

The 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

The initial LAB population in liquid inoculant, dry inoculant with cassava starch, dry inoculant with sago starch, and dry inoculant with combination of cassava and sago starches were $3 \times 10^6$ cfu/ml, $2.6 \times 10^7$ cfu/g, $4.2 \times 10^7$ cfu/g and $4.0 \times 10^7$ cfu/g, respectively. This population is consistant with the minimum target at the intial LAB population approximately 106 cfu/g. Chemical composition of king grass silages are presented in Table 1.

**Table 1.** Chemical composition of king grass silages

| | Treatments | | | | | SEM | P |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | | |
| Dry matter | $20.4^{c}$ | $22.0^{bc}$ | $24.4^{a}$ | $22.9^{ab}$ | $23.1^{ab}$ | 0.6 | * |
| Organic matter | $89.9^{b}$ | $92.7^{a}$ | $92.5^{a}$ | $90.8^{b}$ | $94.0^{a}$ | 0.5 | ** |
| Crude Protein | $8.7^{b}$ | $10.2^{a}$ | $10.8^{a}$ | $9.8^{ab}$ | $9.9^{ab}$ | 0.4 | * |
| NDF | 73.5 | 71.7 | 71.4 | 73.1 | 72.7 | 0.7 | NS |
| ADF | 47.3 | 46.8 | 45.3 | 45.7 | 46.2 | 0.8 | NS |

Means in the same row with different superscript differ significantly (* P<0.05; ** P<0.01; NS: Non Significant)

Dry matter, OM and CP contents of silage were significantly affected by addition of inoculant LAB. Silage C had the higher DM concent than other silages coud be due to cassava starch is hygroscopic and contain more amylopectin chains, thus it is able to absorb more water. The DM content of all silages were lower than the value of 30% for ideal silage as suggested by Chamberlain and Wilkinson (1996). Silage C also contains a higher crude protein than other silage. This indicates that silage C has low degradation of CP to amino acids and ammonia during ensiling. The NDF and ADF contents in silage with addition of LAB inoculants were sligtly lower 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 other experiments using guinea grass and king grass silages (Ando *et al*., 2006; Santoso *et al*., 2009 and Santoso *et al*., 2011).

**Table 2.** Fermentation characteristic of king grass silages ensiled with liquid or dry LAB inoculants additio

| | Treatments | | | | | SEM | P |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | | |
| Lactic acid (g/kg DM) | 25.6$^{c}$ | 52.6$^{b}$ | 105.3$^{a}$ | 52.0$^{b}$ | 52.5$^{b}$ | 3.1 | ** |
| $NH_3$-N (g/kg Total N) | 137.3$^{a}$ | 92.1$^{bc}$ | 72.8$^{c}$ | 109.7$^{b}$ | 86.1$^{bc}$ | 16.5 | ** |
| Acetic acid (g/kg DM) | 45.1$^{a}$ | 35.0$^{bc}$ | 32.3$^{c}$ | 38.8$^{b}$ | 38.9$^{b}$ | 1.4 | ** |
| Propionic acid (g/kg DM) | 4.5 | 5.2 | 4.6 | 5.2 | 4.3 | 1.0 | NS |
| Butyric acid (g/kg DM) | 12.7$^{a}$ | 6.8$^{b}$ | 4.9$^{c}$ | 7.1$^{b}$ | 6.0$^{bc}$ | 0.4 | ** |
| Total VFA (g/kg DM) | 62.4$^{a}$ | 47.1$^{bc}$ | 41.9$^{c}$ | 51.3$^{b}$ | 49.3$^{b}$ | 1.7 | ** |

Means in the same row with different superscript differ significantly (** P<0.01); NS: Non Significant

Silage treated with dry or liquid LAB inoculants (B, C, D, E) had higher (P<0.01) lactic acid concentration than control silage (A). Meanwhile, concentrations of $NH_3$-N, acetic acid, butyric acid and total VFA were lower (P<0.01) than control silage. Silage treated with dry LAB prepared by cassava starch (C) had the highest lactic acid concentration and the lowest concentrations of $NH_3$-N, acetic acid, butyric acid and total VFA. The concentration of lactic acid in silage C was in the ideal range of lactic acid concentration from 80 to 120 g/kg DM. 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 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 and Wilkinson, 1996). Based on $NH_3$-N concentration, all silages 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 and Wilkinson, 1996). Based on data in Table 1, fermentation in silage was more efficient than other silage.

**Table 3.** In vitro dry matter and organic matter digestibility (%) of king grass silages ensiled with liquid or dry LAB inoculants addition

| | Treatments | | | | | SEM | P |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | | |
| IVDMD | 46.3b | 51.7a | 52.1a | 51.9a | 53.1a | 0.6 | ** |
| IVOMD | 52.7c | 57.9b | 61.8a | 63.0a | 59.7ab | 0.5 | ** |

Means in the same row with different superscript differ significantly (** P<0.01).

Addition of both liquid and dry inoculants increased (P<0.01) in vitro dry matter and organic matter digestibility as compared control silage. Increasing IVOMD in silages with addition of LAB inoculant in the present study could be due to the slightly lower NDF and ADF contents. This result was supported by previous study by Ando *et al*. (2006) that addition of LAB increased the digestibility of DM, OM and CP of guinea grass silage. Similar results has been repoorted by Santoso *et al*. (2014) that addition of epiphytic LAB in rice straw-based silage increased in vitro organic matter digestibility. When compared to control silage, the IVDMD and IVOMD in silage treated with LAB increased by average of 12.7% and 15.0%, respectively.

## CONCLUSION

Addition of liquid or dry LAB inoculants increased lactic acid production, in vitro digestibility of dry matter and organic matter, otherwise decreased ammonia N, acetic and butyric acids, and total VFA. King grass ensiled with addition of dry LAB inoculant with cassava as substrate had the best fermentation quality as compared to other treatments.

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