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Fermentation quality of *Pennisetum purpureum* cv. Mott ensiled with *Lactobacillus plantarum* and sugarcane molasses in tropic

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Abstract. Fermentation quality of *Pennisetum purpureum* (Schumach) cv. Mott ensiled with *Lactobacillus plantarum* [(Orla-Jensen) Bergey et al.] and sugarcane molasses in tropical condition was evaluated. Approximately 100 g portion of wilted material chopped into 20 mm lengths packed into plastic film bags and sealed with a vacuum sealer. T0 was untreated silage, T1 silage with 50 µL *L. plantarum* without molasses, and T2, T3, and T4 treated silage with 50 µL *L. plantarum* and 1 %, 2 %, and 3 % molasses respectively. The bag silos were stored in room temperature (average 25 °C) for 30 d of incubation. This study showed the chemical characteristics were not significantly differ among treatments, eventhough T3 and T4 showed higher organic matter (OM) and water soluble carbohydrate (WSC) contents, meanwhile the highest crude protein (CP) was reached in T3. In general, molasses increased population of lactic acid bacteria (LAB) and decreased pathogen (coliform and aerob bacteria). The favour pH for silages (< 4) were only reached in addition 2 % and 3 % of molasses. It could be concluded that in tropical condition, *L. plantarum* it self could not improve silage quality. Addition 1 % to 3 % molasses improved silage quality, so that molasses were needed to improve fermentation quality of the grass silage with *L. plantarum* inoculation.

Keywords: Dwarf elephant grass, *Lactobacillus plantarum* FCC 123, lactic acid bacteria (LAB), silage quality.

1. Introduction

Dwarf elephant grass (*Pennisetum purpureum* (Schumach) cv. Mott) is one cultivar of elephant grass (napier grass, Uganda grass, barber grass) (*Pennisetum purpureum* Schumach) that has high production, nutrient quality, and palatable for ruminants. Their nutritional quality is higher than king grass (*Pennisetum purpureum* cv King Grass). It's crude protein content, especially the leaves reaches 14 % with 70 % digestibility. *P. purpureum* cv. Mott could be planted on different environment, fertilizing responsive, and curenly wide cultivated in Indonesia. It's growth is very fast with cutting intervals between 30 d to 40 d in the rainy season and could be used as cut grass, pasture grass, or silage [1].



In general, their morphology of trunk is short with a shorter distance compared to *P. purpureum*. Their height ranges from 1 m to 2 m with leaves are limp but grow thick, sprout and clump, the trunk is hairy. Stem texture generally is slightly softer than elephant grass causes dwarf elephant grass to be preferred by ruminants, especially dairy cattle [2].

The results analysis of the Indonesian Animal Feed Quality Testing Center (*Balai Pengujian Mutu Pakan Ternak* - BPMPT) in 2012 showed that *P. purpureum* cv. Mott contains 13.22 % crude protein. This protein level generally is higher than the content of *P. purpureum*, according to Ferreira et. al. [3] the crude protein content of *P. purpureum* was only 5.51 %. Highly crude protein levels based on the analysis of BPMPT and according to Syarifuddin [1] must be maintained. On the other hand highly crude protein content in silage making will be an obstacle because protein is a buffer in the ensilage process, so pH value difficult to decline and another microorganisms as competitor will grow together with lactic acid bacteria (LAB). Another problem, sugar levels of tropical elephant grass is generally poor, so it must be improved to obtain a good quality silage. This study will observe the role of molasses in *P. purpureum* cv. Mott silage making as an additive in the process of influence pH values, production of organic acids, and population of microorganisms. pH values, organic acids content especially lactic acid in silage, and microorganisms composition especially LAB (lactic acid bacteria) populations are important indicators of fermentation quality that must be observed.

2. Materials and methods

2.1. Materials

P. purpureum cv. Mott was obtained from grass field. The condition was 40 d, has cut at 5 cm over the ground, and 2 d wilted. It was packed into plastic bags without sealing and then chopped into 20 mm lengths. *Lactobacillus plantarum* FCC 123, was a commercial LAB strain that have long experience been used for silage making.

2.2. Methods

2.2.1. Silage preparation. Silage were prepared in a small-scale system of fermentation [4], packed into plastic film bags (KRIS BR 2205, 22 × 500 mL) and sealed with a vacuum sealer machine (KRIS VS 200). The silos were treated with *L. plantarum* FCC 123 and sugar cane molasses. T0 was untreated silage, T1 was untreated silage with 50 µL *L. plantarum*. T2 was silage with 50 µL *L. plantarum* and 1 % molasses, T3 was silage with 50 µL *L. plantarum* and 2 % molasses, T4 was silage with 50 µL *L. plantarum* and 3 % molasses. The bag silos were stored in room temperature (average 25 °C) for 30 d of incubation

2.2.2. Chemical analysis. Samples were dried in forced-air oven at 65 °C for 48 h and ground to pass a 1 mm screen with a Grinder-mixer (Panasonic MX-AC400, Japan). Contents of DM (dry matter), OM (organic matter), CP (crude protein), EE (ether extract), and CF (crude fiber) were analyzed according to methods 934.01, 942.05, 976.05, 920.39, and 962.09 respectively, of AOAC [5]. Total water soluble carbohydrate (WSC) including glucose, sucrose, and fructose were determined with HPLC (Jasco). The analytical condition were as follows: column, shodex sugar SC1011 (8.0 mm × 30 cm, Shoko); oven temperature, 80 °C; mobile phase, water; detector, 1.0 mL min⁻¹; detector, Jasco RI-1530.

2.2.3. Microbial analysis. Samples (10 g) were blended with 90 mL of sterilized water and then serially diluted in 10⁻¹ to 10⁻⁹. The total lactic acid bacteria (LAB) was measured with plate count on lactobacilli deMan Rogosa Sharp agar (MRS; Difco) incubated at 30 °C for 2 d under anaerobic cultivation (Mitsubishi anaerob box with anaerobic gen kit, Oxoid). Coliforms bacteria was counted on blue light broth agar (BLB; Nissui Ltd.) incubated at 30 °C for 24 h. Yeast and mold were counted on potato dextrose agar (PDA; Difco) and incubated at 30 °C for 24 h. Yeast was distinguished from molds by appearance of morphological colony and cell forming under microscopic observation. Bacilli

was distinguished with aerobic bacteria with incubating at 75 °C for 15 min before incubating in nutrient agar (NA; Difco) at 30 °C for 24 h under aerobic condition. Colonies was counted as viable numbers of microorganisms in colony-forming unit per gram of fresh matter (FM).

2.2.4. Organic acid analysis. Organic acid content were determined from cold-water extracts. Wet material (10 g) was homogenized with 90 mL of sterile distilled water [6]. The pH was measured with a glass electrode pH meter (Echem E-512 ex GR Sci.), and ammonia-N was determined by steam distillation of filtrates [7]. The organic acid contents were measured by HPLC (Simadzu LC 20AD, Japan) according to the methods describes by Nour et al [8]. The analytical condition were as follows: column, C-18 (4.6 mm × 15 cm); oven temperature 40 °C; mobile phase, fosphat solution 50mM; UVdetector (Simadzu, Japan).

3. Result and discussion

3.1. Chemical composition

In general, addition of molasses to treated *P. purpureum* cv. Mott grass silage increase the quality of silages. It was indicated by increasing of OM, CP, EE, WSC, and decreasing of CF content and total WSC. Crude fiber decline in molasses addition indicated that molasses can support the bacteria to ferment and convert some fiber into organic acid.

In line with Bilal [9] study that stated addition of 3 % molasses was found to be the best for *P. purpureum* cv. Mott fermentation. The similar results has been reported by Cai et al., [4] that except a little decreasing of water soluble carbohydrate (WSC) and maintain crude fiber, BAL inoculants not affected nutrients composition of silage on mini silo. The results was also strengtened by Yang et al., [10] that nutrients composition of silage is not different with or without inoculation of *L. buchneri*. The use of inoculant in WSC fermentation is more focused on increasing the speed of formation of lactic acid and decreasing the pH value (11). Conversely, in insoluble polysaccharide fermentation the role of inoculants is intended to suppress the growth of acid-producing bacteria, interrupting the feed and allowing the methanogenic population time to reduce the fatty acid concentration and thus raise the pH (12, 13). The silages nutrients composition of this this study is given on table 1.

Table 1. Chemicals composition

| Treatments | DM (%) | Chemical composition (% DM) | | | | |
|------------|--------|-----------------------------|--------------|-------------|--------------|-----------|
| | | OM | CP | EE | CF | Total WSC |
| T0 | 11.40 | 81.29 ± 0.44 | 13.30 ± 0.26 | 2.27 ± 0.14 | 27.06 ± 0.23 | 1.25 |
| T1 | 10.35 | 81.44 ± 0.35 | 14.40 ± 0.21 | 2.38 ± 0.19 | 26.13 ± 0.27 | 1.15 |
| T2 | 09.35 | 82.38 ± 0.48 | 15.43 ± 0.29 | 2.44 ± 0.30 | 25.30 ± 0.07 | 1.55 |
| T3 | 10.06 | 82.99 ± 0.34 | 16.66 ± 0.11 | 2.58 ± 0.33 | 24.38 ± 0.37 | 1.65 |
| T4 | 09.24 | 83.21 ± 0.14 | 14.74 ± 0.30 | 2.49 ± 0.10 | 22.72 ± 0.20 | 1.75 |

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fibre; WSC, water soluble carbohydrate; T0, untreated silage; T1, untreated silage with *L. plantarum*; T2, silage with *L. plantarum* and 1% molasses; T3, silage with *L. plantarum* and 2 % molasses; T4, silage with *L. plantarum* and 3 % molasses

Forages in the tropics generally have low DM and WSC. Tropical *P. purpureum* grass according to Ferreira et al., [3] contains 26.38 % dry matter, while Mühlbach [14] says that elephant grass at 60 d of harvest only contains 14 % dry matter, while at the age of 105 d it contains 19 % dry matter ingredients. The low DM content of *P. purpureum* it will lead to bad silage fermentation production, therefore need a special treatment to reduce water content and N ammonia content during ensilage, especially in rainy season. Sugar content of tropical grass is generally low (15). WSC content less than 4 % is a problem that must be controlled to obtain good quality silage in the tropics. According to Cao et al., [16] the use of molasses can provide WSC to be fermented into organic acids, produce a lower

pH value of silage, higher lactic acid content, produce a higher water soluble carbohydrate residue, the level of N-ammonia is lower, and decreases the rate of loss of dry matter compared to silage without the addition of molasses.

3.2. Microorganisms composition

Silage quality are fated by existency of homo-fermentative LAB and chemical composition of materials. Several studies reported that LAB was the dominant microbes in silage [17, 10].

Table 2. Microbial compositions (CFU g⁻¹ FM)

| Treatments | Lactic acid bacteria | Coliform | Aerob bacteria | Clostridia | Yeast | Mold |
|------------|----------------------|-------------------|-------------------|------------|-------|------|
| T0 | 2×10^5 | 1.4×10^6 | 2.1×10^7 | nd | nd | nd |
| T1 | 1.4×10^6 | 1.1×10^6 | 1.9×10^7 | nd | nd | nd |
| T2 | 2.0×10^6 | 2.3×10^5 | 1.6×10^6 | nd | nd | nd |
| T3 | 2.7×10^6 | 1.4×10^5 | 2.0×10^5 | nd | nd | nd |
| T4 | 4.0×10^6 | 1.5×10^5 | 1.9×10^5 | nd | nd | nd |

CFU, colony forming unit ; FM, fresh matter; T0, untreated silage; T1, untreated silage with *L. plantarum*; T2, silage with *L. plantarum* and 1 % molasses; T3, silage with *L. plantarum* and 2 % molasses; T4, silage with *L. plantarum* and 3 % molasses; nd, not detected

The addition of *L. plantarum* with or without molasses both increase the population of LAB. LAB play an important role in silage fermentation, and LAB species, number and their characteristics have become a significant factor in predicting the adequacy of silage fermentation. Several studies reported that LAB was the dominant microbes in silage [18, 7, 10, 17]. When LAB, especially lactobacilli, reaches at least 10^5 cfu g⁻¹ of FM, silage can be well preserved. As shown in table 1, the LAB counts with 10^6 present in these study, suggest that high-quality silage.

Molasses and *L. plantarum* slightly decrease the coliform and anaerob bacteria. It has ensured that *L. plantarum* along with molasses could well prepare to *P. purpureum* cv. Mott ensilage. Although coliform and aerob bacteria were unexpectedly still high compare with Napasirth et al. [18], but the number of aerob bacteria equal with Sepala et al.[19]. Clostridia, yeast, and mold are not detected in the silage.

3.3. Organic acid content

The fermentation characteristic of the tropical Elephant grass silages are presented in table 3. Molasses addition improved the nutritive values of the silages by decreasing the pH, whilst 2 % and 3 % that support to reach favorable silages pH (about 4). The pH 4 were also reached by Ferreira et al. [3]. The pH of an ensiled sample is a measure of its acidity, but is also affected by the buffering capacity of the crop.

The favorable pH reachment also were supported by high lactic acid production in T2, T3, and T4 treatments. Lactid acid should be the primary acid in good silage. This acid is stronger than the other acids in silage (acetic, propionic, and butyric), and therefore is usually responsible for most of the drop in silage pH. Further, fermentations that produce lactic acid result in the lowest losses of DM and energy from the crop.

High acetic acid also produced in this silages. Extremely wet silages (< 25 % FM) of the raw material in this study could prolonged fermentations (due to high buffering capacity) than result in silages with high concentrations of acetic acid (> 3 % to 4 % of FM). However, production of acetic acid with a high concentration of acetic acid does not appear to cause negative effects on animal intake.

Table 3. pH value and organic acid content

| Treatments | Moisture (%) | pH | Organic acid (% FM) | | | | VBN (g kg ⁻¹ FM) |
|------------|--------------|------|---------------------|-------------|----------------|----------------|-----------------------------|
| | | | Lactic acid | Acetic acid | Propionic acid | n-butyric acid | |
| T0 | 88.63 | 5.65 | 3.80 ± 0.47 | 1.92 ± 0.43 | nd | 0.36 ± 0.05 | 0.79 ± 0.04 |
| T1 | 89.65 | 6.27 | 3.85 ± 0.39 | 2.06 ± 0.42 | nd | 0.45 ± 0.08 | 0.83 ± 0.05 |
| T2 | 90.65 | 5.58 | 5.52 ± 0.38 | 3.26 ± 0.19 | nd | 0.46 ± 0.11 | 0.85 ± 0.09 |
| T3 | 89.94 | 4.02 | 7.81 ± 0.53 | 3.02 ± 0.38 | nd | 0.43 ± 0.08 | 0.85 ± 0.03 |
| T4 | 90.76 | 3.68 | 8.29 ± 0.64 | 3.26 ± 0.27 | nd | 0.44 ± 0.06 | 0.89 ± 0.01 |

VBN, volatile base nitrogen; FM, fresh matter; T0, untreated silage; T1, untreated silage with *L. plantarum*; T2, silage with *L. plantarum* and 1 % molasses; T3, silage with *L. plantarum* and 2 % molasses; T4, silage with *L. plantarum* and 3 % molasses; nd, not detected.

A high concentration of butyric acid (> 0.5 % of DM) in silages usually indicates that the silage has undergone clostridial fermentation, which is one of the poorest fermentations. In this study, clostridia was not detected but butyric acid contents were almost reach the threshold. Butyric acid was not detected on TMR silages in previous study when moisture content only 55 % to 71 % [20]. Silages high in butyric acid are usually low in nutritive value and have higher ADF and NDF levels because many of the soluble nutrients have been degraded.

The organic acid contents in this study showed much higher than the others, this is strongly assumed to be partly associated with high water content of the grass. One other explanation could be due to its higher value of protein contents.

4. Conclusion

The use of *L. plantarum* it self has not affected the *P. purpureum* cv. Mott silage quality in tropical condition. Addition 1 % to 3 % molasses could improve fermentation process; maintain chemical composition, increase LAB population and lactic acid content, decline pH value, and reduce damage microorganisms. The best of it's silage quality was determined from inoculation of *L. plantarum* and 3 % molasses.

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