The Effect of Various Additive with Different Level on pH, Total Lactic Acid and Total Lactic Acid Bacteria Maize Stover (*Zea Mays* L.) Silage

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Abstract. This research aimed to evaluate the effect of addition different additives and level on pH, lactic acid content and total lactic acid bacteria maize stover (*Zea mays L.*) silage. The research was carried of the Plant Disease Laboratory, Faculty of Agriculture, Universitas Brawijaya. The material consists of maize stover, additives used were molasses, rice bran, pollard and cassava meal. The method used experimental in laboratory in a Completely Randomized Design with four additives, each additive consists of three level, namely T0 maize stover without additive, T1 (10% molasses, 10% rice bran, 10% pollard and 10% cassava meal), and T2 (20% molasses, 20% rice bran, 20% pollard and 20% cassava meal). The variables measured were pH, lactic acid content, and total lactic acid bacteria. Data were analyzed by analysis of variance and if there were any significant differences would be continued with Duncan's Multiple Range Test. The result showed that both additive and level did not give any significant effect ($P > 0,05$) pH 21 days (3.98 ± 0.03), total lactic acid (0.50 ± 0.6) and total lactic acid bacteria ($4.7 \text{ x } 10^4 \pm 2.3$). So, it was suggested to use cheaper and easily available additives for maize stover silage making.

Keywords: bacteria · lactic acid · maize

1 Introduction

Feed is a major aspect in the livestock industry, including ruminant livestock. Diet for ruminants is mostly forage, the majority of which are grasses, legumes and other plants. Forage contains a source of energy in ruminants because it has high crude fiber and Water-Soluble Carbohydrate (WSC), but forage production in the rainy season is abundant and during the dry season decreases, so there is need for an alternative forage substitute, namely using maize stover.

Maize stover is generally used in ruminant farms, is all parts of the corn plant including stems, leaves, and fruit except for the roots [1]. Maize stover has high production potential and good nutrient content. Leaf blade part of maize plant has crude protein content of 9.95%, dry matter content 92.88%, Neutral Detergent Fiber (NDF) content 62.28%, Acid Detergent Fiber (ADF) content 31.12%, calcium 1.01% and phosphorus

E. Widodo et al. (Eds.): ICESAI 2022, ABSR 28, pp. 341–348, 2023.
https://doi.org/10.2991/978-94-6463-116-6_43

0.11% [2]. However, maize stover has a problem, namely the water content is quite high, which is around 80–90% [3] so that it makes maize stover easily rot. Therefore, it is necessary to do preservation to maintain the availability of maize stover production in the dry season, one of which is in the form of silage.

Silage is the preservation of fresh forage through the ensilage process, which is the process of preserving forage using lactic acid fermentation under anaerobic conditions. [4]. The purpose of making silage is as a feed supply that can be used when there is a shortage of forage feed, increasing shelf life, and minimizing loss of forage nutrients. The requirement for making silage is that it must create anaerobic conditions so that *Lactic Acid Bacteria* (LAB) can grow well so that there can be a decrease in pH, the presence of WSC in the material to be silage and the water content of forage to be silage is around 60–70%. A good silage is characterized by the presence of an acidic odor and not a foul smell, has a low pH (around 4), and is slightly brownish in color [3]. Another good silage requirement is the absence of mucus and mold spots on the material being silage A good silage requires adequate tools such as silos that can ensure no air leaks during the ensilage process. Silos are usually made of strong and airtight materials such as plastic, iron, aluminum, and others. In addition to adequate equipment, sufficient WSC is needed to obtain good silage because WSC is a substrate for lactic acid bacteria to grow and produce lactic acid which can prevent spoilage bacteria from growing and make the pH drop to 4. Will inhibit the growth of lactic acid bacteria so that it can inhibit the ensilage process. The addition of energy source additives such as molasses, rice bran, pollard, and cassava meal are common because these materials are easy to obtain, inexpensive, and can significantly increase the WSC of corn meal silage because the high WSC content in additives will increase the performance of bacteria in producing lactic acid. And accelerate the decrease in the pH of silage so that the growth of other bacteria other than lactic acid bacteria is inhibited [5].

2 Materials and Methods

2.1 Research Location

Silage making and pH measurements were carried out in the Field Laboratory, Faculty of Animal Science, Universitas Brawijaya. Analysis of total lactic acid and the number of lactic acid bacteria was carried out in the Laboratory of Plant Diseases, Faculty of Agriculture, Universitas Brawijaya.

2.2 Research Materials

The research material is maize stover (*Zea mays* L.) with a cutting age of 85 days, the additives consist of molasses, rice bran, pollard, and cassava meal.

2.3 Research Methods

The experimental design used was a nested completely randomized design (CRD) with 4 (four) additive treatments, namely: molasses, rice bran, pollard, and cassava meal with additive levels of 0%, 10%, 20% respectively. The treatments tested are as follows:

ML0 = Maize Stover Silage with the addition of 0% Molasses.
ML10 = Maize Stover Silage with the addition of 10% Molasses.
ML20 = Maize Stover Silage with the addition of 20% Molasses.
BL0 = Maize Stover Silage with the addition of 0% Rice Bran.
BL10 = Maize Stover Silage with the addition of 10% Rice Bran.
BL20 = Maize Stover Silage with the addition of 20% Rice Bran.
PL0 = Maize Stover Silage with the addition of 0% Pollard.
PL10 = Maize Stover Silage with addition of 10% Pollard.
PL20 = Maize Stover Silage with addition of 20% Pollard.
GL0 = Maize Stover Silage with the addition of 0% Cassava Meal.
GL10 = Maize Stover Silage with the addition of 10% Cassava Meal.
GL20 = Maize Stover Silage with the addition of 20% Cassava Meal.

2.4 Research Methods

The variables observed in this study were pH, Total Lactic Acid, and Total Lactic Acid Bacteria.

2.5 Research Methods

Data analysis used analysis of variance in a Completely Randomized Nested Design. Test the difference between treatments using Duncan's Multiple Range Test.

3 Results and Discussion

3.1 pH Test

The analysis data for the pH test of the maize stover silage for each treatment are presented in Table 1. Table 1. Shows that the results of statistical analysis of the addition of additives have no significant effect ($P > 0.05$) on the pH value of 21-day silage. Based on the results of research pollard has the lowest score (3.96). The addition of additives to maize stover silage on the pH value of the 21-day silage gave an average score of cassava (3.99) bran (3.99) molasses (3.98) and pollard (3.97). Pollard with an average of (3.97) has the lowest pH value because the pH of each additive is relatively the same, causing the silage pH to be relatively similar. The analysis data for the pH test of the maize stover silage for each treatment are presented in Table 1.

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The addition of cassava meal in the elephant grass ensilage process was able to provide good conditions for the development of lactic acid-forming bacteria so that

Table 1. Average silage pH value 21 days.

Treatment	21 Day Silage pH value				Average
	Additive Type				
	Molasses	Rice Bran	Pollard	Cassava Meal	
L_0	3.99 ± 0.01	4.01 ± 0,01	3.99 ± 0.01	3.99 ± 0.02	3.99 ± 0.02
L_{10}	3.98 ± 0.01	3.97 ± 0,01	3.96 ± 0.01	3.98 ± 0.01	3.98 ± 0.01
L_{20}	3.97 ± 0.01	3.98 ± 0,04	3.97 ± 0.01	3.98 ± 0.01	3.97 ± 0.02
Average	3.98 ± 0.01	3.99 ± 0,02	3.97 ± 0.01	3.99 ± 0.01	

Description: *Result of Analysis at Sumber Sekar Field Laboratory, Faculty of Animal Science, Universitas Brawijaya (2020)

Table 2. Average total lactic acid silage.

Treatment	Total lactic acid silage (%)				Average
	Additive Type				
	Molasses	Rice Bran	Pollard	Cassava Meal	
L_0	0.48 ± 0.45	0.32 ± 0.13	0.40 ± 0.28	0.36 ± 0.20	0.39 ± 0.27
L_{10}	0.56 ± 0.20	0.37 ± 0.42	0.86 ± 0.03	0.56 ± 0.19	0.59 ± 0.21
L_{20}	0.16 ± 0.05	0.44 ± 0.16	0.66 ± 0.36	0.80 ± 0.13	0.16 ± 0.18
Average	0.40 ± 0.23	0.38 ± 0.18	0.64 ± 0.22	0.57 ± 0.17	

Description: *Results of Analysis at the Laboratory of Plant Diseases, Faculty of Agriculture, Universitas Brawijaya (2020).

the pH dropped quickly [6]. The addition of carbohydrate-rich material can accelerate the decrease in silage pH because carbohydrates are a source of energy for lactic acid-forming bacteria [7]. The degree of acidity (pH) which tends to be high will cause spoilage bacteria to thrive and live in that environment, while the low pH value of silage will inhibit the growth of harmful bacteria such as *Clostridium* and *Enterobacterium.* The stages of the silage process, the faster the decrease in pH will be followed by the faster the end of the overhaul of the substrate material, as is known in the aerobic phase the greatest loss of dry matter occurs. When the pH is acidic due to the presence of lactic acid produced by lactic acid bacteria, the reshuffling process stops and the silage becomes stable [8].

3.2 Total Lactic Acid

The results of the measurement of the total lactic acid silage of maize stover for each treatment are presented in Table 2. Table 2 shows that the results of statistical analysis of the addition of additives gave no significant effect ($P > 0.05$) on the total lactic acid silage. Based on the results of research pollard has the highest score (0.86%). The

addition of additives to the maize stover silage to lactic acid silage for 21 days gave the highest average score of pollards (0.64%) followed by cassava meal (0.57%) molasses (0.4%) and rice bran (0.38%).

Additive level showed no significant effect on the total lactic acid silage, the addition of additive level 0% showed the highest score (0.48%) on molasses, additive level 10% showed the highest score (0.86%) on pollard, added additive level 20% showed the highest score (0.66%) on pollard. The type of additive and the level of additive used did not have a significant effect ($P > 0.05$) on the total lactic acid silage. The results of this study are different from the research reported by [9] in that the addition of molasses has a significant effect ($P < 0.05$) with the highest average reaching (10.65%). The higher the level of addition of molasses, the higher the average total lactic acid of elephant grass silage. This difference was caused by the addition of bacterial isolates from cow rumen fluid and different raw materials for making silage. This study only adds additives to the manufacture of silage, the additives are molasses, rice bran, pollard, and cassava meal. Making silage is usually added with additives to increase the content of WSC (Water soluble carbohydrates) and increase the nutritional content of silage. The high WSC content in additives will increase the performance of bacteria in producing lactic acid and accelerate the decrease in silage pH so that the growth of other bacteria other than LAB is inhibited [10].

During the fermentation process, the lactic acid produced will act as a preservative so as to prevent the growth of spoilage microorganisms. The occurrence of acid in silage is caused by the oxidation process of ethanol into acetyl-dehyde which is then oxidized to lactic acid, this condition will cause the atmosphere to become acidic [11]. Lactic acid bacteria can be expected to automatically grow and develop during natural fermentation, but to avoid fermentation failure it is recommended to add homofermentative *Lactic Acid Bacteria* (LAB) inoculums, in order to ensure the continuity of lactic acid fermentation [12].

3.3 Number of Lactic Acid Bacteria

The results of the measurement of the total lactic acid silage of maize stover for each treatment are presented in Table 3. Table 3 shows that the results of the analysis of the addition of additives have no significant effect ($P > 0.05$) on the number of lactic acid bacteria silage. Based on the research results, molasses has the highest score (7.3 x 104 Cfu/gr). Addition of additives to maize stover silage to lactic acid silage for 21 days gave the highest average score of molasses (5.2 x 104 Cfu/gr) followed by pollard (4.8 x 104 Cfu/gr) rice bran (4.63 x 104 Cfu/gr) and cassava meal (4.4 x 104 Cfu/gr).

The additive level showed no significant effect on the total lactic acid silage, the addition of 0% additive level showed the highest score (5.6 x 104) on rice bran, the addition of 10% additive level showed the highest score (7.3 x 104) on molasses, the addition of additives level 20% indicates the highest score (5.4 x 104) on rice bran. The type of additive and the level of additive used did not have a significant effect ($P > 0.05$) on the number of silage lactic acid bacteria. The results of this study differ from the research reported by [13] that the LAB population of complete feed silage with the addition of rice bran, cassava meal, and pollard additives produced as much as 7.10 x 108 Cfu/gr and research from [14] that the silage of sugarcane shoots waste with

Table 3. Average number of silage lactic acid bacteria.

Treatment	Number of Lactic Acid Bacteria Silage (Cfu/gr)				Average
	Additive Type				
	Molasses	Rice Bran	Pollard	Cassava Meal	
L_0	5.5 x 10^4 ± 3.3	5.6 x 10^4 ± 1.6	5.5 x 10^4 ± 1.0	5.5 x 10^4 ± 1.7	5.5 x 10^4 ± 1.9
L_{10}	7.3 x 10^4 ± 1.7	2.9 x 10^4 ± 2.1	3.7 x 10^4 ± 2.8	3.9 x 10^4 ± 2.8	4.5 x 10^4 ± 2.3
L_{20}	2.9 x 10^4 ± 3.0	5.4 x 10^4 ± 3.8	5.2 x 10^4 ± 3.3	3.7 x 10^4 ± 1.0	4.3 x 10^4 ± 2.8
Average	5.2 x 104 ± 2.7	4.63 x 104 ± 2.5	4.8 x 104 ± 2.4	4.4 x 104 ± 1.8	

Description: *Results of Analysis at the Laboratory of Plant Diseases, Faculty of Agriculture, Universitas Brawijaya (2020).

selected lactic acid bacteria contained 10 x 106 Cfu/gr. This difference is caused by the addition of different starters and silage constituents, this research only adds additives to the manufacture of silage, the additives are molasses, rice bran, pollard, and cassava meal. Making silage is usually added with additives to increase the content of WSC (Water-Soluble Carbohydrates) and increase the nutritional content of silage. The high WSC content in additives will increase the performance of bacteria in producing lactic acid and accelerate the decrease in pH of silage so that the growth of other bacteria other than LAB is inhibited [10].

The ensilage process of lactic acid bacteria in the forage will utilize the forage as an energy source and produce organic acids, especially lactic acid, so that the protein undergoes an overhaul. Lactic acid in the ensilage process is produced from organic components, especially carbohydrates, thereby increasing the formation of lactic acid and lowering the pH of silage. The addition of additives to silage is able to accelerate the fermentation process by increasing the use of sugar into lactic acid so that the pH drops quickly, and is able to change the composition of the nutrient silage into better nutrients. Inhibitors are inhibitors of the growth of other unwanted bacteria in the manufacture of silage, for example, propionic acid serves to destroy or inhibit the growth of microbes such as Enterobacter, Clostridia, yeast and fungi.

4 Conclusion

The addition of additives and the level of administration to maize stover silage including molasses, rice bran, pollard, and cassava meal did not have a significant effect on the pH value, lactic acid content and the number of lactic acid bacteria. Based on the results of this study, it is recommended not to use additives in the manufacture of corn silk silage because each treatment does not have a significant effect on the quality of maize stover silage.

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