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Research Paper

EFFECT OF *Trichoderma harzianum* IN *Panicum maximum* cv. *mombaca* ENSILING PROCESS ON PHYSICAL QUALITY, NDF AND ADF

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Abstract

This research purpose was to evaluate the effect of addition mollasses and *Trichoderma harzianum* and incubation time on physical quality, Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) *Panicum maximum* cv. *mombaca* (PMM) silage. The materials used PMM, *Trichoderma harzianum*, and mollasses. The method were laboratory experiment using 4x3 Nested Completly Random Design (CRD Nested) replicated 4 times. Twelve treatments were defferentiated on four treatment namely S_1 (PMM), S_2 (PMM + 5% mollasses), S_3 (PMM + 5% mollasses + 3% *Trichoderma harzianum*) and S_4 (PMM + 5% mollasses + 5% *Trichoderma harzianum*) with three incubations time were 4, 8, and 12 days. The variables observed were physical quality (color, smell, texture, existance of mucus and fungus), NDF and ADF content. Data obtained was analyzed using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The results showed that the addition mollasses and *Trichoderma harzianum* and incubation time had different effect on physical quality and gave highly significant effect ($p<0.01$) on NDF and ADF content. The conclusion was Ensilage of *Panicum maximum* cv. *mombaca* with addition of 5% mollases and 5% *Trichoderma harzianum*'s and 12 days incubation time improve the physical quality, decrease NDF and ADF even though 5% mollasses and 3% *Trichoderma harzianum* with 12 days incubation time was enough to provide an improvement on physical quality, and decreasing of ADF and NDF content.

Key words: *Silage, Trichoderma harzianum, Panicum maximum cv. Mombaca, physical quality, NDF, ADF*.

INTRODUCTION

Panicum maximum cv. *mombaca* is known as *guanea* grass had highly on palatability and quality. The productivity quite high in rainy season about 15.5 – 15.8 tons dry matter/ha/year with stem composition of 2.5 - 3.4 tons of dry material/ha/year and leaves 11.8 - 13, 1 ton of dry matter/ha/year [6]. The nutrient content of *guenea* grass consists of 25.41% dry matter (DM), Ash 12.85%, crude protein (CP) 12.89%, crude fiber (CP) 33.19%, crude fat (EE) 1.34%, total digestible nutrient (TDN) 38 - 61%, and nitrogen free extract (NFE) 40 - 50% [5].

Potential production and nutrient content and abundant production in the rainy season provide solutions for the fulfillment of quality forage in the dry season by utilizing forage feed preservation technology through the ensilage process. Ensilage utilizes lactic acid bacteria which produce lactic acid marked by pH decline. The speed on the ensilage process needed to ensure growth of lactic acid bacteria and inhibit the growth of harmful microorganisms [15] . Modifications to the ensilage process have evolved by utilizing inoculants as agents to accelerate pH reduction [10],[15],[17]. Inoculants can also be used to improve the stability of an aerob conditions in the ensilage process [16].

This research utilizes *Trichoderma harzianum* fungus and mollasses to speed up ensilage process and improve the quality of *Panicum maximum* cv. *mombaca* grass silage. *Trichoderma* sp. was a fungus that can be utilize fiber materials. According to [20] *Trichoderma harzianum* able to produce endoglucase, β-glucosidase and xylanase enzymes. Research of [13] states that the addition of a fiber-breaking enzyme can improve the fermentation process.

MATERIALS AND METHODS

2.1 Materials

The material has been used were *Panicum maximum* cv. *mombaca*, mollasses, *Trichoderma harzianum* with conidia density 11.09 log cfu/g, chopper, glass jar, plastic wrap, scale, and vacuum pump.

2.2 Methodes

The research method was a laboratory experiment using Nested Complately Randomized Design (Nested CRD) 4x3 replicated 4 times. Twelve treatments were deferentiated on four treatment added mollasses and *Trichoderma harzianum* consists of :

- S_1 : *Panicum maximum* cv. *mombaca* (PMM)
- S_2 : PMM + 5% mollasses

- S_3 : PMM + 5% mollasses + 3% *Trichoderma harzianum*
- S_4 : PMM + 5% mollasses + 5% *Trichoderma harzianum*

and three incubation time was 4 (I_4), 8 (I_8), and 12 (I_{12}) days.

Silage making : *Panicum maximum* cv. *mombaca* was harvested manually at 45 – 55 days old regrowth. The grass were chopped 2 – 3 cm length, and wilted for 12 hours in order to reduce the moisture content. Then the grass added *Trichoderma harzianum* 3% or 5% (w/w) and 5% mollasses (w/w). Filling and compacting with the vacuum pump was done stimultaneously to eliminate inherent air. The silage was prepared in 1 kg plastic jar, compressed and sealed with wrap plastics then covered and sealed again with plastic isolation then wrapped again.

2.3 Variables

The variables observed were physical quality of *Panicum maximum* cv. *mombaca* silage (color, odor, texture, exsistence of fungus and mucus), NDF and ADF content.

The assessed quality characteristics were color, odor, texture, pH according modified of [22] . Assessment of color, odor, texture, mucus and fungi assesed by 15 panelist every incubation time with filling the quesioneer based on Table 1

Table 1. *Panicum maximum* cv. *mombaca* silage assesment

Score	Color	Odor	Texture	Mucus	Fungus
1	Dark brown	Very bad	Very hard	Much	Much
2	Light brown Brrown	Bad	Little hard	Medium	Medium
3	Light Yellow	Fresh	Little Firm	Little	Little
4	Olive	Light acid	Medium Firm	Least	Least
5	Fresh green	Acid, light	Very Firm	None	None

2.4 Chemical and data analysis

The Fiber components including Neutral Detergent Fiber (NDF) and ADF (Acid Detergent Fiber) content of the silages were determined according to [20]. Data obtained were analyzed using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). While physical assesment were analyzed as descriptive analysis.

RESULT

3.1. Chemical composition

Chemical composition was considerable variation among raw material of trial before incubation shows in Table 2. Dry matter (DM) content renged from 34 – 36% and the cude protein (CP) content range from 8,96 – 9,55%. The treatment of S_2, S_3 and S_4 that have used molasses tend to increased on DM content.

Table 2. Nutriet Composition Before Incubation

Nutrient	Treatment			
	S_1	S_2	S_3	S_4
DM (%)	34.00	36.00	36.00	35.00
Ash* (%)	12.70	12.44	11.72	12.14
OM* (%)	87.30	87.56	86.62	87.86
CP* (%)	9.51	9.21	8.96	9.55
EE* (%)	3.99	2.97	2.69	3.59
CF* (%)	26.11	20.75	21.00	21.40
NDF* (%)	46.13	39.46	41.50	43.12
ADF* (%)	26.51	22.81	24.57	24.91

Note : *) Based on 100% dry matter,
Analysis result from Nutrition Laboratory, Faculty of Animal Science, University of Brawijaya.
*) DM (dry matter), OM (organic matter), CP (crude protein), EE (extract ether), CF (crude

fiber), NDF (neutral detergent fiber), ADF (acid detergent fiber)

*) S_1 = PMM ; S_2 = PMM + 5% mollasses ; S_3 = PMM + 5% mollasses + 3% *Trichoderma harzianum* ;

S_4 = PMM + 5% mollasses + 5% *Trichoderma harzianum*

3.2. pH of *Panicum maximum* cv. *mombaca* silage

The pH of *Panicum maximum* cv. *mombaca* silage shows in Table 3.

Table 3. The effect of treatment on pH

Treatment	pH
S_1	4.74^b
S_2	4.06^a
S_3	3.96^a
S_4	3.96^a

Note : $^{a-b}$ The different superscripts in the same colomn shows highly significant effect ($p<0.01$)
*) S_1 = PMM ; S_2 = PMM + 5% mollasses ; S_3 = PMM + 5% mollasses + 3% *Trichoderma harzianum* ;
S_4 = PMM + 5% mollasses + 5% *Trichoderma harzianum*

The S_1 treatment had higher pH compare with the other treatment, besides the S_2, S_3 and S_4 treatment had lower pH.

3.2. The effect of treatment on *Panicum maximum* cv. *mombaca* silage pysical quality

Table 4 showed that the effect of treatment on *Panicum maximum* cv. *mombaca* silage pysical quality. The S_1 treatment had the highest color score compare with S_2, S_3 and S_4. The score from high to low respectively S_1 (4.21), S_4 (4.06), S_3 (3.87) and S_2 (3.74). The highest color score tends to fresh green and the lowest tends to olive color. The change in silage color can be made by an utilization of nutrients during ensilage process by lactic acid bacteria and the increasing of temperature in silo. A good silage color according to [12] usually matches with the original color of the forage, when the forage used is green, the silage will be green or yellow.

Table 4. The effect of treatment on pysical quality

Assessment	S_1	S_2	S_3	S_4
Color	4.21 ± 0.336	3.74 ± 0.232	3.87 ± 0.208	4.06 ± 0.199
Odor	4.06 ± 0.152	4.42 ± 0.236	4.24 ± 0.397	4.28 ± 0.433
Texture	2.22 ± 0.474	2.72 ± 0.434	2.71 ± 0.611	2.77 ± 0.599
Mucus	4.98 ± 0.033	4.96 ± 0.033	4.96 ± 0.034	4.96 ± 0.033
Fungus	5.00 ± 0.000	5.00 ± 0.000	5.00 ± 0.000	4.97 ± 0.045

Note : *) The score based on panelist assessment
*) S_1 = PMM ; S_2 = PMM + 5% mollasses ; S_3 = PMM + 5% mollasses + 3% *Trichoderma harzianum* ;
S_4 = PMM + 5% mollasses + 5% *Trichoderma harzianum*

The odor of *Panicum maximum* cv. *mombaca* silage on Table 4 show that the S_2 treatment had the best odor (score 4.42) with the odor tends to acid and light sweet while S_1 had lower odor (score 4.06) with the odor tends to light acid. The ensilage process involves lactic acid bacteria utilization of WSC sources, this lactic acid bacteria produces lactic acid. A good silage should not be rancid, this odor can be caused by the presence of Clostridia fermentation in wet silage [2]. The smell of vinegar must also be avoided in a silage because of the production of acetic acid, this happens in wet silage. While alcoholic odor on silage caused by the presence of yeast that indicates imperfect closure when taking silage then air penetration occurs in the silo [8].

The texture score of the *Panicum maximum* cv. *mombaca* silage shows the higest score on S_4 treatment tends to medium firm texture with the score was 2,77 while the score of S_1 treatment lowest (score 2.22) tends to little hard texture. Silage texture had an important role in determining the quality of animal feed silage. Good silage had a soft texture and the water and the smell did not come out when it squeezed [2]. According to [12] good texture silage had well recognized on the stem or leaf .

Observation of the presence of mucus and fungus showed that descriptively the highest score observed in the *Panicum maximum* cv. *mombaca* without treatment (S_1) while the treatment of *Panicum maximum* cv. *mombaca* + molasses (S_2) had the lowest score and found very little mucus. The silage had a thick and slippery appearance indicates the activity of proliferating of proteolitic microbes [8]. The presence of fungal populations indicates less compaction on silage making process and high on dry mater content [8]. Some fungi such as *Aspergillus* spp., *Fusarium* sp., *Listeria monocytogenes* produce mycotoxins besides dangerous for livestock health, it will be reduce the nutrient quality of silage.

3.3. The effect of incubation time on *Panicum maximum* cv. *mombaca* silage physical quality

Table 5 shows the effect of incubation time on *Panicum maximum* cv. *mombaca* silage pysical quality. The higest color (score 4.58) was in combination of S_1 treatment with 8 days incubation time (S_1I_8), tends to fresh green color. The lowest color (score 3.60) was S_2 with 12 days incubation time (S_2I_{12}), tends to olive color.

The higest odor (score 4.62) were combination of S_4 treatment with 12 days incubation time (S_4I_{12}), tends to acids and light sweet odor. The lowest odor (score 3.73) found on S_3 with 4 days incubation time (S_3I_4), tends to light acid odor.

The highest texture (score 3.45) were combination of S_3 and 12 days incubation time (S_3I_{12}), tends to medium firm texture. While the lowest score texture (score 1.6) were combination S_1 and 4 days incubation time (S_1I_4), tends little hard texture. The physical quality of S_3 treatment with 12 day incubation time is the same research of [3] on *Panicum maximum* grass silage when the grass cut at 84 days with a light yellow color, good odor and soft texture. Using *Trichoderma harzianum* as inoculan and mollasses could be seen had a better texture at 12 days incubation, with textures tends to medium firm.

Table 5 : The effect of incubation time on physical quality

Assesment	Treatment	Incubation time (days)		
		4	8	12
Color	S_1	3.87 ± 0.133	4.58 ± 0.100	4.17 ± 0.200
	S_2	4.00 ± 0.196	3.63 ± 0.159	3.60 ± 0.054
	S_3	4.12 ± 0.100	3.80 ± 0.122	3.70 ± 0.086
	S_4	3.98 ± 0.100	4.08 ± 0.167	4.10 ± 0.310
Odor	S_1	4.02 ± 0.126	3.97 ± 0.086	4.20 ± 0.144
	S_2	4.15 ± 0.158	4.53 ± 0.144	4.57 ± 0.128
	S_3	3.73 ± 0.144	4.42 ± 0.084	4.58 ± 0.100
	S_4	3.78 ± 0.367	4.43 ± 0.139	4.62 ± 0.148
Texture	S_1	1.60 ± 0.054	2.43 ± 0.115	2.63 ± 0.086
	S_2	2.18 ± 0.220	2.92 ± 0.175	3.07 ± 0.122
	S_3	2.10 ± 0.038	2.57 ± 0.296	3.45 ± 0.167
	S_4	1.98 ± 0.033	3.02 ± 0.100	3.30 ± 0.159
Mucus	S_1	5.00 ± 0.000	5.00 ± 0.000	4.93 ± 0.000
	S_2	5.00 ± 0.000	4.93 ± 0.000	4.93 ± 0.000
	S_3	5.00 ± 0.000	4.95 ± 0.033	4.93 ± 0.000
	S_4	5.00 ± 0.000	4.93 ± 0.000	4.93 ± 0.000
Fungus	S_1	5.00 ± 0.000	5.00 ± 0.000	5.00 ± 0.000
	S_2	5.00 ± 0.000	5.00 ± 0.000	5.00 ± 0.000
	S_3	5.00 ± 0.000	5.00 ± 0.000	5.00 ± 0.000
	S_4	5.00 ± 0.000	5.00 ± 0.000	4.92 ± 0.033

Note : *) The score based on panelist assessment

*) S_1 = PMM ; S_2 = PMM + 5% mollasses ; S_3 = PMM + 5% mollasses + 3% *Trichoderma harzianum* ; S_4 = PMM + 5% mollasses + 5% *Trichoderma harzianum*

The little mucus were found in 8 days incubation time of S_2, S_3 and S_4 treatment. While fungus were found in 12 days of incubation time only in S_4 treatment. Fungal and mycotoxin contamination in silage allows the loss of dry matter and when the content more than 1 x 10^4 cfu in the forage will cause disturbance of respiratory, the rumen

fermentation, the reproductive function, kidney disorders, irritation of the skin and eyes, decreases palatability and animal performance [1].

3.3. The Effect of treatment on *Panicum maximum* cv. *mombaca* silage NDF and ADF content

Table 6 shows the effect of adding *Trichoderma harzianum* and mollasses on NDF and ADF content. The treatment shows highly significant effect ($p<0.01$) on NDF and ADF content. The highest NDF was S_1 treatment (50.48%) and the lowest NDF was S_4 (44.50%). The lowest NDF content on S_4 treatment was estimated due to the degradation of the crude fiber by *Trichoderma harzianum*'s enzymes . According [11] *Trichoderma harzianum* can produce the cellulolytic enzymes and increase the activity of other fiber-breaking enzymes such as phasease, β-glucanase, endo glucanase and endoxylanase,otherwise the S_1 treatment did'n have material for crude fiber degradation.

Table 6. Effect treatment on NDF and ADF

Treatment	NDF (%)	ADF (%)
S_1	50.48^{b}	29.47^{d}
S_2	46.68^{ab}	27.13^{a}
S_3	45.58^{a}	29.00^{c}
S_4	44.50^{a}	27.85^{b}

Note : $^{a-b}$ The different superscripts in the same colomn shows highly significant effect ($p<0.01$)
*) S_1 = PMM ; S_2 = PMM + 5% mollasses ; S_3 = PMM + 5% mollasses + 3% *Trichoderma harzianum* ; S_4 = PMM + 5% mollasses + 5% *Trichoderma harzianum*

The NDF content of S_1 treatment almost the same as the research of [3] on *Panicum maximum* grass silage with an incubation period of 47 days with the age of 28 days cutting age (40.30%) while 84 days of cutting age had NDF content of 53.60%. NDF content represents a fiber fraction of the cell wall content consisting of cellulose, hemicellulose, lignin, and proteins bound to the cell wall [20]. The fermentation process of ensilage was possible to stretch the hemicellulose bonds result a decreasing NDF content. Plants had a fiber content that consists of three main components, it was cellulose, hemicellulose, and lignin. Cellulose and hemicellulose can be digested by rumen microbes to produce energy for ruminants. While the lignin component cannot utilized by ruminants because rumen microbes cannot break down lignin to be utilized

as energy. The NDF content also influenced by plant age and season factors such as reaserch of [6]. The NDF content on [15] research was decreased by giving inoculants from 59.20% to be 57.21% in *Panicum maximum* grass silage with 65 days cutting age. Research of [23] showed that the addition of cellulase enzymes and 3 cm chopped treatment of *Panicum maximum* Jacq. did not make a significant difference on NDF content. States of [14] that fertilization using different nitrogen fertilizers affects the increasing NDF content of *Panicum maximum* cv. m*ombaca* silage related to the increasing of larger grass size that requires more fiber to support it.

The ADF content after incubation (Table 6) was increasing in S_1 (25.38%), S_2 (18.94%), S_3 (18.55%), and S_4 (1.80 %) treatment compared with before incubation (Table 2). The lowest ADF content increasing shows the S_4 treatment, this proves that *Trichoderma harzianum* plays a role in producing enzymes to break down crude fiber. An increasing ADF content indicates reducing cell content in *Panicum maximum* cv. *mombaca* grass. This research as same as opinion of [10] and [15] that using inoculants gave lower ADF content.

3.4. The Effect incubation time on *Panicum maximum* cv. *mombaca* silage NDF and ADF content

Table 7 shows the effect of *Panicum maximum* cv. *mombaca* silage incubation time on NDF and ADF content. The incubation time had highly significant effect ($p<0.01$) on NDF and ADF content. The higest NDF content (52.49%) was combination S_1 treatment and 12 days incubation time (S_4I_{12}). While the lowest NDF content (42.20%) was combination of S_4 treatment and 12 days incubation time (S_4I_{12}).

The NDF content influenced by the crop age, as stated by [15] that the NDF content *of Panicum maximum* grass with 35-65 days cutting age was 55.85% - 64.12%, the longer cutting age then NDF content was higher. When The the grass get older it will be followed by the addition of the dry matter and fiber content. The proportion of stems on old grass will be higher than the young grass then the cell content and nutrient value will be decrease with increasing age of grass. Research by [4] in *Panicum maximum* grass silage without treatment had an increase in NDF content.

Table 7. Effect incubation time on NDF and ADF

Variable	Treatment	Incubation Time (days)		
		I_4	I_8	I_{12}
NDF (%)	S_1	46.55 ± 0.738^A	52.40 ± 0.148^B	52.49 ± 0.638^B
	S_2	42.72 ± 0.351^A	50.81 ± 0.322^C	46.52 ± 0.385^B
	S_3	43.69 ± 0.494^A	46.61 ± 0.128^B	46.45 ± 0.127^B
	S_4	42.20 ± 0.209^A	46.26 ± 0.197^C	45.04 ± 0.241^B
ADF (%)	S_1	25.66 ± 0.407^A	29.52 ± 0.310^B	33.24 ± 0.404^C
	S_2	24.93 ± 0.205^A	28.70 ± 0.002^C	27.78 ± 0.230^B
	S_3	26.53 ± 0.371^A	31.35 ± 0.086^C	29.13 ± 0.080^B
	S_4	25.65 ± 0.127^A	30.29 ± 0.002^C	27.63 ± 0.005^B

Note : $^{A-B}$ The different superscripts in the same colomn shows highly significant effect ($p<0.01$)

*) S_1 = PMM ; S_2 = PMM + 5% mollasses ; S_3 = PMM + 5% mollasses + 3% *Trichoderma harzianum* ; S_4 = PMM + 5% mollasses + 5% *Trichoderma harzianum*

The highest ADF content (33.24%) was combination of S_1 treatment with 12 days incubation time (S_1I_{12}) and the lowest ADF was combination S_2 and 4 days incubation time (S_2I_{12}). Even though the best ADF content was combination S_4 and 12 days incubation time (S_4I_{12}) because it's shows increase ADF content at 8 days incubation time (S_4I_8) but decreased at 12 days incubation time (S_4I_{12}). The same pattern seen in the other treatments but S_4I_{12} treatment (27,63%) had lower ADF content compared S_2I_{12} (27.78%).

The deacreasing ADF content can be caused by the degradation of grass crude fiber from an enzyme produced by *Trichoderma harzianum*. The same value was found by [3] almost the same value of ADF content (34.90%) in *Panicum maximum* silage with 47 - 84 days incubation time compare with the combination of S_1 treatment and 12 days incubation time (33.24%). The ensilage process utilizes soluble carbohydrates for lactic acid bacteria growth then will force other pathogens microorganisms not to grow. Carbohydrates generally classified as fiber carbohydrates and non-fiber carbohydrates. Carbohydrate fraction as ADF more difficult to digest compared to NDF therefore ADF useful for evaluating the digestibility of feed ingredients [19]. The decreasing ADF

content in this research estimated because a stretch of lignocellulose bonds by enzyme that have been produce by *Trichoderma harzanum*. This ADF decreasing will result in a decrease of undigested carbohydrate fraction then it would be increase the feed digestibility.

The using *Trichoderma harzianum* in *Panicum maximum* cv. *mombaca* silage showed decreasing the NDF and ADF contents because the fungi can produce β 1,3-glucanase, cellulase, chitinase, proteinase, and phosphatase enzymes [18]. Research of [21] found that *Trichoderma harzianum* able to produce endoglucase, β-glucosidase and xylanase enzymes therefore they can degraded the fiber components in *Panicum maximum* cv. *mombaca* grass. This research is also supported by the opinion of [12] that the addition of a degraded crude fiber enzyme affects the fermentation process. Stated of [15] that the ADF decreasing by giving inoculants from the ADF content of 28.95%, down to 27.21% in *Panicum maximum* ensilage with 65 days cutting age. Research by [7] showed 10.78% decrease of ADF content in *Panicum maximum Jacq*. silage that was given xylanase enzyme treatment from *Trichoderma longibraciatum* fermentation extract. The other research found the deceasing ADF content of *Panicum maximum Jacq*. silage with addition of cellulase enzymes and cutting size of 3 cm from 47.87% to be 46.35% [23].

CONCLUSION

The addition of *Trichoderma harzianum* + molasses has an improved on *Panicum maximum* cv. *mombaca* silage physical quality and decreased NDF and ADF content. The best treatment of this study based on physical quality, NDF and ADF content was found in *Panicum maximum* cv. *mombaca* silage with the addition of 5% molasses + 5% *Trichoderma harzianum* with 12 days incubation time but the addition of 5% mollasses + 3% *Trichoderma harzianum* with 12 days incubation time was enough to provide an increase in physical quality, ADF and NDF.

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