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Nutrient Content of Napier Grass (*Pennisetum purpureum*) Silage Made with Various Additive and Modified Atmosphere in The Silo

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Abstract. During ensilage, anaerob condition must be controlled. Some methods of modified atmosphere in silo were analyzed to compare ensilage characteristics and silage product. So far, there is not been information on the atmosphere condition in the process of silage production. It encourages the researchers to evaluate the condition of ensilage process of *Pennisetum purpureum* by studying atmosphere modification in the silo and the effect of the usage of various additives in the process of silage production. Elephant grass (*Pennisetum purpureum*), molasses, *L. acidophilus* were used. The study was conducted with a Completely Randomized Design (CRD) 3x2 factorial pattern. Atmosphere modification as the first factor consist of : (A0: silage with compaction (conventional) A1: silage with vacuum method, A2: silage with modified CO₂) and two kinds of silage additives as the second factor (B1: indirect additive (molasses); B2: direct additive (Lactic Acid Bacteria). Each treatment combination was repeated 4 times. The objective of the research was to evaluate changes in nutrient content (protein, crude fiber, gross energy). The data obtained were analyzed by analysis of variance, then continued by Honest Significant Differences (HSD) test. Based on the research results it can be concluded that the optimum ensilage can take place, either by compaction methods (conventional), vacuum and the addition of CO₂. While the addition of molasses additive produces silage with better quality than the addition of *L. Acidophilus* inoculant.

Key words: Modified atmosphere, additives, silage

Abstrak. Kondisi anaerob harus dikontrol selama ensilase. Beberapa metode modifikasi atmosfer silo dianalisa untuk membandingkan ciri dan hasil ensilase. Sejauh ini belum ada informasi kondisi atmosfer dalam proses produksi ensilase. Hal ini mendorong peneliti untuk mengevaluasi kondisi proses ensilase *Pennisetum purpureum* dengan mempelajari modifikasi atmosfer silo dan dampak penggunaan bermacam zat tambahan dalam proses pembuatan ensilase. Rumput gajah (*Pennisetum purpureum*), molase, *L. acidophilus* digunakan dalam penelitian dengan Rancangan Acak Lengkap (RAL) 3x2 pola faktorial. Modifikasi atmosfer sebagai faktor pertama terdiri dari: (A0: silase dengan pemadatan (konvensional), A1: silase dengan metode vakum, A2: silase dengan modifikasi CO₂) dan dua jenis silase tambahan sebagai faktor kedua: B1: tambahan langsung (molase); B2: tambahan langsung (Bakteri Asam Laktat). Setiap kombinasi perlakuan diulang 4 kali. Tujuan penelitian adalah mengevaluasi perubahan dalam kandungan nutrisi (protein, serat kasar, energi kasar). Data yang diperoleh dianalisis dengan analisis variansi, dilanjutkan uji Beda Nyata Jujur (BNJ). Kesimpulan penelitian adalah ensilase optimal tercapai baik dengan metode pemadatan (konvensional), vakum, maupun penambahan CO₂. Sedangkan tambahan molase menghasilkan silase dengan kualitas yang lebih baik daripada dengan *L. Acidophilus* inoculant.

Kata kunci: modifikasi atmosfer, zat tambahan, silase

Introduction

Provision of quality forage in feed sustainability for ruminant livestock farms is still a problem for farmer in Indonesia. This is due to the provision of forage sometimes constrained by the seasons, on the other hand is very abundant in certain seasons. Therefore

to maintain the continuous availability of forage required preservation of adequate technology. Preservation of forage in the form of silage is one attempt to maintain the availability of quality feed to maintain the nutrient value of feed to be used as a buffer stability of livestock production. The principle of making silage is

the forage fermentation by microbes that produce a lot of lactic acid (Ridwan et al., 2005) on condition that occurs in anaerobic conditions. Conventionally to create anaerobic conditions at field scale can be pursued by methods that minimize the compaction of silage material space between particles of feed material in the silo. However the process of respiration in plant cells sometimes still occur, so that although in small quantities would still occur in the decomposition process of silage material. The weaknesses that occur in the conventional process of making silage is less subdued anaerob condition, so it still provides an opportunity to grow aerobic microbes. This condition greatly affects the characteristics of the resulting silage, as reported Kilzilsimsek et al. (2005) that the type of raw material and affect the quality of silage silo are physically and chemically. Speed factor achieving vacuum conditions in the atmosphere largely determines nutritional silo silage. The more oxygen in the silo, causing respiratory process carried out by aerobic bacteria, so the longer it will produce more effluent. The effluent contains dissolved organic materials derived from materials made silage. It is known that the initial packing density affects silage fermentation (Ruppel et al., 1995; Muck and Holmes 2000) but there is little published information about its effect on silage quality. Johnson et al. (2005) reported that in the experiments reported here with vacuum-packed bags, no attempt was made to constrain the herbage during fermentation (as it would be in rigid-walled vessels such as glass), so the packing density decreased over time as gases accumulated. It was apparent that more efficient removal of air at initial packing improved the rate of pH decline and lactate accumulation. However, it was also apparent that increased vacuum settings caused more crushing of herbage and thus greater release of sap.

Several methods of optimization of anaerobic conditions need to be studied, to compare the characteristics of silage and results obtained. So far there has not been sufficient information on the modification of atmospheric conditions in silage making. In addition, to improve the quality of silage required the addition of BAL at the time ensilage inoculum (Bureenok et al., 2006) or the addition of carbohydrate sources fermentable additive. The addition of soluble carbohydrates (WSC = water soluble carbohydrate) can quite quickly be exploited by BAL as nutrients for growth (Ridwan et al., 2005; Kurnianingtyas et al., 2012).

Based on these studies, it was necessary to process conditions ensilage on elephant grass by studying the application of methods of modifying the atmosphere in the silo and the effect of adding various additive in the manufacture of elephant grass silage in terms of protein content, crude fiber and gross energy.

Materials and Methods

Preparation materials. Samples of fresh cut napier grass at the age of 40 days, then cut with a chopper with a length of 3-5 cm, then wilted for 24 hour at room temperature. Set up a silo of size 1 kg of plastic and plastic rope. Additive mixture of grasses and ready to put in a plastic bag. The addition of direct additive as 0.1% (v/w) or about 106 cfu/g forage (Weinberg et al., 2003). Inoculation performed immediately after the feed is inserted in the plastic/silo. The addition of indirect additive/molasses as much as 3% performed at the time of mixing the forage. Atmospheric modification method is as follows: For the A0 performed before compaction of plastic tied. For A1, after a mixture of grass and molasses, then inserted in the plastic silo and directly tied. Before spending tied air is then performed with a

vacuum pump (during 25-30 second) until all the air out of the bag. For the A2 treatment, after a mixture of grass and molasses put in a plastic bag, without any compaction, then tied, then drained of CO₂ into the bag until the entire cavity filled with CO₂ (10 kg/m² during 25- 30 second). Subsequently incubated at room temperature. Observations of nutrient content (dry matter basis) performed on the incubation period of 14 days and 21 days is completed.

Experimental design and statistical analysis.

The research was conducted with Completely Randomized Design (CRD) 3x2 factorial pattern. Atmosphere modification as the first factor consist of: (A0: silage with compaction (conventional); A1: silage with vacuum method; A2: silage with modified CO₂) and two kinds of silage additives as the second factor (B1: indirect additive (molasses); B2: direct additive (Lactic Acid Bacteria). Each treatment combination was repeated 4 times, so there are 24 units of the experiment. Variables were observed: the content of nutrients (protein, crude fiber, energy) is carried out according to AOAC methods (AOAC, 2005). Furthermore, the data obtained were analyzed by analysis of variance, if there were significant effect then continued by HSD test (Steel and Torrie, 1993).

Results and Discussion

To maintain and enhance the nutritional content of the silage can be the addition of additives such as a bacterial culture (lactic acid bacteria), the source of water-soluble carbohydrates, organic acids, enzymes, and nutrients (urea, ammonia, mineral) (McDonald, 1991). Effect of modified atmosphere in the silo and different additive in this research result shown at Table 1. Combination between modified atmosphere (A0, A1 and A2) and molasses additive (B1) showed higher protein content at 14 and 21 days respectively than combination between modified atmosphere

(A0, A1 and A2) with *L. acidophilus* additive (15.13-15.62% vs 11.97- 14.59%). In contrast with crude fiber, while the gross energy content more various.

Crude Protein

During ensilage, partially degraded protein from grass (proteolysis) either by protease enzymes into plant and microbial compounds NPN (non-protein nitrogen), especially amino acids and ammonia (McDonald, 1991), amide, acetic acid, butyric acid, and water. The formed water made the anaerobic state difficult to occur (Reksohadiprodjo, 1988). At the young forages containing high protein, so that there is fermentation of protein (Ristianto et al., 1979). Results of analysis of variance showed that at 14 days, the interaction between air modification and the addition of additive does not significantly affect silage crude protein content, whereas the treatment effect modification of air were significant ($P < 0.05$), HSD test showed that of compaction treatment (A0) is different with vacuum method (A2) ($P < 0.05$). In the vacuum method silage crude protein content is lower. The addition of additive treatment showed highly significant effect on silage crude protein levels ($P < 0.01$). Protein content of silage with molasses additive (B1) is markedly higher than the crude protein content of silage inoculants with the addition of lactic acid bacteria ($P < 0.01$). This suggests overhaul of crude protein was higher in silage inoculants with the addition of BAL. Without the addition of carbohydrates fermentable on ensilage process with direct inoculation of lactic acid bacteria is thought to cause overhaul nutrients to support bacterial growth is higher. in line with Chen et al. (2014) that Molasses application increased lactic acid content and improved aerobic stability of TMR silage. Shown on this research, the addition of Lactic acid bacteria inoculants, the bacterial population in the early ensilage relatively high, so when we do not particularly protein

Table 1. Nutrient quality of napier grass silage with different modified atmosphere and additif during ensilage

Treatment	Crude Protein (%)		Crude Fiber (%)		GE (Kkal/kg)	
	14 days	21 days	14 days	21 days	14 days	21 days
A0B1	15.62±1.06	16.48±1.22 ^a	36.91±1.27	38.43±6.70	2644±399	2056±474
A0B2	14.59±1.71	13.47±0.87 ^b	40.98±1.11	42.60±3.30	2250±322	2026±67
A1B1	15.13±0.64	15.80±0.61 ^a	38.37±2.81	39.27±3.21	2523±356	2131±478
A1B2	11.97±0.59	12.47±0.66 ^{bc}	43.81±5.07	42.29±2.77	3608±238	2190±220
A2B1	15.44±1.09	16.65±0.80 ^a	34.95±0.62	39.68±6.15	3379±668	2158±125
A2B2	12.44±0.51	11.65±0.41 ^c	45.58±1.95	39.91±3.08	2160±180	2439±543

Values bearing different superscript at the same column shows significant ($P<0.05$).

A0: silage with compaction (conventional); A1: silage with vacuum method; A2: silage with modified CO₂; B1: indirect additive (molasses); B2: direct additive (lactic acid bacteria).

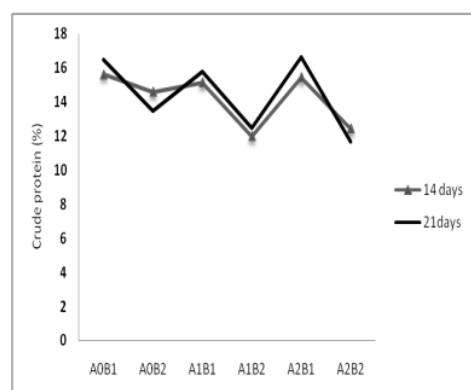


Figure 1. Crude protein content of silage during incubation time (A0B1: silage with conventional compaction and indirect additif/molasses; A0B2: silage with conventional compaction and direct addition/LAB; A1B1: silage with vacuum method and indirect additive (molasses), A1B2: silage with vacuum method and LAB; A2B1: silage with modified CO₂ and indirect additive (molasses); A2B2: silage with modified CO₂ and direct additive/LAB

nutrients to support growth that exist in the Napier grass as the material is preserved to be used, so that at the end of the process ensilage decreased levels of protein are higher. Kung (2010) stated that high concentrations of ammonia (> 10 to 15% of CP) are a result of excessive protein breakdown in the silo caused by a slow drop in pH or clostridial action. Owens et al. (2002) also stated that during ensiling protein is degraded to peptides and free amino acids by plant proteases. This

research is consistent with Yuan et al. (2012) that reported the proteolysis-inhibited effect of WHDG (wet hullless-barley distillers' grains) in the silage mixtures was pronounced during the ensiling especially the initial stages of ensiling. It is generally recognized that protein is degraded into amino acids by microorganisms and plant enzymes, and further broken down into ammonia or amines, resulting in a loss of silage protein.

Crude Fiber

In the process of formation of silage (ensilage), overhauled the plant carbohydrates into fatty acids that is flying lactic acid, acetic acid, butyric acid, carbonic acid, and alcohol in small amounts. Ensiling acid production during fermentation is a result of the WSC, but it is also a structural carbohydrate substrate extras that can be used. Measurement of loss of WSC is definitely hard to do, as a samples suspected release of sugar by fermentation is partly a result of hydrolysis of structural carbohydrates in plants, such as cellulose, and pectin hemicellulose (McDonald et al., 1991), Santoso et al. (2011), Yahaya et al. (2004); Yamamoto et al. (2004); Antaribaba et al. (2009) dan Santoso et al. (2009). then stated also that nearly half of hemicelluloses can be degraded. There are three possible causes hemicelluloses solution, namely: (1) degradation by enzymes hemicelluloses plants, (2) degradation by

enzymes hemicelluloses bacteria and (3) hydrolysis by organic acids produced during the fermentation process. In this study, hydrolysis of carbohydrates presented as crude fiber content changes during ensilage. The results are shown in Figure 2.

On the 14th day, the results of analysis of variance showed that the interaction between the modifications of the air with the addition of additive does not significantly affect the content of crude fiber. Similarly, treatment modifications do not significantly affect air crude fiber content of silage, but the addition of very real additive effect on crude fiber content of silage ($P < 0.01$). HSD test results showed that treatment of silage by the addition of molasses additive (B1) containing the crude fiber content was lower than the addition of inoculants treatment BAL (B2). This shows that the overhaul of carbohydrates in the treatment B1 higher than B2. Also point out that due to reshuffle the relatively higher carbohydrate causes the formation of lactic acid more rapidly

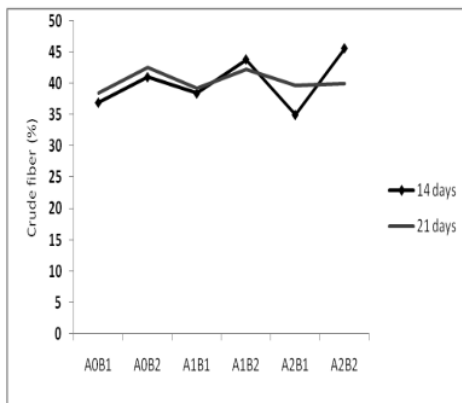


Figure 2. Fiber content of silage rough time during incubation time (A0B1: silage with conventional compaction and indirect additif/molasses; A0B2: silage with conventional compaction and direct addition/LAB; A1B1: silage with vacuum method and indirect additive (molasses), A1B2: silage with vacuum method and LAB; A2B1: silage with modified CO₂ and indirect additive (molasses); A2B2: silage with modified CO₂ and direct additive/LAB

so that the pH optimum for silage becomes more rapid. In accordance with the statement of Jones et al. (2004) that the process of fermentation is the biological activity of lactic acid bacteria convert simple sugars into acids (mainly lactic acid). Sugar components utilized to begin the initial phase ensilage stable phase characterized by the dominance of lactic acid bacteria and pH decrease does not happen again. Accordance to Jay (2000) report that group lactic acid bacteria fermented carbohydrates into energy and lactic acid. Caplice and Fitzgerald (1999) ; Jay (2000); and Kuipers et al. (2000) reported that metabolic pathways the case will be different when glucose as carbon source, homofermentatif bacteria like *Lactococcus* and *Streptococcus* generate two lactate from one molecule glucose, while heterofermentatif bacteria such *Leuconostoc* and *Weissella* changing the glucose molecule to lactate, ethanol and carbon dioxide.

On day 21st, the results of analysis of variance showed that the interaction between the modifications of the air with the addition of additive does not significantly affect on the content of crude fiber. Likewise, the influence of air modification or addition of additive. This suggests that changes in crude fiber content are relatively low or no carbs again reshuffle at the end ensilage. It was consistent with Johnson et al. (2005) that reported reported the use of a vacuum at the laboratory scale plastic silo with inoculum resulted in a pH 3.94 ($P < 0.001$) and without inoculum 4:21. This shows that the inoculum was instrumental in silage fermentation process.

Gross Energy

Changes in the gross energy content (dry matter base) of silage showed the presence of nutrients in the degradation of this organic material is a major component of energy in feedstuffs. McDonald et al. (1991) states that the loss of nutrients during fermentation

depends on the nutrients which are fermented and the responsible organisms. In general, some fermented products have higher gross energy than the substrate. Loss of dry matter is greater than the energy lost during fermentation. Results of analysis of variance showed that there was no interaction effect with the additive, as well as the influence of air and additive single modification either on day 14 or day-to-21. This suggests that no significant energy loss during the ensilage process. Loss of DM and gross energy would be greater if the *Clostridia* or *Enterobacter* dominant in fermentation, and in contrast when the dominant lactic acid bacteria. Although not significant, the results showed that the largest energy change is the modification of the treatment method of vacuum and air with the addition of inoculum's BAL (presented in the graph). At day 14th, silage with modified CO₂ and direct additif (LAB inoculum) have the lowest gross energy, but at

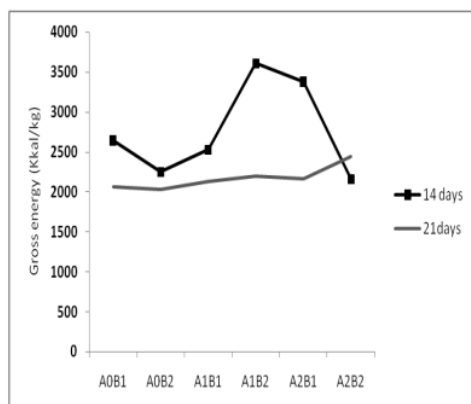


Figure 3. Gross energy content of silage during Incubation Time (A0B1: silage with conventional compaction and indirect additif/molasses; A0B2: silage with conventional compaction and direct addition/LAB; A1B1: silage with vacuum method and indirect additive (molasses), A1B2: silage with vacuum method and LAB; A2B1: silage with modified CO₂ and indirect additive (molasses); A2B2: silage with modified CO₂ and direct additive/LAB

day 21st gross energy were similar at all treatment. It showed that production of lactic acid due to ensilage stability. The high amount of effluent produced is also the cause of high loss because GE did not participate effluent measured gross energy. High volume of effluent that showed a high amount of dissolved organic matter, besides causing loss of high dry matter anyway. In accordance with the statement of Davies (2007) during the dry ingredients ensilage loss occurs when charging 5%, the liquid silage 3%, during the fermentation process 5%, damage due to air 10 and 4% loss in the field. This loss indicates that the lactic acid bacteria utilize a number of nutrients for the production of acid.

Conclusion

Based on the research results can be concluded that the optimal ensilage can take place, either by compaction methods (conventional), vacuum and the addition of CO₂. According to the nutrient content, addition of molasses additive produce better silage than the addition of *L. acidophyllus* without the addition of other additive.

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