The Effect of *Lactobacillus plantarum* Addition and Fermentation Periods on Nutritive Value Dwarf Elephant Grass (*Pennisetum purpureum* cv Mott) Silage

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**Abstract.** This research aimed to examine the effect of different *Lactobacillus plantarum* addition and fermentation periods on its physical quality, pH, nutritive value (dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), extract ether (EE)). This research also to find out the best treatments of dwarf elephant grass silage by *Lactobacillus plantarum* addition with different fermentation periods. Completely Randomized Factorial Design was used in this experiment, with two factor ie. *Lactobacillus plantarum* inclusion (0, 0.3; 0.6; 0.9 %) and fermentation periods (0, 7, 14, 21 days). Each treatment was replicated three times. Addition 6 % molasses of fresh weight grass was employed in all the treatment. The result indicated that different of *Lactobacillus plantarum* addition, fermentation periods and their interaction were highly significant effect on pH, OM, CF and EE value (p<0.01). Interaction of two factors also highly significant influence to DM and CP content of dwarf elephant grass silage. *Lactobacillus plantarum* addition factor has highly significant effect on CP content and fermentation period was a significant effect on CP value of dwarf elephant grass silage. Physical quality of dwarf elephant grass with various *Lactobacillus plantarum* addition and fermentation periods shown a good quality of silage with fresh and acidic odor, good texture, a little bit browny color, and no fungi. Average pH of silage was around 3.90 – 4.30 after incubation 7 – 21 days. Average of nutritive value dwarf elephant grass silage were 11.55 – 13.15 % DM, 81.13- 82.32 % OM, 10.48 – 12.00 % CP, 22.31 – 25.27 % CF and 1.21 – 2.98% EE. It can be concluded that *Lactobacillus plantarum* addition can faster ensilage fermentation and the best silage was on treatment 0.6 % *Lactobacillus plantarum* addition and 7 days fermentation periods.

**1. Introduction**

Scarcity of forage in dry season already no doubt that become a common problem for ruminant farmer. A viable option to obtain forage during dry season is by making a silage in wet season. Silage is forage preservation method by decreasing the pH within minimum fermentation period to maintenance the nutritive value of forage as much as can. [1] stated that successful silage fermentation depends on achieving both anaerobic conditions and a low pH. The low pH is usually accomplished through the fermentation of sugar in the crop to lactic acid by lactic acid bacteria, which decreases plant enzyme activity, prevent the proliferation of detrimental anaerobic microorganism, especially Clostridia and Enterobacteria.

Dwarf elephant grass (*Pennisetum purpureum* cv Mott) was popular grass in current time, especially at East Java, Indonesia because of its productivity and palatability. [2] stated that Mott grass is high
quality forage that maintain its quality over long regrowth interval, has potential to provide fodder in one slump period when other traditional fodders are inadequate, and if preserved as silage during that time and during second slump period, quality fodder supply can be ensured. According to [3] dwarf elephant grass has low concentration of fermentable carbohydrates, it supported with [4] water soluble carbohydrate (WSC) of dwarf elephant grass only 4.1 %. As a result, fermentation process is difficult, and produce a poor quality of silage [5]. Thus, ensiling practices such as wilting and use of additives to increase dry matter concentration may be used to improve the fermentation process and silage quality [3] and [6]. Using molasses as additive can increase the amount of fermentation end products due to fermentation of the available sugars. Molasses enrich the fresh material with carbohydrates and fill the gaseous pores thereby reducing the influx of oxygen in the silage [7].

Not only molasses that can improve quality of silage, [8] noted that application of lactic acid bacteria (LAB) also a common practice in the United States and Europe. Homofermentative LAB such as Lactobacillus plantarum, Enterococcus faecium and Pediococcus spp are used, with the goal to faster fermentation, lower pH, raised lactate: acetate ratio, lower ethanol and ammonia nitrogen concentration and improve DM recovery [9]. In addition to improved silage characteristics, LAB inoculants may acts as probiotics in the rumen [10]. Improved animal performance has been demonstrated even when the silage inoculant did not affect quality of silage fermentation [11].

According that, this research aimed to examine the effect of different Lactobacillus plantarum addition with different fermentation periods on its physical quality, pH, and nutritive value (dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), extract ether (EE). This research also to find out the best treatments of dwarf elephant grass silage by Lactobacillus plantarum addition with different fermentation periods.

2. Material and methods
This research was carry out at Animal Feed and Nutrition Laboratory Faculty of Animal Science, University of Brawijaya. Dwarf elephant grass were harvest at 50 days, chopped into 4 - 5 cm of the length and then wilted for 24 – 48 hours to decrease the water content of dwarf elephant grass. After wilted, it already for silage making. Before adding Lactobacillus plantarum (1.85 x 10^7 cfu per ml) it add with 6 % molasses from fresh weight of grass. The mixture were put in polypropylene plastic bag, compacted to expel the air using vacuum tool, then seal with rope and black tape. After that, it put in plastic bag again and seal again. It was repeat ones again for the same mixture and it done for all the treatments. The silage was incubate in Fermentation Room according to fermentation periods of silage. Completely Randomized Factorial Design was used in this experiment, with two factors ie. Lactobacillus plantarum inclusion (0, 0.3; 0.6; 0.9 %) and fermentation periods (0, 7, 14, 21 days). Each treatment was replicate three times. Parameters of this experiment were its physical quality, pH and nutritive value (DM, OM, CP, CF, EE). pH of silage measured with pH meter. Nutritive value of silage was determine by proximate analysis according [12]. The collected data were analyze by two-way ANOVA with the fixed effects of Lactobacillus plantarum, ensilage time (fermentation periods) and their interaction using GLM procedure. DMRT was used to compare the differences between the treatment means and the significance was declared at p < 0.05 or p<0.01.

3. Results and discussion
Physical quality of dwarf elephant grass with various Lactobacillus plantarum addition and fermentation periods shown a good quality of silage with fresh and acidic odor, good texture, a little bit browny color, and no fungi. The colour of silage was a little bit browny because of molasses addition for all the treatments. The texture of dwarf elephant grass was smoother in 21 days fermentation, but still in chopped grass form. The odor of silage with different of Lactobacillus plantarum addition give a slightly different of acidic odor, except on 0 day, because there was not fermented yet. Average of pH and nutritive value of dwarf elephant grass silage with various Lactobacillus plantarum addition and fermentation periods are shown in Table 1 to 6.
Table 1. Average pH of dwarf elephant grass silage with various Lactobacillus plantarum addition (%) and fermentation periods

| Lactobacillus plantarum addition (%) | Fermentation periods (days) | Average |
|-------------------------------------|-----------------------------|---------|
| 0                                  | 0  | 7  | 14 | 21 |         |
| 0                                  | 6.10±0.17b | 3.90±0.20a | 3.90±0.00a | 3.97±0.12a | 4.47±3.27a |
| 0.3                                | 6.33±0.06b | 3.93±0.06a | 3.97±0.06a | 4.33±0.06a | 4.64±3.43b |
| 0.6                                | 6.50±0.10b | 4.10±0.10a | 4.13±0.06a | 4.30±0.00a | 4.76±3.49c |
| 0.9                                | 6.80±0.10a | 4.03±0.06a | 4.07±0.12a | 4.17±0.06a | 4.77±4.07c |
| Average                            | 6.43±0.88c | 3.99±0.27a | 4.02±0.31a | 4.19±0.49b |         |

Values with different superscripts within a row each factor differ highly significant (p<0.01)

The result indicated that different of Lactobacillus plantarum addition, fermentation periods and their interaction were highly significant effect on pH, OM, CF and EE value (p<0.01). Interaction of two factors also highly significant influence to DM, CP content of dwarf elephant grass silage (p<0.01). Lactobacillus plantarum addition factor has highly significant effect on CP content (p<0.01) and fermentation period was a significant effect on CP value of dwarf elephant grass silage (p<0.05).

Here the average pH of dwarf elephant grass with various Lactobacillus plantarum addition (%) and fermentation periods were shown at Table 1.

According to Table 1, the addition of molasses and Lactobacillus plantarum on dwarf elephant grass silage make faster decreasing of pH, on 7 days the pH already on 3.90 – 4.10. All the treatment almost have the same value, because of addition 6 % molasses on each treatment, including on P0 without Lactobacillus plantarum inclusion. Addition of molasses can increase WSC of dwarf elephant grass and support the growth of Lactobacillus plantarum then pH of silage decreased faster. It was supported [7] Molasses enrich the fresh material with carbohydrates and fill the gaseous pores thereby reducing the influx of oxygen in the silage. Average pH value of this experiment are 3.90-4.30; slightly higher with the result of [2], which is the pH of dwarf elephant grass silage (45 days stage of cut) with adding 5 % molasses between 4.01 – 4.08, but already fulfill the pH of good silage criteria. Attainment of low pH is one of the important determinants for silage fermentation quality [1] and [17]. DM and OM of dwarf elephant grass silage with various Lactobacillus plantarum addition (%) and fermentation periods are shown in Table 2 and 3.

Table 2. Dry matter (DM) of dwarf elephant grass silage with various Lactobacillus plantarum addition (%) and fermentation periods

| Lactobacillus plantarum addition (%) | Fermentation periods (days) | Average |
|-------------------------------------|-----------------------------|---------|
| 0                                  | 0  | 7  | 14 | 21 |         |
| 0                                  | 12.47±0.12a | 12.14±0.36a | 12.38±0.70a | 13.45±1.65b | 12.61±1.74 |
| 0.3                                | 12.02±0.73a | 11.93±0.44a | 12.64±0.11a | 11.26±0.47a | 11.96±1.70 |
| 0.6                                | 12.20±0.33a | 12.81±0.02b | 12.28±0.20a | 11.74±0.26a | 12.26±1.32 |
| 0.9                                | 13.15±0.6b | 11.78±0.06a | 11.55±0.50a | 11.76±0.85a | 12.06±2.20 |
| Average                            | 12.46±1.48 | 12.17±1.36 | 12.21±1.40 | 12.05±2.89 |         |

Values with different superscripts within a row each factor differ highly significant (p<0.01)
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According to [13] that there was a positive relationship between RAC with pH. RAC was needed by LAB (lactic acid bacteria) to decrease pH of silage to become 3.5 [14], inhibit the growth of the detrimental anaerobic microorganism [15] and hydrolysis of plant proteins by plant enzymes [16]. [17] stated that silage additives is using at ensilage to ensure the fermentation process to produce well preserved silages, reduce ensiling losses and improve aerobic stability of silages during the feed-out period. It was proved on DM content (Table 2) of all the treatments, which is no differ on fermentation periods and Lactobacillus plantarum inclusion, except on both interaction factors with average DM content of the silage are 11.26 – 13.45 %. This result was lower with [2], which is the DM content of dwarf elephant grass silage (45 days stage of cut) with adding 5 % molasses between 16.80 – 17.67 %. It can be happened because of different soil fertility, varieties of dwarf elephant grass, stage of cut, climate etc.

OM content (Table 3) of all the treatments were highly significant (p < 0.01) influenced by different of Lactobacillus plantarum addition, fermentation periods and their interaction. Average OM content of the silage are 81.13 – 83.38 %. This result was a little bit lower with [18], which is the OM content of dwarf elephant grass silage with adding 20 % rice bran was 83.64%.

There was slightly improvement in CP content (Table 4) due to Lactobacillus plantarum inclusion when compared with control (no Lactobacillus plantarum inclusion). The possible reason of increasing CP content during ensilage may be a proteolytic activity during fermentation produces NH₃ but due the efficient fermentation and early stability of silage, this proteolysis activity is inhibited and the produced NH₃ helps in getting the aerobic stability because of its fungicidal properties [19]. Another reason may be protein sparing activity, as by day 7, pH has been reduced sufficiently to inactive the plant proteolytic enzymes [20]. Average CP content of this study were 10.48 – 12.00 %. This value was lower compared with [2], which is the CP content of dwarf elephant grass silage (45 days stage of cut) with adding 5 % molasses between 13.95 – 14.16 %. CP content of this silage were in line with the result [18] which were the CP content of dwarf elephant grass silage with adding 20 % rice bran was 11.35 % and dwarf elephant grass silage no addition was 9.89 %. The difference of CP content dwarf elephant grass silage could be influenced by a lot factor, such as cutting age, leaves: stem ratio, soil fertility, varieties of dwarf elephant grass, stage of cut, climate etc.

In brief, CF and EE content of dwarf elephant grass silage (Table 5 and 6) in this experiment have the same trends, increasing fermentation periods resulted in increasing CF and EE value. Although the CF value a little bit higher at 21 days fermentation, texture of silage are more smoother but still in its forms. It means that fiber was fermented, become more in its quantity and will be more easily to degrade by rumen microbe. EE value at 21 days fermentation also higher, it shows good fermentation by lactic acid bacteria to produce lactic acid and decrease pH of silage. CF and EE content of this experiment are lower with the result [18] which is using 20 % rice bran in dwarf elephant grass are 30.32 % and 3.99 %. It shows that CF in this experiment are more easily to degrade by microbe rumen than [18].
Table 4. Crude Protein (CP) of dwarf elephant grass silage with various *Lactobacillus plantarum* addition (%) and fermentation periods

| *Lactobacillus plantarum* addition (%) | Fermentation periods (days) | Average  |
|---------------------------------------|-----------------------------|----------|
|                                       | 0                           | 7        | 14      | 21      |
| 0                                     | 10.48±0.38<sup>a</sup>      | 11.21±0.15<sup>b</sup> | 11.18±0.06<sup>b</sup> | 11.66±0.12<sup>b</sup> |
| 0.3                                   | 11.84±0.13<sup>bc</sup>     | 11.21±0.08<sup>b</sup> | 11.46±0.16<sup>b</sup> | 11.09±0.03<sup>b</sup> |
| 0.6                                   | 11.51±0.05<sup>b</sup>      | 12.00±0.11<sup>bc</sup> | 11.74±0.09<sup>bc</sup> | 11.11±0.06<sup>b</sup> |
| 0.9                                   | 11.51±0.11<sup>b</sup>      | 11.25±0.13<sup>b</sup> | 11.14±0.11<sup>b</sup> | 11.11±0.02<sup>b</sup> |
|                                       | 11.34±1.78<sup>a</sup>     | 11.42±1.17<sup>b</sup> | 11.38±0.83<sup>b</sup> | 11.24±0.84<sup>b</sup> |

Fermentation period was a significant effect on CP value of dwarf elephant grass silage (p<0.05). Values with different superscripts within a row each factor ( *Lactobacillus plantarum* addition and their interaction of two factor) differ highly significant (p < 0.01).

Table 5. Crude fibre (CF) of dwarf elephant grass silage with various *Lactobacillus plantarum* addition (%) and fermentation periods

| *Lactobacillus plantarum* addition (%) | Fermentation periods (days) | Average  |
|---------------------------------------|-----------------------------|----------|
|                                       | 0                           | 7        | 14      | 21      |
| 0                                     | 22.48±0.65<sup>a</sup>      | 22.31±0.03<sup>a</sup> | 23.24±0.44<sup>a</sup> | 25.27±0.29<sup>ab</sup> |
| 0.3                                   | 23.38±0.12<sup>ab</sup>     | 22.57±0.76<sup>a</sup> | 24.48±0.05<sup>bc</sup> | 23.84±0.18<sup>b</sup> |
| 0.6                                   | 23.43±0.73<sup>ab</sup>     | 23.50±0.39<sup>ab</sup> | 24.82±0.26<sup>bc</sup> | 24.02±0.43<sup>ab</sup> |
| 0.9                                   | 24.41±0.41<sup>ab</sup>     | 22.85±0.05<sup>a</sup> | 23.16±0.43<sup>a</sup> | 23.73±0.01<sup>ab</sup> |
|                                       | 23.55±2.45<sup>a</sup>     | 22.81±1.53<sup>bc</sup> | 23.92±2.54<sup>c</sup> | 24.21±2.13<sup>d</sup> |

Values with different superscripts within a row each factor differ highly significant (p<0.01).

Table 6. Ethy extract (EE) of dwarf elephant grass silage with various *Lactobacillus plantarum* addition (%) and fermentation periods

| *Lactobacillus plantarum* addition (%) | Fermentation periods (days) | Average  |
|---------------------------------------|-----------------------------|----------|
|                                       | 0                           | 7        | 14      | 21      |
| 0                                     | 1.22±0.01<sup>a</sup>       | 2.21±0.02<sup>bc</sup> | 2.35±0.09<sup>bc</sup> | 2.98±0.03<sup>c</sup> |
| 0.3                                   | 1.27±0.06<sup>a</sup>       | 1.84±0.17<sup>bc</sup> | 2.37±0.06<sup>bc</sup> | 2.66±0.10<sup>bc</sup> |
| 0.6                                   | 1.21±0.05<sup>a</sup>       | 2.31±0.03<sup>bc</sup> | 2.39±0.08<sup>bc</sup> | 2.82±0.01<sup>c</sup> |
| 0.9                                   | 1.62±0.06<sup>b</sup>       | 2.09±0.03<sup>bc</sup> | 1.95±0.15<sup>bc</sup> | 2.52±0.01<sup>bc</sup> |
|                                       | 1.33±0.58<sup>a</sup>       | 2.11±0.60<sup>bc</sup> | 2.27±0.63<sup>bc</sup> | 2.74±0.59<sup>c</sup> |

Values with different superscripts within a row each factor differ highly significant (p<0.01).

Addition of *Lactobacillus plantarum* in this experiment (Table 5 and 6) also influence the CF and EE content of dwarf elephant grass silage. Increasing level of *Lactobacillus plantarum* inclusion give an impact of a little bit increasing of CF content and decreasing of EE contents. Declining of EE content showed that EE content are needed for *Lactobacillus plantarum* to multiple and produce membran cell.

4. Conclusion
It can be concluded that *Lactobacillus plantarum* addition can faster ensilage fermentation and the best silage was on treatment 0.6 % *Lactobacillus plantarum* addition and 7 days fermentation periods.
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