

Effect of Fresh Rumen Content Plus Carbohydrate Source Additives and Incubation Time in Ensilage Process on pH, Physical Quality, and Total of Lactic Acid Bacteria of Dwarf Elephant Grass (*Pennisetum Purpureum Cv. Mott*) Silage

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Abstract— The purpose of this research was to test the effect of using fresh rumen cattle contents as an inoculant combined with carbohydrate source additives (cassava and rice bran) in making silage on the pH, physical quality, and total of lactic acid bacteria of dwarf elephant grass silage (*Pennisetum purpureum cv Mott*). This research was conducted with an experimental method using a randomized block design (RBD) with a nested pattern where the second factor was nested into factor 1 with 3 groups of replications. Factor 1: level of addition of fresh rumen cattle content as an inoculant mixed with additives as much as 7.5% of the weight of dwarf elephant grass, S0 = fresh dwarf elephant grass 1 kg + additive 75 g (control), S1 = S0 + inoculant as much as 37.5 g, S2 = S0 + 75 g of inoculant. Factor 2: The incubation time (ensilage) was 0, 1, 2, 4, 12, and 21 days. The results showed that the best dwarf elephant grass silage was the lowest pH (3.97), the least fungus and the best color, texture, odor, and the highest total colony of lactic acid bacteria, namely 6.92 log₁₀ cfu/g, which was shown in S2 treatment, namely dwarf elephant grass silage made with the composition of 1 kg dwarf elephant grass, plus 75 g of carbohydrate additives and 75 g of fresh rumen cattle inoculant.

Keywords— Silage, dwarf elephant grass, inoculant, physical quality, pH, total colonies of lactic acid bacteria.

I. INTRODUCTION

Making silage is the preservation of forage in the fresh form to ensure the availability of sufficient and guaranteed feed at all times. Silage making has long been known in countries with cold climates. The principle of making silage is to create anaerobic conditions when storing fresh forage in a special place called a silo. Piltz and Kaiser (2004) stated that at the beginning of the ensilage process when oxygen was still available (aerobic conditions), the cells of the silage material were still actively carrying out the respiration process and aerobic bacteria would grow by utilizing food substances contained in the silage material. Both processes will produce heat and break down the food substances contained in the silage material, causing a bad flavor and also the growth of mushrooms. Therefore, the availability of oxygen (aerobic conditions) in the manufacture of silage must be minimized so that the conditions in the silage can rapidly change to anaerobic. Anaerobic conditions are needed to support lactic

acid bacteria which play a role in producing lactic acid by utilizing dissolved carbohydrates. The lactic acid in the process of making silage plays a role in reducing the pH of the silage as quickly as possible. The low pH in silage can kill all bacteria and stop the fermentation process in the silage, thus making the silage in a stable condition. The delay in reducing the pH of the silage can cause the fermentation process and damage to the silage material. To accelerate the decrease in pH and achieve a stable silage condition as soon as possible, it is necessary to add lactic acid bacteria or lactic acid bacteria source material. The contents of the beef rumen are waste from slaughtering cattle which can be used as an inoculant source of lactic acid in the process of making silage. The contents of the beef rumen contain various kinds of microbes that can accelerate the process of breaking down carbohydrates in silage under anaerobic conditions and produce various acids, especially lactic acid and VFAs. The resulting acid compound can accelerate the pH drop in the silo. In addition to pH, the physical quality of silage can be assessed from several indicators such as color, aroma, presence of fungi, and texture which can be observed through organoleptic tests. Silage color is one indicator of the physical quality of silage, the color which is like the original color is good quality silage, and silage that deviates from the original color is low-quality silage (Kurniawan et al, 2015). Wati, et al. (2018) stated that silage with aroma fresh and slightly acidic indicates good silage quality. The incubation time affects the aroma of the silage to be slightly acidic due to the fermentation process which can produce lactic acid. The pattern of changes in the increasingly acidic odor is certainly in line with the lower silage pH. Good silage is slightly sour, free from sweet odors, ammonia odors, and H₂S odors. Silage with or without the addition of starter tends to be sour so that each different treatment does not affect the smell of silage. The presence of mushrooms is one indicator of success in making silage because the silage process is carried out anaerobically, so adding the contents of the rumen cows as an inoculant combined with rice bran additives and cassava flour as an additive source of carbohydrates in making mini

elephant grass silage is expected to play a role in avoiding failures in silage making and can produce good quality silage.

II. MATERIAL AND METHODS

This research was conducted with an experimental method using a randomized block design (RBD) with nested patterns where the second factor was nested into factor 1 with 3 replication groups. Factor 1: the level of addition of fresh rumen cattle content as an inoculant mixed with additives as much as 7.5% of the weight of dwarf elephant grass, S0 = 1 kg fresh dwarf elephant grass + 75 g additives (control), S1 = S0 + inoculants as much 37.5 g, S2 = S0 + 75 g inoculant. Factor 2: The incubation time (ensilage) was 0, 1, 2, 4, 12, and 21 days.

TABLE 1. Assessment of the physical quality of silage (Syarifudin, 2008)

Physical Observation	Score		
	3	2	1
Odor	Acid	Not sour	Rotten
Color	Dark green	Brownish green	Brownish
Texture	Hard	It's a little harsh	Soft
Visible Molds	Nothing	Enough	Lots

This research uses dwarf elephant grass at the age of 40-50 days which is cut one day before the time of making silage with purpose of withering or reducing the water content in elephant grass. The water content contained in the fresh rumen cattle content is obtained from the Slaughterhouse of Jalan Mojopahit Selatan, Mojorejo, Kec. Junrejo, Batu City. The additive used in this study was a mixture of rice bran (*Oryza sativa*) and cassava flour (*Manihot esculenta*) with a weight ratio of 1: 1. Mixing is done by weighing 2 kg of rice bran: 2 kg of cassava flour then mixed until homogeneous. The parameters observed included pH and physical quality of silage (color, odor, texture, and visible molds). The chemical quality of pH using a pH meter and physical quality was carried out by 8 untrained panelists with a scored assessment. The assessment for each physical quality observation criterion using a score can be seen in table 1.

A. Silage Making Procedure

Weighed the dwarf elephant grass was chopped manually, weighed as much as 1 kg, plus 75g additives, mixed evenly as a control treatment (S0). Silage treatment S1 and S2 were each made with S0 added with 37.5 g and 75.0 g fresh rumen contents then mixed evenly. The S2 treatment was 1 kg dwarf elephant grass + 75 g of additives + 75 g of fresh rumen contents then mixed well. The silage material that has been evenly mixed is put into a plastic bag as a silo then sucked using a vacuum machine then tightly laced, then incubated with incubation times of 0, 1, 2, 4, 12, and 21 days.

The data were analyzed using variance (ANOVA) according to randomized block design (RBD) with nested patterns and if the results obtained were different, it was followed by Duncan's distance test. The mathematical model used in the analysis is (Sudarwati et al., 2019).

III. RESULT DISCUSSION

The results of pH measurements of dwarf elephant grass silage made with several levels using the rumen contents as an inoculant plus additives and incubation time are presented in table 2. The data in table 2 shows that the addition of inoculants with rumen contents plus additives in making mini elephant grass silage can significantly (P <0.01) reduce silage pH. Silage from all S0-S2 treatments on the 4th day of incubation showed the ideal silage pH <4.5. Ohshima et al. (1997) stated that good silage can occur when the silage pH has reached less than 4.5. Silage in S2 treatment is silage made from mini elephant grass 1 kg plus 75 g of additives (a mixture of cassava flour and rice bran) and 75 g of fresh rumen content, which is very significant (P <0.01) shows the lowest pH (4, 23) followed by silage treatment S1 (4.33) and S0 (4.37). The condition of pH sequence from the smallest of the S2, S1, and S0 treatments continued until the 21-day incubation. This proves that the addition of inoculants with beef rumen content and additives for carbohydrate sources, a mixture of cassava flour and rice bran can support the success of the ensilage process. Observation of pH in the ensilage process is closely related to the availability of LAB and soluble carbohydrates. The availability of LAB is very helpful in accelerating the formation of lactic acid so that the ideal pH can be achieved by the opinion (Utomo, 2013) which states that the silage of beef rumen content provides sufficient substrate for bacteria lactic acid to form lactic acid so that a decrease in pH occurs. The faster the decline pH of silage to reach the ideal pH is <4.5, which means that the faster the silage reaches a stable condition which means it also reduces the occurrence of damage to the silage material, suppresses the growth of molds, avoids bad odors and silage texture. The results of the color analysis of dwarf elephant grass silage made with several levels of the rumen contents as an inoculant plus additive and incubation time are presented in table 3. One of the determinants of the physical quality of silage is the color quality. Silage that does not change color from the beginning of silage production has good silage quality. Based on the results of the organoleptic test, the incubation time for each treatment, there was no significant difference (P > 0.05) in the color quality of mini elephant grass silage. The longer the incubation day, the decreasing the color quality of the mini elephant grass silage, it can be seen in the table on day 4 that the color quality began to decline from 2.22 (brownish-green) to 1.79 (brownish).

TABLE 2. pH of dwarf elephant grass silage made with different levels of rumen content plus additives and different incubation time.

Treatment	Ensilage duration (days)					Average	
	0	1	2	4	12		21
S0	6,10±0,17 ^d	5,37±0,15 ^c	4,97±0,15 ^b	4,37±0,31 ^a	4,10±0,10 ^a	4,07±0,12 ^a	4,83±0,77 ^b
S1	6,13±0,12 ^d	5,07±0,38 ^c	4,63±0,21 ^b	4,33±0,15 ^{ab}	4,17±0,15 ^a	4,00±0,10 ^a	4,72±0,76 ^{ab}
S2	6,13±0,06 ^d	5,07±0,21 ^c	4,63±0,15 ^b	4,23±0,23 ^a	3,97±0,12 ^a	3,97±0,12 ^a	4,67±0,79 ^a

Note: Different superscript values (a, d) indicate very significant differences (P <0.01).

TABLE 3. The Color of dwarf elephant grass silage made with different levels of rumen contents plus additives and different incubation time.

Treatment	Ensilage duration (days)						Average
	0	1	2	4	12	21	
S0	2,50±0,45	2,21±0,36	1,92±0,14	1,38±0,22	1,88±0,76	1,46±0,26	1,89±0,54
S1	2,75±0,22	2,21±0,19	2,08±0,07	1,83±0,31	1,71±0,14	1,58±0,19	2,03±0,43
S2	2,83±0,19	2,21±0,26	2,67±0,26	2,17±0,62	1,75±0,22	1,33±0,07	2,16±0,59

Note: The values are not significantly different (P> 0.05).

Assumed color values:

- 1: brownish
- 2: brownish green
- 3: dark green

McDonald (1981) states that respiration occurs at the beginning of making silage which will produce CO₂, air, and heat, if this process takes too long, the temperature in the silo will be high so that it will damage the green color. The anaerobic phase can be quickly achieved in mini elephant grass silage which is added with rice bran and cassava flour because lactic acid-producing bacteria utilize the soluble carbohydrates in the accelerator to lower the silage pH so that the resulting silage color is dark brown to light brown. A good silage color has a color like the original color (Suyatno et al., 2011), a good silage color is like the original color. Based on the literature, S2 showed the best results for silage color. According to (Reksohadiprodjo, 1998) states that the changes that occur in plants that undergo an ensilage process are caused by the aerobic respiration process that takes place as long as oxygen supplies are still there. Sugar will be oxidized to CO₂ and air, heat is generated in this process so that the temperature rises.

The results of the odor analysis of dwarf elephant grass silage made with several levels of the rumen contents as an inoculant plus additives and incubation time are presented in table 4. The distinctive smell of silage is acid because the

fermentation process occurs in the ensilage process. Based on the results of the organoleptic test, it can be seen that there is no significant effect (P> 0.05) of the incubation time in each treatment on the smell of mini elephant grass silage. However, the longer the incubation time, the more distinctive smell of silage will be. The acidic atmosphere occurred on the 2nd day of the incubation period. The smell of acidity, such as the smell of tape, is the hallmark of good silage. The smell of silage comes from the acids produced during the ensilage (Lado, 2007). The addition of an accelerator to cassava flour and rice bran produces good silage in terms of smell, which is sour like typical tape, this is because cassava flour contains starch. (Safarina, 2009) stated that during the process of starch ensilage contained in cassava flour, it is converted into sugar through a satirization process before the fermentation process. According to (Stefani et al., 2010) the results of aerobic reactions that occur in the initial phase of silage fermentation produce volatile fatty acids so that the addition of a fermentation starter will accelerate the occurrence of an acidic atmosphere and result in a decrease in silage pH which results in a sour smell.

TABLE 4. The smell of dwarf elephant grass silage made with different levels of rumen contents plus additives and different incubation time.

Treatment	Ensilage duration (days)						Average
	0	1	2	4	12	21	
S0	2,00±0,00	2,46±0,26	2,88±0,13	2,96±0,07	2,92±0,14	3,00±0,00	2,70±0,39
S1	2,00±0,00	2,79±0,14	2,92±0,07	3,00±0,00	3,00±0,00	3,00±0,00	2,78±0,37
S2	2,00±0,00	2,67±2,67	2,92±0,14	3,00±0,00	3,00±0,00	2,96±0,07	2,76±0,38

Note: The values are not significantly different (P> 0.05).

Odor value assumption:

- 1: foul
- 2: not sour
- 3: acid

TABLE 5. Texture of dwarf elephant grass silage made with different levels of rumen contents plus additives and different incubation times.

Treatment	Ensilage duration (days)						Average
	0	1	2	4	12	21	
S0	2,96±0,07	2,92±0,07	2,83±0,07	2,33±0,26	2,04±0,14	1,92±0,14	2,50±0,45
S1	3,00±0,00	2,96±0,07	2,88±0,00	2,29±0,31	2,13±0,13	1,96±0,31	2,53±0,46
S2	3,00±0,00	2,96±0,07	2,88±0,00	2,50±0,33	2,17±0,14	2,04±0,07	2,59±0,41

Note: The values are not significantly different (P> 0.05).

Assumed texture value:

- 1: soft
- 2: a little harsh
- 3: hard

The results of the texture analysis of dwarf elephant grass silage made with several levels of the rumen contents as an inoculant plus additive and incubation time are presented in table 5. Organoleptic tests for texture on silage are one of the determinants of the quality of silage made, the denser the

texture of the silage shows the good quality of silage. the softer the silage texture, the quality level is not good according to the opinion (Kurniawan et al., 2015) which states that texture is one indicator to determine the physical quality of agricultural waste silage, the resulting solid texture shows that

the silage is of good quality. Based on the results of the organoleptic test, it can be seen that the interaction of incubation time in each treatment was not significantly different ($P > 0.05$) on the texture of dwarf elephant grass silage. It can be seen that the quality of the silage texture begins to change on the 4th day, namely (S0: 2,33, S1: 2,29, S3: 2,50) with the assumption that the texture is slightly soft. The decrease in texture quality during the incubation period in silage occurs due to the addition of inoculant contents of the beef rumen which contain high levels of lactic acid bacteria so that the process of converting glucose into CO₂, water, and heat will also increase. This is in line with the opinion (McDonald, 1981) the use of lactic acid bacteria 60 ml so that with more lactic acid bacteria it produces more CO₂, water and heat because lactic acid bacteria can convert glucose into CO₂, air and heat. During the process, there is a decrease in dry matter content and an increase in air content caused by the first ensilage stage, where respiration is still sustainable, sugar is converted into CO₂, H₂O, and heat.

The results of the analysis of visible molds of dwarf elephant grass silage made with several levels of rumen contents as inoculants plus additives and incubation times are presented in table 6. The results of the organoleptic tests carried out showed that the incubation time for each treatment was the addition of inoculants with rumen contents plus additives in making silage dwarf elephant grass significantly ($P < 0.05$) affected the presence of silage molds. At the level of the addition of inoculant content of the rumen content of cattle, S0 treatment (control) was the treatment with the least presence of molds. This can be assumed because of the treatment without the addition of inoculants from the contents of the cattle rumen. The contents of the rumen cattle contain not only good bacteria but also pathogenic bacteria that can affect the quality of silage. In the average incubation time, there was an increase in the presence of fungi on the 4th day of incubation, namely (S0: 2,21, S1: 2,29, S2: 2,67)

(sufficient) and continued to increase until the 21st day of incubation. Additives and inoculants containing rumen cattle are intended to help the rate of the ensilage process so that the quality is good and prevent molds and pathogenic bacteria in silage. This is supported by research (Kurnianingtyas et al., 2012) which states that the molds produced by banana stem silage added with molasses are no molds, while banana stem silage added with rice bran and cassava flour produces silage with sufficient molds. This is because the anaerobic phase can be achieved quickly. After all, lactic acid-producing bacteria (*Lactobacillus*) take advantage of the addition of rice bran accelerators, molasses, and cassava flour to lower the pH so that fungi and bacteria do not develop

A. Total Content of Lactic Acid Bacteria

The data in table 7 shows that the incubation time of each treatment, rumen content has a very significant effect ($P < 0.01$) on the total colony of lactic acid bacteria in dwarf elephant grass silage. Treatment S2 showed the highest total colony of lactic acid bacteria was 6.92 log₁₀ cfu / g, significantly different from treatment S1 (6.46 log₁₀ cfu / g) and S0 (6.52 log₁₀ cfu / g). The increase in the number of colonies of lactic acid bacteria was caused by the treatment given, namely the addition of food sources of alcoholic carbohydrates and the content of rumen cattle inoculants. S2 treatment is the best treatment with the highest lactic acid bacteria colony value because the composition of the silage constituents has been fulfilled so that it can support the fermentation process, there is dwarf elephant grass silage which causes the growth of lactic acid bacteria to increase along with the addition of lactic acid. The growth of lactic acid bacteria is also supported by the degree of acidity of the silage, table 2 shows a phenomenon between 4.67 - 4.83 where this atmosphere is effective for lactic acid bacteria to develop.

TABLE 6. Visible molds in dwarf elephant grass silage made with different levels of rumen contents plus additives and different incubation times

Treatment	Ensilage duration (days)						Average
	0	1	2	4	12	21	
S0	3,00±0,00 ^a	2,92±0,14 ^a	2,71±0,51 ^a	2,21±0,36 ^a	1,96±0,19 ^a	1,67±0,51 ^a	2,41±0,59 ^a
S1	3,00±0,00 ^a	2,96±0,07 ^a	2,75±0,43 ^a	2,29±0,40 ^a	2,00±0,00 ^a	1,79±0,26 ^a	2,47±0,53 ^a
S2	3,00±0,00 ^a	2,89±0,19 ^a	2,96±0,07 ^a	2,67±0,26 ^a	2,21±0,19 ^a	2,13±0,22 ^a	2,64±0,39 ^b

Note: The superscript values (a, b) are significantly different ($P < 0.05$).

Assumed value for visible molds:

- 1: a lot
- 2: enough
- 3: nothing

TABLE 7. Number of lactic acid bacterial colonies (log₁₀ cfu / g) of dwarf elephant grass silage made with different rumen contents plus additives and different incubation time.

Treatment	Ensilage duration (days)						Average
	0	1	2	4	12	21	
S0	5,41±0,21 ^a	5,54±0,25 ^a	5,82±0,09 ^a	5,75±0,15 ^a	7,93±0,30 ^b	8,65±0,33 ^b	6,52±1,33 ^a
S1	5,49±0,39 ^a	5,93±0,90 ^a	5,70±0,50 ^a	6,74±0,04 ^b	7,13±0,54 ^b	7,74±0,05 ^b	6,46±0,94 ^a
S2	5,54±0,29 ^a	5,87±0,96 ^a	6,90±0,72 ^b	7,60±0,35 ^b	7,92±0,37 ^b	7,71±0,06 ^b	6,92±1,05 ^b

Note: The mean value with the notation (a, b) shows a very significant difference ($P < 0.01$).

This is by the opinion (Nurjana, 2016) that a low pH value (pH <3.7) encourages the number of dead microbial cells including lactic acid bacteria so that the growth of lactic acid bacteria is inhibited. (Cai et al., 1999) reported that reported

silage was of good quality when the population of lactic acid bacteria was at least 105 (cfu / g). Table 7 shows that the total incubation time of lactic acid bacteria colonies increased from day 1 and continued to increase until day 21. This is supported

by a decrease in silage pH (Table 2). According to (Harahap, 2009) controlling the concentration of lactic acid bacteria is also related to the faster the decrease in pH. This decrease in pH causes lactic acid bacteria to have microbial activity.

IV. CONCLUSION

The best silage of dwarf elephant grass was the lowest pH (3.97), the least molds and the best color, texture, odor and the highest total colony of lactic acid bacteria, namely 6.92 log₁₀ cfu / g, which was shown in S2 treatment, namely dwarf elephant grass silage. made with the composition of 1 kg dwarf elephant grass added with 75 g of carbohydrate source additives and 75 g of fresh rumen cattle inoculant.

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