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EVALUATION OF MAIZE STOVER SILAGE QUALITY PREPARED WITH DIFFERENT LEVEL OF *PEDIOCOCCUS ACIDILACTICI* BACTERIA AS INOCULANT

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ABSTRACT

Maize stover has potential as ruminant feed because it has good nutritional value and is abundant. Its perishable nature makes maize stover cannot stand for a long time. It is important to conserve maize stover especially in from of silage and utilize it during the shortage of forages period. This experiment aimed to find out the best level of *Pediococcus acidilactici* bacteria in the maize stover silage making. The materials used were maize stover, molasses and *Pediococcus acidilactici* bacteria 1×10^5 cfu/g and 1×10^6 cfu/g. The experiment used randomized block design of 3 treatments and 4 blocks. The treatments were of T₁: maize stover+10% molasses; T₂: maize stover+10% molasses+*Pediococcus acidilactici* 1×10^5 cfu/g; and T₃: maize stover+10% molasses+*Pediococcus acidilactici* 1×10^6 cfu/g. The ensilage process was carried out for 21 days. The results showed that T₃ resulted the highest quality of maize stover silage with highest DM and OM degradability (67.93% and 67.58%, respectively); 96 hours *in vitro* gas production (117.51 ml/500 mg DM); DM and OM digestibility (67.96% and 69.45%); NH₃ concentration (19.23 mM) and microbial protein synthesis (39.56 g microbial N/kg fermented RDOM). It can be concluded that maize stover added with 10% molasses and *Pediococcus acidilactici* bacteria 1×10^6 with 21 days incubation can produce good quality of maize stover silage.

KEY WORDS

Degradability, *in vitro*, microbial protein synthesis, product fermentation, silage.

The main problem in the ruminant production is availability of the forages. The condition is worst during the dry season, since during those season forages production is low. Maize stover is forage from corn plants that are generally harvested at the age of 65-75 days after planting (Herniwati and Sariubang, 2011). Maize stover has some advantages as forage, among others, it has a wide degree of adaptation in the dry season so that it can replace the available green grass, has high productivity, namely 20 tons/ha/harvest, contain high water-soluble carbohydrates, has high palatability and has a high buffering capacity in the rumen (Izhar and Safitri, 2016). However, maize stover contains high moisture and perishables, then for next time uses, maize stover needs to be preserved.

Ensiling is a wet preservation technique of high moisture forages that is commonly applied in ruminant production. The ensilage process must take place in the anaerobic condition with lactic acid bacteria as inoculant. In the anaerobic conditions, the lactic acid bacteria ferment readily available carbohydrates (RAC) in the maize stover and produce lactic acids in faster way. High production of lactic acid in faster way is key role for the success of ensilage process, as with high and fast lactic acid production, all biological and chemical processes in the silage material will stop quickly and the silage is on the stable conditions for long period preservation. Thus there are at least three important requirements for the success of ensilage processes, i.e. the anaerobic conditions, enough availability of RAC and the presence of enough quantity of lactic acids bacteria. Therefore, in this experiment it is tested the addition of lactic acid bacteria *Pediococcus acidilactici* in maize stover silage making.

MATERIALS AND METHODS OF RESEARCH

The research was conducted in Sumbersekar Research Station, Faculty of Animal Science, Brawijaya University Malang for silage making and at Laboratory of Feed and Animal Nutrition, Department of Animal Feed and Nutrition, Faculty of Animal Science, Brawijaya University Malang for silage sample evaluation and analysis. The study was conducted on March 10, 2020 until June 21, 2020.

Material and Methods

The materials used in this study were maize stover aged 65-75 days, molasses, and starter bacteria *Pediococcus acidilactici*. The experiment used randomized block experimental design of three treatments and 4 replications. The treatments were silage making using maize stover and molasses as material with addition of different concentration of *Pediococcus acidilactici* bacteria as inoculant as coded below:

- T_0 = Maize stover with the addition of 10% molasses;
- T_1 = T_0 plus 1% *Pediococcus acidilactici* bacteria of 1×10^5 cfu/g;
- T_2 = T_1 plus 1% *Pediococcus acidilactici* bacteria of 1×10^6 cfu/g.

The variables measured were:

- Nutrient content including DM, OM, CP, CF and non fiber carbohydrates (NFC) that were analysed using AOAC method (2005);
- In vitro degradability test parameters using procedure of Makkar *et al* (1995), including DM and OM degradability in the rumen, rumen ammonia concentration and efficiency of rumen microbial protein synthesis (Blümmel and Ørskov 1993);
- In vitro digestibility test parameters including DM and OM digestibility using procedure of Tilley and Terry (1963).

The data were analyzed using a one-way-analysis of variance of randomized block design consisting of 3 treatments and 4 blocks. The mean difference between treatments was analyzed using Duncan's new Multiple Range Test. All statistical analysis calculations were processed using Microsoft Excel 2016.

Maize stover silages were prepared according to procedure below:

- Weighing maize stover, 1.5 kg per treatment unit and chopping into two to three mm length;
- Wilting the stover to reach approximately 60% moisture content;
- Adding molasses to maize stover, 10% of the stover weight;
- Adding *Pediococcus acidilactici* bacteria, 1% of the stover weight of 1×10^5 cfu/g and 1×10^6 cfu/g for treatment T_2 and T_3 , respectively;
- Mixing very well the silage materials of each treatment unit;
- Putting the silage materials of each treatment unit into plastic bag, compacted as much as possible and tied tightly using rubber band;
- Storing/incubating all silages for 21 days;
- Observing each silage for pH and organoleptic test;
- Taking silage sample from each treatment unit as much as 700 g then put in the oven 60°C for 48 hours and ground to pass 1mm mesh size and used for proximate analysis and in vitro degradability and digestibility tests.

RESULTS AND DISCUSSION

In addition to feed palatability or intake, nutrient content and digestibility are other two main parameters that determine quality of a feedstuff for livestock. Based on proximate analysis, nutrient content of maize stover silage of different treatments in this experiment is presented in Table 1.

Based on the results of the proximate analysis in Table 1, it can be seen that all nutrients content of maize stover silage in all treatments were not significantly different. However CP content of the silage prepared with *Pediococcus acidilactici* showed slightly lower than those of control silage (without *Pediococcus acidilactici*) and CF content of silage on

treatment T₃ was lower than those on treatment T₁ and T₂. This was presumably because the bacteria were active in breaking down the protein and fiber contained in the silage material. According to Santoso and Hariadi (2008) during ensilage process either directly or after withering, hydrolysis process will still continue to take place within the first 24 hours of silage incubation. CP and CF in the diet have an important role in the rumen. CP will undergo a hydrolysis process into peptides by proteolytic enzymes produced by proteolytic microbes. Peptides will be degraded into amino acids which will later be deaminated into ammonia to compose microbial proteins (Suprpto *et. al.*, 2013). While CF functions as bulky for rumen mate, to induce rumination and saliva production to maintain rumen pH and as substrates for rumen microbes, especially fiber-degrading bacteria.

Table 1 – Nutrient content of maize stover silage prepared using different concentration of *Pediococcus acidilactici* bacteria as inoculant

Treatment	DM (%)	Ash (% DM)	OM (% DM)	CP (% DM)	CF (% DM)
T ₁	26.81	7.85	92.15	9.03	26.39
T ₂	26.66	7.48	92.52	8.10	26.42
T ₃	26.73	7.88	92.12	8.16	24.51

The results showed that the digestibility of organic matter in each feed silage treatment was directly proportional to the digestibility of the dry matter produced. The organic matter are part with dry matter. When, it's high it also increase the result of the organic matter. This statement is reinforced by (Suardin, Sandiah and Aka, 2015) which states that the high digestibility of organic matter is in line with high dry matter digestibility or vice versa. According to (Tillman *et. al.*, 1998) the dry matter and organic matter digestibility are correlated each other, which is representeting result of digestibility.

Table 2 – Dry matter digestibility and organic matter digestibility of maize stover silage *in vitro*

Treatment	Dry matter digestibility (%)	Organic matter digestibility (%)
T ₁	66.53±1.49 ^a	68.04±1.21 ^a
T ₂	67.72±0.80 ^b	69.45±0.55 ^b
T ₃	67.96±0.77 ^b	69.45±0.35 ^c

^{a,b,c} different superscripts at the same row indicate significant differences ($p < 0.05$).

Based on the results of the analysis of variance in Table 2 it can be seen that the use of *Pediococcus acidilactici* bacteria as a starter with different levels in each treatment had significant effect ($p < 0.05$) against the digestibility of dry matter. The dry matter digestibility value of silage 21 days incubation has a normal range, namely between 66-69%, this is confirmed by (Sutardi, 1980) the dry matter digestibility range are at 50-60%. During the ensiled process the changes might from the crude fiber that hard to digest. The highest average dry matter digestibility value was produced by T₃, namely the treatment of maize stover with the addition of 10% molasses and starter bacteria *Pediococcus acidilactici* level 1×10^6 cfu/g at 21 days incubation of 67.72%, followed by T₁, namely maize stover treatment with the addition of 10% molasses at 21 days incubation was 67.96%, while the lowest dry matter digestibility value was produced by T₂, namely the treatment of maize stover silage with the addition of 10% molasses and starter bacteria *Pediococcus acidilactici* level 1×10^5 cfu/gr of 66.53% at 21 days incubation. This is caused by factors that affect digestibility, one of which is the difference in the crude fiber content of each treatment. Based on the results of the proximate analysis, T₃ has the lowest crude fiber content, namely 24.51%, so the lower the value of crude fiber content, the higher the digestibility value of a feed ingredient and vice versa. This is because the higher the crude fiber, the stronger the cell wall, which means that the more difficult the feed material is to degrade. According (Tillman *et al.*, 1998) stated that crude fiber is a factor of chemical composition that has the greatest influence on digestibility and generally, the higher the crude fiber content, the lower the digestibility and the rate of degradation of feed stuffs in the rumen (Anggorodi, 1990).

Digestibility of organic matter is an important factor in determining the quality of the ration. Each type of ruminant animal has rumen microbes with different abilities to degrade rations, resulting in differences in rumen digestibility (Fatmasari, 2013). Based on the results of the analysis of variance in Table 1, it shows that the addition of starter bacteria *Pediococcus acidilactici* with different levels in each treatment had a significant effect ($p < 0.05$) at 21 days incubation on digestibility of organic matter. The highest digestibility value of organic matter at 21 days incubation was found in the T_3 of 69.45% then followed by T_2 of 69.45% and the lowest treatment was T_1 of 68.04%. These results indicate that the increase in the digestibility value of dry matter is directly proportional to the increase in the value of the digestibility of organic matter, this is because organic matter is a constituent component of dry matter. This statement is in accordance with the opinion of (Sutardi and Adawiah 2003) which states that organic matter is closely related to dry matter because organic matter is the largest part of dry matter. Andayani (2010) adds that the value of organic matter digestibility is in line with the value of dry matter digestibility, this is because organic matter is part of dry matter.

Based on observations, it shows that gas production increasing incubation time. The results of statistical analysis showed that the value of gas production between treatments had a significant effect ($p < 0.05$) on the 96 hour, which are shown in Table 3.

Table 3 – In vitro gas production of maize stover silage incubation 21 days

T	Gas production (ml/500 mg DM) at the incubation period									
	2	4	8	12	16	24	36	48	72	96
T_1	2,79±0,50	3,77±0,55	16,69±0,46**	25,73±1,66**	39,94±0,64**	66,65±1,35***	88,88±0,22***	101,88±2,36**	109,01±2,65**	114,70±1,31**
T_2	3,06±0,51	4,86±0,71	18,19±0,7ab*	29,48±1,14b*	43,24±1,13ab*	70,18±1,96ab**	91,24±1,90ab**	106,59±1,76ab*	110,68±1,98ab*	115,79±0,72ab*
T_3	4,39±0,97	4,99±0,32	19,60±1,43c*	30,33±1,16b*	43,23±1,72b*	73,85±1,00b**	99,97±1,06b**	110,04±2,09c*	111,95±1,89c*	117,51±1,00c*

T: Treatment; DM: Dry Matter; ^{a,b,c} different superscripts at the same row indicate significant differences ($p < 0.05$) and highly significant ($p < 0.01$); *significant different, **highly significant different.

The results of the observations from the graphs in Figures 1 show that the gas production in all treatments has the same pattern during the 96 hour incubation, where the gas production produced continuously increases from the first 2 hours to the 96 hour incubation time. The gas volume at the beginning of the incubation at the 2 and 4 hours has increased gradually but not significantly, it is suspected that at that hour the microbial experience an adaptation phase or a lag phase, which means that the microbial are experiencing an adjustment to their environment and the available substrate. At 8, 12, 16, 24, 36 and 48 hours the volume of gas began to increase significantly, this was confirmed by the results of statistical analysis (Tables 3) which showed that the treatments at 8, 12 and 16 hours respectively had a significant effect ($p < 0.05$), 24 and 36 hours had a very significant effect ($p < 0.01$) and the 48 hour had a significant effect ($p < 0.05$) to the value of gas production. This is probably due to the fact that microbial are experiencing a logarithmic phase (log phase), where in this phase there is a very fast growth and proliferation of microbes as well as a maximum metabolic process and cell division. It is in this phase that the microbial begin to degrade the feed, break down carbohydrates into simpler structures and produce gas from the organic matter breakdown. Furthermore, at 72 and 96 hours there was still an increase in gas volume but statistically there was no significant effect ($p > 0.05$) on the value of gas production. It is assumed that at that hour the microbial had entered a stationary phase, which means that the growth rate was the same as the death rate. The stationary phase is characterized by constant growth between living and dead bacteria, this is due to reduced nutrients and the formation of metabolic compounds that tend to be toxic to bacteria. After 96 hours it is likely that the microbial have undergone a death phase, where the mortality rate is higher than the growth rate.

Gas production during 96 hours incubation showed the lowest yield was at T_1 , namely 2.79 ml/500 gr DM, while the highest yield was at T_3 which was 117.51 ml/500 gr DM. The higher the gas produced, the higher the microbial activity of the rumen so that more feed is

degraded. This is supported by (Ella *et. al.*, 1997) which states that gas production is a parameter to see rumen microbial activity in degrading feed, if gas production is high, the microbial activity in the rumen is also high. In addition, gas production can also provide an overview of the amount of organic matter that can be digested in the rumen. The difference in the value of gas production is thought to be caused by the crude fiber content between different treatments, namely T_1 has the highest crude fiber content of 26.39% which is relatively the same as T_2 , while T_3 has the lowest crude fiber content of 24.51%, so it can be concluded that the high production gas indicates a relatively low effect of crude fiber. This is supported by (Wahyuni *et. al.*, 2014) that the level of gas production is influenced by fiber components consisting of cellulose and lignin so that it takes a long time to degrade. Edwards *et.al.* (2012) added that fiber and lignin components have the ability to inhibit fermentation in vitro. The maize stover that ensiled for 96 hours are reflected of the result of dry matter. The increasing of the dry matter, it will be curvilinear decline the result of the gas production. The season of the result might be content of crude fiber. Reported (Agricultural Research Council, 1980) the digestibility are relay on the ruminal activity. Increase the number digestibility the ruminal activity also increased.

Figure 1 – Graph of the difference in the volume of 21 days incubation gas production

Feed degradation in the rumen especially for most of CF and in small part of CP and soluble carbohydrates is very important to support microbial growth in the rumen which then important to supply of nutrients to the host animal. High rumen microbial growth and population are important for ruminant, especially for degrading fiber bacteria. The bacteria are essential for CF degradation contained in the forages, which are as the main feed for ruminant. The forage fiber is degraded by rumen microbes into VFAs, which are as main energy sources for ruminants. In addition, high rumen microbial population give opportunity to increase number of rumen microbes flowing into the abomasum and small intestine. Where, in the abomasum the micobes will lysis and release all nutrients contained in their cells, including protein that is as main component ($\pm 65\%$) of the microbial cells biomass. The protein is then enzymatically digested in the abomasum and also in the small intestine into amino acids which then ready for absorbtion to supply most amino acids requirement by ruminants. Dry matter and organic matter degradability of maize stover silage prepared using different concentration of *Pediococcus acidilactici* bacteria as innoculant measured in this experiment are presented in Table 4.

The results of analysis of various DDM and DOM from residual gas production in vitro incubation of 96 hours in Table 4 show that the use of *Pediococcus acidilactici* bacteria with different levels on mize stover had a significant effect ($p < 0.05$) on 21 days incubation of DDM and DOM. T_1 , T_2 , and T_3 had an average DDM value of 65.56%; 67.13%; 67.93% at 21 days incubation. Table 4 shows that the higher the amount of bacteria content in the treatment, the higher the dry matter degradability value, possibly this is due to an increase in

crude protein content. According to Arora *et. al* (1989), protein will be hydrolyzed into oligopeptides by proteolytic enzymes produced by microbes in the rumen, then the oligopeptides will be hydrolyzed into amino acids. These rumen microbes will become a source of protein for the host, besides that the host can also take advantage of small molecules from oligopeptides, amino acids, alpha keto acids and hydroxy acids which may not degrade in the rumen. In addition, a long incubation time of 96 hours can also affect the increase in DDM value, this is in accordance with the opinion of Sofiana (2018) which states that the longer the incubation time (72 hours) of mixed plus feed and fermented straw makes the DDM value increase. Mehrez *et. al* (1977) added that the longer the incubation time, the higher the degradation of feed by rumen microbes. The increase in feed degradation occurred due to microbial activity in the rumen which resulted in reduced feed substrate along with increasing incubation time (Zulkarnain *et. al.*, 2014).

Table 4 – Degradability Dry Matter (DDM) and Degradability Organic Matter (DOM) of maize stover silage after 96 hours incubation in the rumen in vitro

Treatment (T)	DDM (%)	DOM (%)
T ₁	65.56±0.32 ^a	64.80±1.03 ^a
T ₂	67.13±0.53 ^b	66.51±0.80 ^b
T ₃	67.93±0.77 ^c	67.58±2.04 ^c

^{a,b,c} different superscripts at the same row indicate significant differences ($p < 0.05$).

The level of degradation of organic matter is thought to be due to differences in crude fiber content in each treatment. The lower crude fiber content in T₃ of 24.51% caused DOM to be higher because rumen microbes could more easily degrade feed, while the crude fiber content in T₁ was 26.39% at 21 days incubation tends to be higher to produce the lowest DOM. This is in accordance with the research of (Fajri *et. al.*, 2018) which produced the best degradability value in the silage treatment of odot grass with the addition of 10% pollard and 10% bran which was caused by the low content of crude fiber in odot grass, namely 29.74%. The low crude fiber content makes feed easier to digest in the digestive tract so that it is easier for bacteria to penetrate into the feed ingredients for the digestive process (Permana *et. al.*, 2013). The increase in the value of DDM is in line with the increase in the value of the DOM, this is in accordance with the opinion of Afdhal & Erwan (2013) that an increase in the value of DOM causes the value of DDM to also increase or vice versa.

The concentrations of NH₃ produced during 24 hours of in vitro incubation in maize stover silage with the addition of 10% molasses and starter *Pediacoccus acidilactici* by different levels are presented in Table 5.

Table 5 – Concentrations of NH₃

Treatment	Concentrations of NH ₃ (mM)
T ₁	18.44±0.78
T ₂	18.80±1.30
T ₃	19.23±1.58

Based on Table 4, the concentration of NH₃ from the degradation of maize stover silage using 10% molasses and starter bacteria *Pediacoccus acidilactici* at different levels, varies with the range 18.44%-19.23%. Ammonia concentration (NH₃) in each treatment increased at incubation for 24 hours. The highest increase occurred in T₃ of 19.23%, while the lowest value was found at T₁ of 18.44% however, statistically each treatment had no significant effect ($p > 0.05$) on the NH₃ concentration. (Tanuwiria *et. al.*, 2005) added that high NH₃ production reflects the amount of ration protein that is easily degraded by rumen microbes. The amount of NH₃ in the rumen will be beneficial for rumen microbes as the main source for microbial protein synthesis so that the population is getting higher. The enzymatic activity increase when the number of digestibility increases.

This shows the importance of increasing the protein content in feed to accelerate the work of microbes so that digestibility increases. Ammonia is one of the fermentation products

in the rumen that comes from protein degradation which will be used by rumen microbes for their growth. Rumen microbes will utilize ammonia as a source used in protein synthesis. The resulting protein can act as a structural protein in the formation of cell and functional components in the form of enzymes. The use of a starter causes LAB to develop properly, which causes the pH to be low. The low pH value causes protein decomposition bacteria to be suppressed so that it is assumed that the substrate protein will not change relatively, thus the protein supply from the substrate will be more degraded by rumen bacteria into NH_3 . (McDonald *et al.*, 2002) explained that a high concentration of NH_3 can show that the feed protein degradation process is faster than the microbial protein formation process, so that the resulting NH_3 accumulates in the rumen.

Rumen microbial protein synthesis is influenced by the concentration of NH_3 where, the higher the concentration of NH_3 , the growth of rumen microbes will be fast so that the digestibility of the feed is also high. The results of statistical analysis showed that the treatment had a significant effect ($p < 0.05$) at 21 days incubation on the value of rumen microbial protein synthesis, show in table 6.

Table 6 – Microbial protein synthesis

Treatment	Microbial protein synthesis (g microbial N/kg fermented RDOM)
T ₁	37.94±0.64 ^a
T ₂	38.43±0.52 ^b
T ₃	39.56±0.29 ^c

^{a,b,c} different superscripts at the same row indicate significant differences ($p < 0.05$).

Based on Table 6, it can be explained that each of the T₁, T₂, and T₃ treatments had an average microbial protein synthesis value of 37.94; 38.43; 39.56 g microbial N/kg fermented RDOM at 21 days incubation. The value of microbial protein synthesis tended to be high in the T₃ while the lowest value was found in the T₁ with 21 days incubation. According to (Arora *et al.*, 1989), microbial protein synthesis depends on the speed of breakdown of feed nitrogen, microbial needs for amino acids and the type of rumen fermentation which is influenced by the type of feed. The range of values for microbial protein synthesis in this study was 37.48-39.56 g microbial N/kg fermented RDOM and included in the optimal range. Compared founded with study from (Thirumalesh and Krishnamoorthy, 2013) the optimal value of microbial protein are in the range 14-60 g microbial N/kg fermented RDOM.

CONCLUSION

The use of *Pedococcus acidilactici* bacteria level 1×10^6 cfu/g as a starter by adding soluble carbohydrates (molasses) in the ensilage process incubated for 21 days can maintain the quality of maize stover silage.

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