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The Effect of Ensiled Maize Stover Combined with Additives as an Animal Feed

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ABSTRACT

The research purpose was to determine the effect of addition starter *Pediococcus pentosaceus* with different level in the maize stover silage on the *in vitro* digestibility, fermentation product, and microbial protein synthesis. The treatments consist of T_1 = maize stover silage + molasses 10%, T_2 = maize stover silage + molasses 10% + *P. pentosaceus* ($1x10^5$ cfu/g), and T_3 = maize stover silage + molasses 10% + *P. pentosaceus* ($1x10^6$ cfu/g), fermented for 0 and 21 days. The research method was an experiment by using Randomized Block Design (RBD), with 3 treatments and 4 blocks. The result showed that addition of starter *P. pentosaceus* in the maize stover silage gave significant effect (P<0.05) on dry matter (T_1 (65.67±1.56%), T_2 (65.78±0.60%), and T_3 (67.54±1.11%)), and organic matter digestibility (T_1 (66.93±0.48%), T_2 (68.15±1.34%), T_3 (68.68±1.04%)) at 21 days incubation. The result of statistical analysis showed that treatment gave significant effect (P<0.05) on gas production at 96 hours of silage with 0 day incubation, T_1 (114.74±0.60 ml/500mg DM), T_2 (116.12±1.44 ml/500mg DM), T_3 (116.97±1.12 ml/500mg DM) and 21 days incubation, T_1 (124.27±0.59 ml/500mg DM), T_2 (125.27±1.49 ml/500mg DM), and T_3 (126.16±1.26 ml/500mg DM). All treatments not gave significant effect (P>0.05) on degradability, NH_3 and microbial protein synthesis on 0 days incubation. However, did it gave significantly effect (P<0.05) in 21 days incubation silage, dry matter degradability (T_1 (66.16±0.42%), T_2 (65.70±0.58%), T_3 (66.66±0.21%)), organic matter degradability (T_1 (65.67±1.56%), T_2 (65.78±0.60%), and T_3 (67.54±1.11%)), microbial protein synthesis (T_1 (37.72±0.13 g/N/kg OM apparently fermented), T_2 (37.84±0.64 g/N/kg OM apparently fermented), T_3 (38.82±0.56 g/N/kg OM apparently fermented). It can be concluded that addition of starter *P. pentosaceus* ($1x10^6$ cfu/g) on maize stover silage with 21 days incubation can produce silage with good quality on the *in vitro* digestibility, fermentation product, and microbial protein synthesis.

Keywords: Digestibility, Fermentation product, In vitro, Maize stover, Microbial protein synthesis, *Pediococcus pentosaceus*

Introduction

The demand for livestock product such as meat, eggs, and milk are increasing along with population growth and improvement in economic condition, for that we need a new breakthrough to increase livestock productivity in order to be able to meet animal food for humans and in effort to avoid the predicted food crisis. Aside from genetic and environmental factors, feed is an important key in livestock productivity.

High demand for ruminant animal feed, forcing farmers to be more innovative in providing forage feed in order to anticipate the limited forage in the dry season. Availability of forage varies widely, in the rainy season the availability of forage is quite abundant, however in the dry season it is very limited, so that farmers find it difficult to get good quality forages. One of the means that can be done by breeders is utilizing maize stover which Indonesia has abundant potential, about 20 tons/Ha/season (Karimuna *et al.*, 2009). Maize stover contains nutrients that are quite good (ash content 7.56%, crude protein 7.25%, and crude fiber 29.6% (Rahayu *et al.*, 2017), but has a high enough water content (80.35%) so it is easily rotten and damaged. One of the things that can be done for processing and preserving it, namely the silage method.

Silage is an alternative feed preservation technology which aims to maintain nutrient content (Widodo, 2014). Ensiling is a wet preservation technique that is commonly applied in various feed materials. The silage condition must be on the anaerobic condition with lactic acid bacteria is inoculant.

In general, all part of the maize stover can be used as silage. Maize stover is characterized a high fiber content and thus is susceptible to increasing during the silage process. The lactic acid bacteria are commonly used for ensuring good silage as inoculant. The strain commonly used are *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus bucheneri*, *Pedioccocus acidilactici*, *Pediococcus pentosaceus* and *Enterococcus faecium* which can cause the pH of silage to rapidly drop (Nusio, 2005).

One type of bacteria that can be used in this research is *P. pentosaceus* starter. The basic characteristics of this starter in ensilage are being able to adapt to materials with high water content, high ambient temperature, produce bacteriocins, and act as probiotics (Ohmomo *et al*., 2002). *P. pentosaceus* bacteria are lactic acid bacteria from the homofermentative group, in the process of ensilage, it is able to lower pH and increased the concentration of lactic acid (Weinberg, *et al.,* 1999). Homofermentative lactic bacteria single or in combination with heterofermentative lactic acid bacteria, can improve the aerobic stability of silage by inhibiting the activity of fungi and yeast. Addition of a starter *P. pentosaceus* bacteria it is silage.

Materials and Methods

Materials

A sample of maize stover was freshly collected with a harvest age of 65-75 days obtained in the dau district, Malang regency. The part of maize used are leaves, stems, flowers, and fruits. The inoculant used are *P. pentosaceus* (level $1x10^5$ and $1x10^6$ cfu/g), molasses, and ruminal fluid was taken from slaughtered house of Gadang, Malang. The ensiling procedure was performed following the method of Holik *et al*. (2019) for 21 days.

Methods

After being ensiled, the fermented of maize stover are cutting into smaller piece through 1 mm sieve size. Then incubated are conducted at intervals 0, 2, 4, 8, 12, 16, 24, 36, 48 and 96 hours (Makkar *et al*., 1995). The treatments consist of:

T_1: maize stover silage + molasses 10%

T_2: maize stover silage + molasses 10% + *P. pentosaceus* ($1x10^5$ cfu/g)

T_3: maize stover silage + molasses 10% + *P. pentosaceus* ($1x10^6$ cfu/g)

The research method was an experiment by using Randomized Block Design (RBD), with 3 treatments and 4 blocks.

Observed variables

1. Dry matter and organic matter digestibility (Tilley and Terry, 1963).
2. *In vitro* gas production (Makkar *et al*., 1995).
3. Degradability (Makkar *et al*., 1995).
4. NH_3 (Conway, 1957).
5. Microbial protein synthesis (Blummel *et al*., 1997).

Data analysis

The data were analyzed with an analysis of variance using Microsoft Excel. Duncan test were performed for any variables that showed significant different (Steel and Torrie, 1993).

$$Y_{ij}k = \mu + \tau_i + \varepsilon_{ijk}$$

Explanation:

$Y_{ij}k$ = observed value

μ = population mean value

τ_i = treatment effect *i*

ε_{ijk} = effect of the error

i = treatment 1,2,3,4 and 5

k = repeat 1,2,3 and 4.

Results and Discussion

Dry matter and organic matter digestibility

The result showed that all treatments at 0 days incubation had no significant effect (P>0.05) of dry matter and organic matter digestibility of maize stover silage, and otherwise all treatments at 21 days incubation had a significant effect (P<0.05) of dry matter and organic matter digestibility. The result of research and statistical analysis obtained the result of dry matter and organic matter digestibility which are presented in Table 1.

The mean value of dry matter digestibility of maize stover silage with 0 day incubation in this study was around 66.84–67.57%, while at 21 days incubation had a higher average value, which was around 67.47–68.86%. The different in value is due to the fact that all treatments at the 21 day incubation underwent a fermentation process at the time of ensilage, which remodeled the feed to make it easier to digest. This is in accordance with Nusio (2005) opinion, which states that the fermentation process is able to break down complex organic compounds into simpler compounds so that they can be digested properly by livestock.

The result shows that maize stover silage with the addition molasses 10% and starter *Pediococcus pentosaceus* bacteria (level $1x10^6$ cfu/g) with 21 days incubation (T3) had the highest dry matter digestibility value of 66.66% compared to other treatments, this is due to differences in nutrient comparisons in each treatments (especially the crude fiber and crude protein content). The content of crude fiber and crude protein T_3 was higher (9.89 % and 24.71%) when compared to T_1 (9.03% and 26.39%) and T_2 (9.39% and 25.69%). This is in accordance research of Andayani (2010) which states that the digestibility of the feed has positive correlation with the content of crude fiber and crude protein, the lower of crude fiber content, the higher the digestibility of the feed. According to Astutik *et al*. (2019), the level of digestibility is influenced by the amount of lignin that blinds cellulose and hemicellulose in the feed, the higher the amount of lignin, the lower the digestibility of the feed. This is presumably because the microbes could not optimally digest the crude fiber components contained in the feed.

Table 1. Average of dry matter and organic matter digestibility

Incubation	T	Dry matter digestibility (%)	Organic matter digestibility (%)
0 day	T_1	66.84±0.47	66.86±0.95
	T_2	67.28±1.29	68.24±0.42
	T_3	67.57±0.84	68.30±1.75
21 day	T_1	67.47±0.60[a]	66.93±0.48[a]
	T_2	67.87±0.93[b]	68.15±1.34[b]
	T_3	68.86±0.76[b]	68.68±1.04[b]

T_1: maize stover silage + molasses 10%, T_2: maize stover silage + molasses 10% + *P. pentosaceus* ($1x10^5$ cfu/g), and T_3: maize stover silage + molasses 10% + *P. pentosaceus* ($1x10^6$ cfu/g).
[a,b] different superscripts at the same row indicate significant differences (P<0.05).

The lowest organic matter digestibility value at 21 days incubation was the control treatments T_1 (65.67±1.56%) and increases at T_2 (65.78±0.60%) and T_3 (67.54±1.11%). The organic matter digestibility value in this study was higher than the dry matter digestibility value, this is in accordance with the opinion of Riswandi *et al*. (2015) which states that organic matter digestibility has a higher value that dry matter digestibility, this is because the dry material still contains ash content, so materials without ash content are relatively easy to digest.

The high value of dry matter and organic matter digestibility in maize stover silage with 21 days incubation is thought to be because molasses contains carbohydrates (sucrose) which is a disaccharide group that can accelerate proliferation of ensilage so that not much of the silage's nutrients are dissolved. Addition of molasses has an effect on digestibility, because molasses can be increase ration consumption in the treated feed.

Gas production

The end product of fermentation process are gas production. Gas production is the result of a fermentation process which describes the amount of organic matter that can be digested in the rumen. The level of gas production reflects the effectiveness of the fermentation process, the higher the value of gas production, better the fermentation process. The pattern of increasing gas production maize stover silage with 0 and 21 days incubation can be seen in Figure 1 and 2.
The curve of increasing gas production in Figure 1 and 2 occurs in accordance with the activity of rumen microbial growth. In general, there are 4 phases of microbial growth, namely lag, log, stationary and death phase at the time eight hours incubation production gas are increase, this stated later called lag phase. Another statement called adaptation phase. Continued at 24, 36, and 48 hours incubation time are curve linear increase.
The increase of this phase from activity of microbial on the stover. Last at 72 and 96 hours the result are slightly decreasing during incubation time.

The results of statistical analysis of maize stover silage gas production with 0 and 21 days incubation are presented in Table 2 and 3. The result of research showed that silage not had significant effect (P>0.05) at the incubation 2, 8, 16, and 96 hours. At the incubation 14 and 72 hours, showed a significant effect (P<0.05), and at incubation 24, 36, and 48 hours showed highly significant effect (P<0.01) on *in vitro* gas production. Total value *in vitro* gas production 96 hours in silage with 0 day incubation that is T_1 (114.74±0.60 ml/500 mg DM), T_2 (116.12±1.44 ml/500 mg DM), and T_3 (116.97±1.12 ml/500 mg DM). Silage with 21 days incubation, lowest gas production value at T_1 (124.27±0.59 ml/500 mg DM) that is treatments without addition starter *P. pentosaceus* bacteria, then increased on T_2 (125.27±1.49 ml/500 mg DM), and highs on T_3 (126.16±1.26 ml/500 mg DM) that is maize stover silage with addition molasses 10 % and starter *P. pentosaceus* bacteria level $1x10^6$ cfu/g. The level of gas production are reflected from fermentation process. The correlation of fermentation is depend on the microbial activity.

The low *in vitro* gas production value of 96 hours at T_1, both at 0 day and 21 days incubation, is thought to be due to the high crude fiber

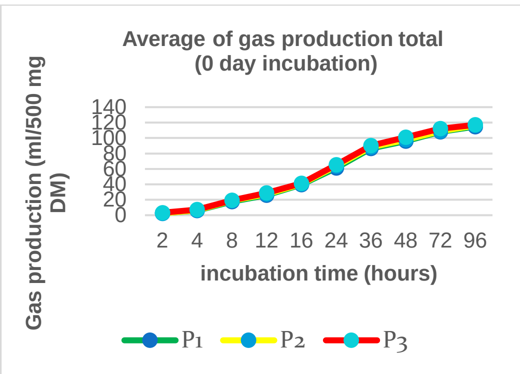

Figure 1. Volume of increase in gas production between incubation maize stover silage 0 days incubation.

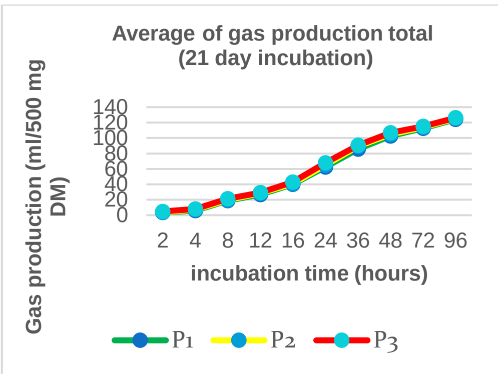

Figure 2. Volume of increase in gas production between incubation maize stover silage 21 days incubation.

Table 2. Average value of *in vitro* gas production 96 hours maize stover silage 0 day incubation

	Gas production (ml/500 mg DM) with 0 day incubation									
T	2	4	8	12	16	24	36	48	72	96
T_1	2.58±0.44	5.93±0.70^{a*}	17.47±1.12	26.10±0.86^{a*}	39.61±0.91	61.15±1.75^{a**}	86.33±1.16^{a**}	96.14±1.45^{a**}	107.83±1.36^{a*}	114.74±0.60^{a*}
T_2	2.40±0.23	6.34±0.13^{a*}	18.81±0.71	27.18±0.80^{a*}	40.24±0.91	63.70±0.44^{b**}	88.02±0.34^{b**}	97.82±0.69^{b**}	108.94±1.58^{b*}	116.12±1.44^{b*}
T_3	3.26±0.19	7.33±0.25^{b*}	19.39±0.27	28.69±0.39^{b*}	41.38±0.99	65.40±0.61^{c**}	90.35±0.39^{c**}	100.97±0.59^{c**}	112.18±0.88^{c*}	116.97±1.12^{c*}

T_1: maize stover silage + molasses 10%, T_2: maize stover silage + molasses 10% + *P. pentosaceus* (1x10^5 cfu/g), and T_3: maize stover silage + molasses 10% + *P. pentosaceus* (1x10^6 cfu/g).
^{a,b,c} Differences in superscripts in the same column showed significantly different result (p<0.05) and a very significant effect (p<0.01).
* Significant different, ** highly significant different.

Table 3. Average value of *in vitro* gas production 96 hours maize stover silage 21 day incubation

	Gas production (ml/500 mg DM) with 21 days incubation									
T	2	4	8	12	16	24	36	48	72	96
T_1	3.60±0.36	6.06±0.77^{a*}	18.92±0.89	26.58±1.35^{a*}	40.18±0.93	62.45±2.04^{a**}	85.60±1.35^{a**}	102.80±1.13^{a**}	112.44±1.18^{a*}	124.27±0.59^{a*}
T_2	3.82±0.35	7.38±0.42^{b*}	19.75±0.94	28.35±0.33^{a*}	41.00±1.99	64.86±0.29^{b**}	89.27±0.33^{b**}	104.54±0.26^{b**}	113.78±0.38^{b*}	125.27±1.49^{b*}
T_3	4.91±0.50	7.95±0.54^{b*}	21.24±0.98	29.29±1.02^{b*}	42.95±1.19	67.64±0.31^{c**}	90.41±0.61^{c**}	106.81±1.04^{c**}	115.04±0.43^{c*}	126.16±1.26^{c*}

T_1: maize stover silage + molasses 10%, T_2: maize stover silage + molasses 10% + *P. pentosaceus* (1x10^5 cfu/g), and T_3: maize stover silage + molasses 10% + *P. pentosaceus* (1x10^6 cfu/g).
^{a,b,c} Differences in superscripts in the same column showed significantly different result (p<0.05) and a very significant effect (p<0.01).
* Significant different, ** highly significant different.

content in silage. At T_3, the *highest in vitro* gas production was obtained with a crude fiber content of 24.87% (0 day incubation) and 24.81% (21 day incubation), while T_1 has the lowest gas production value with crude fiber content 24.55% (0 day incubation) and 26.39% (21 day incubation). Syahrir *et al*. (2009) states that gas production indicates the fermentation process of organic matter. Decreased gas production is also thought to be due to the decreasing digestibility value of the treated feed. This is in accordance with research of Assakur (2013) which states that the resulting gas production illustrates the microbial activity of the rumen in digesting feed.

Degradability

The results of the research and statistical analysis of dry matter and organic matter degradability in the rumen *in vitro* are presented in Table 4. Table 4 shows that all treatments at 0 day incubation had no significant effect (P>0.05), on the contrary, each silage treatment at 21 days incubation had a significant effect (P<0.05) on dry matter and organic matter degradability. The average dry matter degradability value at 0 day incubation ranged from 62.40–62.87% and organic matter degradability ranged from 62.84–62.76%. This mean has a lower value when compared to the treatment at 21 days incubation, namely dry matter degradability ranged from 66.16–66.66% and organic matter degradability ranged from 65.67-67.54%. At 21 days incubation, the lowest organic matter degradability value was at T1 (65.67%) increased in T2 (65.78%) and T3 (67.54%). This is due to the increase in nutrient content (especially crude protein content) when of the ensilage, so that the feed degradation process in the rumen is higher and digestibility increases.

Dry matter and organic matter degradability values of each treatment both at 0 day and 21 days incubation had an increase from T_1 then increased in T_2 and T_3. This is due to the addition of 10% molasses and lactic acid starter bacteria with different levels. According to Astutik *et al*. (2019), molasses contains carbohydrates (sucrose) which is a disaccharide group that can accelerate the ensilage process, so that not much silage is dissolved. Nusio (2005) added that the addition of molasses can accelerate the formation of lactic acid and provide energy for bacteria, through the fermentation process it is able to break down complex organic compounds into simpler compounds so that the feed is easily digested.

NH_3

The concentration of NH_3 is the main product of fermentation of feed protein in the rumen by rumen microbes. The results of the statistical analysis of NH_3 concentrations from these silages are presented in Table 5. The results of statistical analysis in Table 5 show that each treatment at 0 day and 21 days incubation had no significant effect (P>0.05). At 0 and 21 days incubation, the lowest NH_3 concentration value was at T_1 then increased in T_2 and T_3. The increase in NH_3 concentration was closely related to the crude protein content which continued to increase from T_1 to T_3. Sutardi (1978) stated that rumen NH_3 production would increase in line with the increase in crude protein content and the increase in the digestibility of the organic matter of

Table 4. Average value of dry matter and organic matter degradability residual gas production 96 hours

Incubation	T	Dry matter degradability (%)	Organic matter degradability (%)
0 day	T_1	62.40±0.82	62.84±0.93
	T_2	62.82±0.09	62.47±0.49
	T_3	62.87±0.95	62.76±0.97
21 day	T_1	66.16±0.42^b	65.67±1.56^a
	T_2	65.70±0.58^a	65.78±0.60^b
	T_3	66.66±0.21^c	67.54±1.11^c

T_1: maize stover silage + molasses 10%, T_2: maize stover silage + molasses 10% + *P. pentosaceus* (1x10^5 cfu/g), and T_3: maize stover silage + molasses 10% + *P. pentosaceus* (1x10^6 cfu/g).
^{a,b,c} Different superscripts at the same row indicate significant differences (P<0.05).

Table 5. Average NH_3 concentration values

Treatment	NH_3 concentration (mM)	
	0 day incubation	21 day incubation
T_1	17.51±0.68	18.10±0.47
T_2	18.14±0.35	18.79±0.86
T_3	18.27±0.11	19.17±0.87

T_1: maize stover silage + molasses 10%, T_2: maize stover silage + molasses 10% + *P. pentosaceus* ($1x10^5$ cfu/g), and T_3: maize stover silage + molasses 10% + *Pediococcus pentosaceus* ($1x10^6$ cfu/g).

Table 6. Average values of microbial protein synthesis

Treatment	Microbial protein synthesis (g N/kg OM apparently fermented)	
	0 day incubation	21 day incubation
T_1	37.99±0.31	37.72±0.13^b
T_2	37.70±0.37	37.84±0.64^a
T_3	38.79±0.85	38.82±0.56^c

^{a,b,c} Different superscripts in the same column showed significant differences (p<0.05).

the feed. Feed protein content can affect microbial reproduction, if the protein content increases, the rumen microbes will increase faster. Increasing the number of microbes in itself will cause an increase in rumen microbial activity in remodeling feed protein into ammonia so that the resulting NH_3 production will also increase.

Microbial protein synthesis

The results of the research and statistical analysis of the average value of Microbial Protein Synthesis are presented in Table 6. The results showed that all treatments with an incubation time of 0 days had no significant effect (P>0.05), on the contrary, all treatments with an incubation time of 21 days had a significant effect (P<0.05) on microbial protein synthesis, in this study ranged from 37.70–38.79 g N/kg apparently OM fermented for 0 days incubation silage treatment and 37.72–38.82 g N/kg apparently OM fermented for treatment with 21 days incubation silage. ARC (1984) explained that the average microbial protein for all feed ingredients in ruminants fermented in the rumen, ranged from 15.45 g N/kg OM apparently fermented per day had a significant effect (P<0.05) on microbial protein synthesis.

Table 6 shows that T_3 has the highest average Microbial protein synthesis value, both at 0 day incubation (38.79±0.85 g N/kg OM apparently fermented) and at 21 days incubation silage (38.82±0.56 g N/kg OM apparently fermented). According to Arora (1989) microbial protein synthesis depends on the speed of nitrogen breakdown of feed, the absorption rate of ammonia and amino acids, the microbial needs for amino acids, and the type of rumen fermentation which is influenced by the type of feed.

T_3 treatment feed had the highest value compared to T_1 and T_2, this could be due to differences in the level of starter lactic acid bacteria in the treated feed ingredients, where in T_3 was added a starter of lactic acid bacteria which was higher ($1x10^6$ cfu/g) compared to other treatments, T_1 (without bacterial starter) and T_2 ($1x10^5$ cfu/g), this is because the lactic acid bacterial starter itself is actually a source of protein, so that when the lactic acid bacteria ensilage process will release the binding protein which is converted into available protein. Protein in feed is degraded in the rumen and will produce N-NH_3 which is needed by rumen microorganisms to synthesize their body protein (Suryani *et al*., 2014).

Conclusions

Addition of molasses and *Pediococcus pentosaceus* bacteria (level $1x10^6$ cfu/g) with an incubation period of 21 days when making maize stover silage can be produce good quality silage to *in vitro* digestibility, fermentation product, and microbial protein synthesis. The result of the study have a positive impact on farmers (how to meet forages need in the dry season by using maize stover).

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