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Effect of Dried Rumen Content and Carbohydrate Sources in Ensiling Process on Corn Stover Silage (*Zea mays*) to Increasing Nutrient Contents and InVitro Digestibility

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***Abstract*— *The purpose of this study was to determine the effect of addition of inoculant (dried rumen content) and/or additives (rice bran and cassava flour) and incubation time on nutrient content of Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Neutral Detergen Fiber (NDF), in vitro DM digestibility and in vitro OM digestibility of corn stover silage. Corn stover silage was made 100% corn stover with inoculant and/or additives with treatments P_0 (without addition as control), P_1 (4% additives), P_2 (4% mixed of inoculant and additives (1:0,5 weight ratio), and P_3 (4% inoculant + 4% additives) with incubation time i.e. 2, 4 and 21 days. The incubation time is 0 days for comparison before the ensilage process. The variables observed were DM, OM, CP, NDF, in vitro DM digestibility and in vitro OM digestibility. Data were analysis of variance (ANOVA). The method used in the nutrient content test is a 4x3 factorial Completely Randomized Design (CRD) with 4 replications and continued with Duncan's Multiple Range Test (DMRT). The method used in the digestibility test is an experimental method with a CRD with 4 treatments and 4 replications. The results showed that addition of inoculant and/or additives gave highly significant effect ($P<0.01$) on DM, OM, CP, NDF, significantly effect ($P<0.05$) on DM digestibility, but not significantly different OM digestibility. The incubation time showed gave highly significant effect ($P<0.01$) on DM, OM, CP, and NDF. Interactions showed not significantly effect in DM, OM, CP and NDF. The results of the study can be concluded with addition of inoculants and/or additives were effect on increased of DM, OM, CP, DM digestibility, and reduced on NDF, and not affect on OM digestibility in corn stover silage (Zea mays).*

***Keywords*— *Additives, inoculant, nutrient content, incubation time*.

I. INTRODUCTION

Fresh forage fodder is a feed support to ruminant productivity. The obstacle faced by farmers is the availability of forage fodder which is limited by the season. Availability of forage fodder in the rainy season, whereas in the dry season is very difficult. Efforts to preserve fresh forage called silage are expected to be a solution to overcome the problem of forage fodder deficiency in the dry season. Manufacture this silage an effort to preserve forage fodder in the rainy season. Manufacture silage is expected to maintain and increase the quality of forage fodder for livestock productivity.

The principle of making silage is the process of forage fodder fermentation by lactic acid-producing microorganisms in anaerobic conditions so as to inhibit the production of spoilage microorganisms. The addition of inoculants can support the fermentation process. The rumen contents is by-products of slaughterhouses which can be used as inoculants for corn stover silage because it still contain lactic acid bacteria (LAB). The rumen contents used are better dried so that the ensilage process optimally.

The low content of dry matter and water soluble carbohydrate (WSC) in fresh forage fodder in the tropics causes low quality of fermentation. Therefore, did wither process before the manufacture of corn stover silage. Increase of WSC can was by addition additives form cassava flour and rice bran which is a source of soluble carbohydrates as a substrate for the development of LAB in lactic acid production so inhibit the production of spoilage microorganisms. The purpose of this study was to determine the effect of addition of inoculant and/or additives and incubation time on nutrient content of DM, OM, CP, NDF, in vitro DM digestibility and in vitro OM digestibility.

II. MATERIALS AND METHODS

A. Located of research

The research was conducted in the Sumbersekar Field Laboratory, Dau, Malang, East Java, Indonesia for the manufacture of corn stover silage, Laboratory of Nutrition and Animal Feed, Faculty of Animal Science, Brawijaya University, Malang, East Java, Indonesia for proximate analysis (DM, OM and CP), NDF analysis, and in vitro DM digestibility and OM digestibility(according to [22].

B. Material

The material used in this study was corn stover silage with addition of a dried rumen content as inoculant and a mixture of rice bran and cassava flour (ratio 3: 1) as an additive. Corn stover is obtained from corn farmer near the Sekar Sumber Field Laboratory, Faculty of Animal Science, Universitas Brawijaya, Malang, East Java, Indonesia. Corn stover is cut at aged between 70-80 days after planting then discarded parts of the cob and flowers to homogenize and then chopped using chopper machine after it was withered for 24 hours. Sixteen kg of fresh rumen content collected from Local Slaughterhouse

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located in Malang from 8 beff cattles. All rumen content then put in an oven 60^o C for 2-3 days [6]. Additives used cassava flour and rice bran with grade "A" obtained from local market near Malang.

C. Research Methods

1. Nutrient Content of DM, OM and CP

The method used in the nutrient content test is a 4x3 factorial Completely Randomized Design (CRD) with 4 replications and continued with Duncan's Multiple Range Test (DMRT). Factor A is the addition of inoculant and/or additives with treatments P_0 (without addition as control), P_1 (4% additives), P_2 (4% mixed of inoculant and additives (1:0,5 weight ratio), and P_3 (4% inoculant + 4% additives). Factor B is incubation time i.e. 2, 4 and 21 days. The incubation time is 0 days for comparison before the ensilage process.

2. In Vitro DM Digestibility and In Vitro OM Digestibility (according to [22])

The method used is an experimental method with a Completely Randomized Design (CRD) 4 treatments and 4 replications. The incubation time is only used for 21 days. The treatments used are P_0 (without addition as control), P_1 (4% additives), P_2 (4% mixed of inoculant and additives (1:0,5 weight ratio), and P_3 (4% inoculant + 4% additives).

III. Result and Discussion

A. DM Content

Data of DM content of corn stover silage in various treatments and incubation times were presented in Table 1.

TABLE 1. DM content (%) of corn stover silage in various treatments and incubation times

Treatments	Incubation Time				Mean
	0 day	2 day	4 day	21 day	(2 – 21 day)
P_0	24.53 ± 1.27	21.67 ± 0.86	21.77 ± 1.06	22.70 ± 0.36	22.05 ± 0.89^p
P_1	25.32 ± 1.00	23.66 ± 1.03	23.91 ± 0.34	25.41 ± 0.30	24.33 ± 1.00^q
P_2	25.40 ± 1.90	22.34 ± 2.70	24.39 ± 1.10	25.53 ± 0.43	24.09 ± 2.07^q
P_3	27.57 ± 1.06	26.68 ± 0.70	26.64 ± 0.90	28.28 ± 0.44	27.20 ± 1.02^r
Mean	25.70 ± 1.68	23.58 ± 2.42^x	24.18 ± 1.96^x	25.48 ± 2.07^y	

Notes :
$^{p-r}$) Value with different superscript showed highly significant effect (P<0.01) of treatments
$^{x-y}$) Value with different superscript showed highly significant effect (P<0.01) of incubation time
Source : Result test in Laboratory of Nutrition and Animal Feed, Faculty of Animal Science, Brawijaya University, Malang, East Java, Indonesia (2019)

Data on Table 1 showed that the treatment and incubation time give highly significant effect (P <0.01) on the DM content. Data on Table 1 showed the highest of DM content in P_3 (27.20%), compared with P_1 (24.33%) and P_2 (24.09%). The addition of additives as an accelerator in the form of cassava flour and rice bran can absorb water in manufacture of silage, so the dry matter will be high and can reduce the water content contained in silage. This is consistent with the statement [19] that the addition of accelerators in the form of non-structural carbohydrates can increase dry matter which is useful for reducing water content, increasing nutrient content. The lowest DM content is in the treatment P_0 (22.05%). This causes the ensilase process to less optimally. The maximum ensilage process can be supported by the addition of dried rumen content and/or high additives to increase the production of lactic acid produced by anaerobic microorganisms so as to inhibit the growth of pathogenic bacteria. This is caused in a low DM content in silage without inoculants and additives (P_0). The results of a study conducted by [12] reported that a higher DM content in manufacture of silage was inoculated with a mixture of several lactic acid bacteria compared to silage without the addition of inoculants.

Data on Table 1 showed that the lowest DM content of silage is incubation time at 4th day (23.58%) and the 2nd day (24.18%). The decrease in DM content is caused by the energy requirements used by the dried rumen content to fermentation process to produce lactic acid. According to explanation by [25] explains that the increase in water content in putak pulp during the fermentation process is caused the decomposition of dry matter used for energy sources by the *Aspergillus oryzae* inoculum so as to reduce the DM content in silage. The highest of DM content is in the 21st day incubation time that is 25.48%. This is due to the low pH of the 21st day incubation time causing the polysaccharide hydrolysis process to increase rapidly so that it absorbs a lot of starch so that it can increase the DM content and reduce the water content of the material. According to report by [3] that the longer the fermentation process, the water content of silage will decrease due to the degradation process by microorganisms during the fermentation process which causes the ability of the material to retain water retention so that the dry matter content of silage will increase. This is reinforced by the opinion [21] that in the long time of fermented tofu pulp at the 48th hour decreased due to the ability of the material to retain water decreases so that the dry matter is increasing.

B. OM Content

Data of OM content of corn stover silage in various treatments and incubation times were presented in Table 2.

Data on Table 2 showed that the treatment and incubation time have highly significant effect (P<0.01) on the OM content. Data on Table 2 showed that the highest OM content was in treatment P_1 with a content of 91.03% DM. This is caused by the use of additives in the form of cassava flour and rice bran with a total addition of 4%. This showed that the fermentation process optimally because LAB microorganisms utilize OM to the maximum with the use of cassava flour and rice bran with a total of 4% to do activities to produce organic acids. The result of raw material of cassava flour test has a OM content of 97.77% DM and a rice bran OM content of 92.89% DM. This is indicated that the high additives raw

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material in the use of silage affects the OM content in silage. According to the results of the report [7] that the addition of cassava flour in the manufacture of vegetable waste silage can increase the content of organic matter because cassava flour has a high OM content. Low of OM content is P_2 (89.61% DM) and P_3 (8.62% DM). This is because the percentage of addition of additives and inoculants is small, so that soluble carbohydrates and inoculants are insufficient the needs of LAB microorganisms to do fermentation processes in producing lactic acid, LAB microorganisms will utilize corn forage in carrying out their activities. This is consistent with the statement [7] that the low levels of organic matter in the silage of vegetable waste causes LAB microorganisms not be able to utilize soluble carbohydrates in their activities, so that they only utilize enzymes from vegetable waste in the fermentation process.

TABLE 2. OM content (%DM) of corn stover silage in various treatments and incubation times

Treatments	Incubation Time				Mean
	0 day	2 day	4 day	21 day	(2 – 21 day)
P_0	90.24 ± 0.05	90.86 ± 0.17	90.25 ± 0.25	90.70 ± 0.28	90.60 ± 0.35^q
P_1	89.98 ± 0.67	91.33 ± 0.74	90.66 ± 0.30	91.10 ± 0.09	91.03 ± 0.51^r
P_2	90.12 ± 0.41	89.42 ± 0.25	89.54 ± 0.46	89.88 ± 0.19	89.61 ± 0.36^p
P_3	90.40 ± 0.38	89.91 ± 0.75	89.40 ± 0.15	89.55 ± 0.33	89.62 ± 0.49^p
Mean	90.18 ± 0.42	90.38 ± 0.92^y	89.96 ± 0.60^x	90.31 ± 0.68^x	

Notes :
$^{p-r}$) Value with different superscript showed highly significant effect (P<0.01) of treatments
$^{x-y}$) Value with different superscript showed highly significant effect (P<0.01) of incubation time
Source : Result test in Laboratory of Nutrition and Animal Feed, Faculty of Animal Science, Brawijaya University, Malang, East Java, Indonesia (2019)

The OM content fluctuates with the length of incubation. Data on Table 2 showed the highest OM contents in the incubation time to 2nd days with content 90.38% DM. This refers to the incubation time of 2nd and 21st is the optimal time for microorganisms to degrade organic matter used to support the fermentation process. The lowest OM content was in the 4th day incubation time (89.96% DM) and 21 days (90.31% DM). Reduced of OM content is caused by the overhaul of organic matter containing soluble carbohydrate by dried rumen content to energy needs in the growth of microorganisms so as to increase the composition of the ingredients. This is in accordance with the opinion [24] that the cause of the decrease in organic matter is due to the use of organic matter which support the fermentation process in organic matter components containing soluble carbohydrate, so that lactic acid increases and pH decreases.

C. CP Content

Data of CP content of corn stover silage in various treatments and incubation times were presented in Table 3.

TABLE 3. CP content (%DM) of corn stover silage in various treatments and incubation times

Treatments	Incubation Time				Mean
	0 day	2 day	4 day	21 day	(2 – 21 day)
P_0	9.03 ± 0.35	9.17 ± 0.35	9.11 ± 0.59	9.06 ± 0.14	9.12 ± 0.37^p
P_1	10.41 ± 0.16	9.78 ± 0.51	11.63 ± 1.85	9.70 ± 0.27	10.37 ± 1.38^q
P_2	9.85 ± 0.55	8.97 ± 0.46	10.39 ± 1.32	9.67 ± 0.35	9.68 ± 0.97^{pq}
P_3	9.73 ± 0.41	9.20 ± 0.54	12.12 ± 2.08	9.70 ± 0.51	10.34 ± 1.76^q
Mean	9.76 ± 0.62	9.28 ± 0.53^x	10.81 ± 1.85^y	9.53 ± 0.42^x	

Notes :
$^{p-q}$) Value with different superscript showed highly significant effect (P<0.01) of treatments
$^{x-y}$) Value with different superscript showed highly significant effect (P<0.01) of incubation time
Source : Result test in Laboratory of Nutrition and Animal Feed, Faculty of Animal Science, Brawijaya University, Malang, East Java, Indonesia (2019)

The results of the CP content analysis showed highly significant effect (P<0.01) on treatment and incubation time. Data on Table 3 showed the highest CP content in the P_1 treatment (10.37% DM) and P_3 (10.34% DM), but the effect was not significantly different from P_2 (9.72% DM). The lowest CP content is in the P_0 treatment as much as 9.12% DM. The high and low CP content in each treatment is caused by the raw material and the percentage of the addition of inoculant and/or additives given. This is evident that the proximate test of raw mattter in manufacture corn stover silage produces the highest CP content to the lowest, namely in rice bran, corn stover, inoculant and cassava flour which have CP content, respectively 12.54% DM, 9.76% DM, 9.14% DM and 2.53% DM. The higher the addition of additives and inoculant will increase the CP content in silage. The increase in CP content in fermented corn stover silage is due to a change in the proportion of the nutrient component, ie carbohydrates, while protein has increased. This is due to the fact that the treatment has more optimal microorganism condition factors which are balanced with substart conditions the needs of microorganisms that affect the performance of microbes in fermenting soluble carbohydrate material. This is what causes an increase in protein in corn stover silage. The results of another study conducted [4] reported that fermentation using the addition of soluble carbohydrate sources in the form of rice bran can increase the highest protein, because rice bran contains high protein, vitamins and minerals needed by microorganisms in microbial protein synthesis. This is reinforced by the opinion [2] that the amylase enzyme activity can increase the CP content, the higher the amylase activity, the higher the protein content produced because the amylase enzyme plays a role in providing simple sugars as a basic ingredient for protein synthesis.

Data on Table 3 showed the average CP content of corn stover silage at the highest on day 4th incubation time with a content of 10.81% DM. The incubation time can increase CP content in corn stover silage. This indicates that the incubation period of the 4th day is the optimal time for proteolytic bacteria to degrade proteins. According to [11] reported that along with the incubation process, the microorganisms will utilize the nutrient content of the substrate to synthesize the body's own microorganism protein so that proteoilytic bacteria will multiply and can increase the protein content in the feed. The lowest CP content was in the incubation period of 2nd day (9.28% DM) and 21 days (9.53% DM). CP content decreased when compared with the length of incubation on the 0th day before the ensilase took place. This is because the process of protein degradation carried out by proteolytic microorganisms into amino acids and NH_3 during the storage process is used by other microorganisms. The use of nitrogen sources in fermentation media for protein synthesis in cells. According to a statement [14] states that the absorption of cells by nitrogen sources causes the protein content in the media decreases along with the length of the fermentation process.

D. NDF Content

Data of NDF content of corn stover silage in various treatments and incubation times were presented in Table 4.

TABLE 4. NDF content (%DM) of corn stover silage in various treatments and incubation times

Treatments	Incubation Time				Mean
	0 day	2 day	4 day	21 day	(2 – 21 day)
P_0	69.01 ± 2.10	70.57 ± 0.95	71.06 ± 3.76	66.63 ± 1.52	69.42 ± 3.00^q
P_1	64.97 ± 2.56	69.03 ± 2.59	66.33 ± 2.87	61.97 ± 0.81	65.78 ± 3.67^p
P_2	69.00 ± 1.90	71.52 ± 1.85	71.74 ± 3.74	66.92 ± 0.74	70.06 ± 3.21^q
P_3	64.18 ± 1.74	66.50 ± 2.51	67.52 ± 3.93	63.60 ± 0.34	65.87 ± 3.00^p
Mean	66.79 ± 2.97	69.41 ± 2.70^y	69.16 ± 3.99^y	64.78 ± 2.31^x	

Notes :
p-q) Value with different superscript showed highly significant effect (P<0.01) of treatments
x-y) Value with different superscript showed highly significant effect (P<0.01) of incubation time
Source : Result test in Laboratory of Nutrition and Animal Feed, Faculty of Animal Science, Brawijaya University, Malang, East Java, Indonesia (2019)

Data on Table 4 showed NDF content have highly significant difference (<0.01) for the treatment and incubation time. Data on Table 4 showed that the treatment had the highest NDF content of corn stover silage in P_2 (70.06% DM) P_0 (69.42% DM). This indicates that the lignocellulosic binding is still strong so it is difficult to digest. This is reinforced by reports [23] that if the NDF content is high, the bonding of cell walls during storage, especially lignocellulose is still strong so that feed is difficult to digest by livestock. High NDF content in plants according to [20] that will be less good if used as feed because it can inhibit the digestive process optimally by livestock. The lowest NDF content was in P_3 (65.87% DM) and P_1 (65.78% DM). The addition of inoculant and/or additives in the form of cassava flour and rice bran with a percentage of 4% each can support the fermentation process of corn forage silage can reduce NDF. The decrease in NDF content is thought to be due to the performance of microorganisms that break the bond of crude fibers in silage. According to statement [13] reported that the decrease in NDF content after feed treatment showed the breakdown of cellulose cell walls so that the feed was easily digested. This is reinforced by [1] that a decrease in NDF content caused during the fermentation process will result in the breaking of lignohemiselulosa and lignocellulose bonds.

Data on Table 4 showed a low NDF content that is at the 21st day incubation time (64.78% DM). This indicates that the incubation time of the 21st day is an increase in the activity of microbial enzymes that can secrete cellulase enzymes that can degrade fiber fractions. The results of a study conducted [20] reported that the addition of silage with soy sauce additive on storage time for 21st days decreased NDF content due to loosening of the fiber fraction that had reached optimal conditions. The more substrates and the longer the incubation time, the more the content of NDF, ADF and lignin are dissolved. The highest NDF content was at the second incubation time (69.41% DM) and the 4th day (69.16% DM). This is presumably because the microorganism has not yet reached the reshuffle process for the fiber fraction, so that the cell wall bonding during the fermentation process, especially the lignocellulose bond is still strongly bound. According to the results of the report [9] reported that the increase in NDF content in sugarcane shoot silage caused by organic matter which is easily digested has been overhauled by LABduring the ensilage process, so that what remains is organic matter which is not easily digested such as crude fiber.

E. In Vitro DM Digestibility and In Vitro OM Digestibility

Data on in vitro DM digestibility and in vitro DM digestibility (%) of corn stover silage in various treatments and 21 days incubation time were presented in Table 5.

TABLE 5. In vitro DM digestibility and in vitro DM digestibility (%) of corn stover silage in various treatments and 21 days incubation time

Treatments	DM Digestibility*	OM Digestibility
P_0	47.61 ± 4.29^a	48.11 ± 4.03
P_1	51.77 ± 3.53^{ab}	53.12 ± 3.72
P_2	48.77 ± 2.69^{ab}	50.30 ± 2.62
P_3	52.87 ± 1.78^b	52.47 ± 3.34

Note : a-b) Value with different superscript showed highly significant effect (P<0.05) of treatments
Source : Result test in Laboratory of Nutrition and Animal Feed, Faculty of Animal Science, Brawijaya University, Malang, East Java, Indonesia (2019)

Data on Table 5 showed that the addition of dried rumen content and/or additives have significant effect (P <0.05) on DM digestibility. Data on Table 5 showed that the highest of

DM digestibility in the P_3 treatment, but the effect was not significantly different in the P_1 and P_2 treatments. The high DM digestibility is caused by the addition of inoculant and/or additives to the treatment with a level of 4%. The addition of inoculant and additives causes high WSC content in corn stover silage thereby increasing the activity of LAB in degrading feed into organic acids, especially lactic acid. According to the statement [17] that the addition of additives in the form of rice bran as a source of soluble carbohydrates can be utilized by LAB for growth nutrition so that it can produce lactic acid. According to a statement [15] reported that the addition of feed ingredients that contain high soluble carbohydrates in the process of manufacture of silage can increase digestibility. High or low digestibility is influenced by the composition of the protein contained in the silage and NDF content. According to statement [10] that the lower the NDF, ADF, and lignin content in a feed ingredient will increase the digestibility value. This is evidenced in the research data Table 4 with the lowest NDF content in P_3 of 65.87% DM and the highest in P_0 (69.42% DM) and P_2 (70.06% DM). A high NDF content at P_0 indicates a low DM digestibility. A low DM digestibility value is showed in the P_0 treatment silage with a value of 47.61%. This is suspected because microbes are not able to digest the fiber components contained in silage optimally. This is reinforced by the results of the study [5] that DM digestibility in the treatment of odot grass silage control with the addition of 6% molasses has the lowest DM digestibility value caused by increased crude fiber, lignin and hemicellulose content so that microbes are unable to digest fiber components resulting in decreased digestive value.

Data on Table 5 showed showed that the addition of dried rumen content and/or additives did not have a significant effect (P>0.05) on OM digestibility. Data on Table 5 showed the average OM digestibility of corn stover silage in P_0, P_1, P_2 and P_3 range 48.11 - 53.12%. This is influenced by the content of organic matter and protein is almost the same in each treatment. This is proven by research data on OM content (Table 1) and CP (Table 2), that OM content of silage is around 89.74 - 90.77% DM and CP content of silage is around 9.09 - 10.38% DM. This is reinforced by the statement [8] that the digestibility of organic matter is influenced by the protein contained in the feed, because the protein has a different level of solubility and degradation that will affect the overhaul of protein in the rumen and post rumen. The digestibility value of feed was reported by [18] that it is influenced by the chemical composition of feed and fibrous feed fraction.

VI. CONCLUSIONS

The results of the study can be concluded with addition of inoculants and/or additives were effect on increased of DM, OM, CP, DM digestibility, and reduced on NDF, and not affect on OM digestibility in corn stover silage (*Zea mays*).

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