The Use of *Trichoderma sp*. as a Starter of Fermentation Dry Teak Leaves (*Tectona grandis*) as Animal Feed

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ABSTRACT: There were many starter that has been produced for the fermentation of forages. The aim of this study is to determine the effectiveness of starter *Trichoderma sp*. within fermentation to compare to other starter to increase the physical quality of dry teak leaves as animal feed. This study conducted Completely Randomized Design (CRD) experimental method, consisted of 4 treatments (T0: Control; T1: SOC; T2: EM4 and T3: *Trichoderma sp*.) and 3 replications. This study conducted a the fermentation for 14 days. Observations of the temperature and pH are conducted once in every two days. Bacteria and fungi are calculated on day seven and fourteen using total cell count method. The results showed that *Trichoderma sp*. increase the physical quality of dry teak leaves (color, smell, texture and fungi) significantly (P <0.05). The highest *Trichoderma sp*. obtained T3 (4,5 x 10^4 cfu/g) while the number of bacteria achieved by T1 and T2. In aplying the fermentation for 14 days is recomended. The conclusion of this study indicates that *Trichoderma sp*. is the suitable starter for fermentation of dry teak leaves.

Keywords: Fermentation, Physical quality, Teak leaves, and *Trichoderma sp.*

INTRODUCTION

Development of local feed-based technology innovation is expected to improve the competitiveness of farm products, because of the contribution of feed and seed production costs of about 70-80% or more (Diwyanto and Priyanti, 2009). By-product of food crops and agro-industry are potential sources of ruminant feed ingredients, one of them is dry teak leaves. Although most of agriculturel by-products have limited nutritive value (high fiber, low availeble nutrient and digestibility), contain various anti-nutritional compounds such as selica, tannins, theobromine, cyanide, and ceratyne which can lead to decrease productivity of livestock, thus they become a limiting factor in utilization in rations, but fermented can improve the nutritive value of the by-product (Tamada *et al*., 1999 and Mudita *et al*., 2011).

Dry teak leaves have strategic value is used as feed material for the abundant availability during the dry season or when forage is limited. Dry teak leaves containing 89.07% Dry Materials, Organic Materials 71.83% and 4.9% and Crude Protein; 26.04% Crude fiber and 5.59% Crude Fat (Mintarso, 2008). The use of dry teak leaves in complete feed showed the best in vitro digestibility (KcBK = 61.04% and KcBO = 59.49%) is the composition of 70% concentrate and 30% BK BK forage (20% and 10% field grass dry teak leaves).

Lamid *et al*. (2013) reported that the inoculation of *Actinobacillus sp.* Painokulasi *Actinobacillus sp.* On the teak leaf fermentation can lower crude fiber content, increase the crude protein content. Dose efficient to use bacterial fermentation teak leaves *Actinobacillus* sp. is 10%. Significant differences (P <0.05) for crude fiber and crude protein P3 and P2 compared to controls. Effective solution microorganisms 4 (EM4) was first discovered by Prof. Dr. Teruo Higa of the University Ryukyus, Japan, is a starter for fermentation of feed that many well known farmers in Indonesia. EM4 containing the microorganism fermentation. Total microorganism fermentation in the EM4 very much, about 80 genera. The selected microorganisms that can work effectively in fermenting organic matter. Of the many microorganisms, there are five principal categories, namely: photosynthetic bacteria, *Lactobacillus sp*, *Streptomyces sp*, yeast and *actinomycetes* (Indriyani, 2009).

Fermentation causes changes in the organic elements of feed, so that the components in the feed becomes simpler. The highest value of protein digestibility of feed obtained at concentrations of starter solution of EM-4 15% in the amount of 83.29%. The use of EM-4 at concentrations of starter solution of 20% (P4) is not effective, the value decreased protein digestibility of feed that is 75.26%. This occurs because microorganisms EM-4 in decomposing the organic elements of feed too much compared with the available substrate, thereby reducing the speed of the growth of microorganisms (Winedar et. al. 2006).

SOC (Liquid Organic Supplement) is Organic Bio Nutrients developed by PT Life Bright Prosperous (HCS). SOC among others, can balance the microorganisms in the rumen of animals and increase appetite. During the dry season the majority of farmers are having trouble getting feedstuffs. Cattle ranchers in Gunung kidul Yogyakarta, already widely utilize dry teak leaves and bamboo leaves as feed material fermented with SOC, the composition of the fermentation with 10 kg of dry leaves, 2 kg rice bran, 2.5 cassava flour, 10 grams of salt, 5 spoons of molasses, 6 liters of water and 3 spoons of liquid organic supplements (SOC), a result that maintained PO cattle can grow well (Anonymous, 2013).

Besides EM4 and SOC, *Trichoderma sp* also widely used as a starter to ferment the feed. Supriyati *et al*. (2013) suggest the changes of Nutritive values during fermentation of rice straw using *Trichoderma* viride as the starter was observed. The fermentation did not influence percentage of NDF (P> .05) but influenced the percentages of ADF, CP and ash (P <0.01) in. Its could be concluded that the highest nutrient percentage of fermented rice straw using Trichoderma viride were Obtained at days of 8th.

This resarch was conducted to determine the effectiveness of starter *Trichoderma sp*. within fermentation to compare to other starter to increase the physical quality of dry teak leaves as animal feed.

MATERIALS AND METHODS

An experiment was carried at Brahmaputra Animal Husbandry Academy, Yogyakarta. Dry teak leaves obtained from Brahmaputra garden, collected and chopped into a length of approximately 1-2 cm. and then put in 12 fermentation of plastic cans. Each can contain 2.5 kg of dry teak leaves. From the 12 cans was divided into 4 treatments. Each treatment was replicated 3 time. Treat 1 as a control (T0) cans filled teak leaves and distilled water 1.5 liters. Treatment 2 (T1) filled cans of dry leaves, distilled water 1,5 liters and starter SOC, Treatment 3 (T2) cans filled dried leaves and starter EM4 (2% and 1.5 liters of distilled water). Treatment 4 (T3) charged 1.5 liter of distilled water and a solution of *Trichoderma sp* inoculum. Inoculum was obtained from the Laboratory of Biotechnology, Faculty of Agriculture Gadjah Mada University.

All of treatment incubated anaerobically for 14 days. Observations of the temperature and pH are conducted once in every two days. Bacteria and fungi are calculated on day seven and fourteen using total cell count method. data recorded includes physical quality teak leaves fermented leaves (color, smell, texture and fungi) are calculated using the 20 testers.

All data were statistically analysed by one-way analysis of variance (ANOVA) and followed by Duncan's New Multiple Range Test for significant difference between treatments (Ghozali, 2011).

RESULTS AND DISCUSSIONS

Development of local feed-based technology innovation is expected to improve the competitiveness of farm products from Indonesia, because of the contribution in the cost of feed production reaches 70-80% or more (Diwyanto and Priyanti, 2009). as one example is the use of dry teak leaves fermentation. Potential leaves of teak in Indonesia is very large as the forest on the island of Java alone managed forestry in 1989 was 3,007,222 ha (22.8% of total land area), and teak forests reached 1.0693 million ha (Siregar, 2005). Teak leaves as ruminants feeding has many advantages, but high of crude fiber and tannin and low digestibility, therefore the fermentation is an effort to optimize the use of teak leaves as ruminants feeding. Because fermentation effect on the physical and chemical circumstances that will affect the quality and feed palability, then the research with various fermentation starter is expected to produce physical and chemical feed in accordance with the needs of livestock.

The study of teak leaf fermentation without starter T (T0), starter SOC (T2), starter EM4 (T3) and *Trichoderma* (T4) is given in Table 1. The results of statistical anasilis showed The color of teak leaves are fermented without starter is different from that given sarter, the more black dark brown, like other decaying leaves. According Sianipar and Simanihuruk (2009), silage without adding inukulum have a darker color, because the fresh material to be fermented has a living tissue that occurs in the early phase of active aerobic respiration produces water, CO2 and heat. The increasing temperature, affecting the dark color of the silage.

Fermented smell assessment scores in this study indicate that smell at T2, T3 and T4 is lower than T0 (P <0.05), indicating that the administration of a fermented starter produce better quality with the smell / aroma fresh. Likewise, the smell and texture as well as the presence of fungal fermentation results show significant differences. Ridla *et al*, (2007) which cited Zachariah *et al*, (2015) claimed that a good quality silage has a soft texture, not slimy and no smell. Teak leaf texture fermented at T3 showed the most gently. This can be caused by T3 contains many *Trichoderma sp*. (Table 3). This fungus is able to produce cellulase enzymes that can decrease crude fiber, by changing selulusa become more simple carbohydrates. *Trichoderma sp*. prolific producing extracellular proteins and is best known for its ability to produce enzymes that can degrade cellulose and chitin (Harman, *et al*., 2004).

The existence of Trichoderma in the fermentation process is to inhibit the growth of other fungi, seen in Table 1. That the presence of the fungus in the most low T3. *Trichoderma sp*. is a fungus that can be antagonistic biocontrol agent due to other fungi, especially those that are pathogenic. Antagonist activity in question may include competition, parasitism, predation, or the formation of toxins such as antibiotics. For the purposes of biotechnology, biocontrol agents of Trichoderma can be isolated and used to address the issue of crop damage due to pathogens. Ability and mechanism of *Trichoderma sp*. in inhibiting the growth of pathogens in detail varies on each species. The difference is due to the ability of the ecological factors that make the production of metabolites also varies *Trichoderma sp*. produce metabolites that are volatile and non-volatile. Non-volatile metabolites are more effective than those volatile. Metabolite produced by *Trichoderma sp*. can diffuse through the dialysis membrane then can inhibit the growth of some pathogens. One example of these metabolites are monooxygenases which appears when the contact between species of *Trichoderma sp*, and the optimal pH 4. The absence of these metabolites will not change the morphology of Trichoderma but it will only reduce the ability of pathogen inhibition (Hasanuddin, 2003). Meanwhile, according to Prayuwidayati, (2009) mycelium of *Trichoderma sp* can produce an enzyme that is diverse, including the enzyme cellulase, glucanase and chitinase.

The observation of the pH in the fermentation process showed that T0 is higher than T1, T2 and T3 (P <0.05) (Table 3). This can be caused by the T0 acid-producing bacteria such as lactic acid is less developed, while giving starter / inoculum, support lactic acid bacteria that have the potential to improve the quality of fermentation (Hippen *et al*., 2010). PH good during the fermentation ranges between 3.8 to 4.2. when PH is higher than 4.8 indicates a failure fermentation (Ranjit and Kung, 2000). In this study, the pH of the fermentation treatment of teak leaves are added starters ranged from 4.33 to 3.93. It showed in this study the fermentation process goes well, but in the TO fermentation imperfectly.

Table 1. Physical quality teak leaves fermented leaves

Parameter	T0	T1	T2	T3
Color	3.22 ± 0.12^a	2.72 ± 1.04^b	2.88 ± 0.32^b	2.72 ± 1.04^b
Smell	3.30 ± 0.05^a	2.93 ± 0.24^b	3.13 ± 0.15^b	2.22 ± 0.33^b
Texsture	1.02 ± 0.29^a	2.75 ± 0.10^b	3.22 ± 0.03^c	3.37 ± 0.03^d
Fungi	3.12 ± 0.07^a	2.35 ± 0.05^b	2.36 ± 0.03^{ab}	2.12 ± 0.03^c
pH	5.33 ± 0.57^a	4.33 ± 0.57^b	4.33 ± 0.57^b	3.93 ± 0.12^b
CF	28.40 ± 1.62^a	25.23 ± 1.37^b	25.95 ± 1.04^b	25.14 ± 1.01^b

[abc] Means with different superscript on horizontal row were significantly (P<0,05)

Color : 1. (tawny), 2. (brown), 3. (dark brown), 4. (dark) Smell : 1. (freshly acid), 2. (acid), 3. (less acid), 4. (spoiled) Texture : 1. (very rough), 2. (rough). 3. (rather soft), 4. (soft) CF : Crude Fiber

Table 1. It can be seen that the quality of the texture of fermented increased becomes increasingly gently due to lower crude fiber content. Inoculan use EM4 produce cellulase enzymes to digest crude fiber advantageous because the bacteria do not produce crude fiber in the activity, making it more effective in lowering crude fiber (Santosa and Aryani, 2007).

Table 2. Total of bacteria

Treatments	T0	T1	T2	T3
R1	6,1x106	4,6x106	4,7x106	2,2x106
R2	5,0x106	1,4x106	5,0x106	2,2x106
R3	5,1x106	6,1x106	5,9x106	6,4x106
Rata-rata	5,4x106ns	4,0x106ns	5,2x106ns	3,6x106ns

ns : Non Significant

Total bacteria at the end of fermentation (day 14) showed no real difference among the treatments. In this study, no added nutrients other than teak leaves and stater so nutrients needed are not available in sufficient quantities. The fall in the growth of the bacteria are seen in the decreasing temperature as shown in chart 1. Decrease in the average - average occurs after the eighth day.

Table 3. Total of *Trichoderma sp* (Colony Forming Unit/CFUx104/g)

Treatments	T0	T1	T2	T3
R1	0.55	0.44	0.20	3.5.00
R2	0.35	0.45	0.45	6.00
R3	0.40	0.35	0.45	4.00
Mean	0.43^{a}	0.41^{a}	0.37^{a}	4.50^{b}

abc Means with different superscript on horizontal row were significantly ($P<0,05$)

The presence of fungi during the fermentation process showed significant differences ($P <0.05$). At T3 produce the highest of *Trichoderma sp*. The existence of this fungus was found to produce silage with color, smell and good texture. From the results of this study indicate the use of an inoculum of *Trichoderma sp* as a fermentation starter teak leaves produce the best physical quality. The use of *Trichoderma sp*. can reduce other harmful fungi in the fermentation process, making good quality silage fuisik, and produce cellulase enzymes that can degrade selulusa in teak leaves, resulting in lower crude fiber content.

When this study was applied to the development of Indonesian animal husbandry, to achieve self-sufficiency in meat, especially in the utilization of by-products for ruminant feed, then dried teak leaves as animal feed is recommended to be fermented. During the fermentation can be given additional material source of carbohydrates and protein to improve the working of microbial perombak seratkasar in teak leaves and improve the quality of the fermented feed (silage).

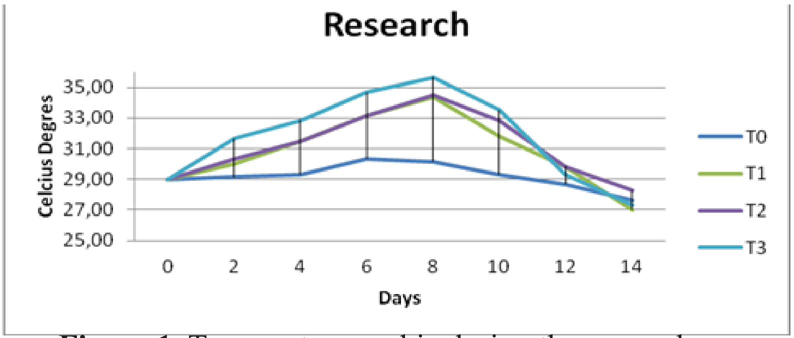

Figure 1. Temperatur graphic during the research

CONCLUSION

The results showed that *Trichoderma sp*. increase of the physical quality of dry teak leaves (color, smell, texture and fungi) Significantly. The highest *Trichoderma sp*. Obtained T3 (4.5 x 104 cfu / g) while the number of bacteria Achieved by T1 and T2. In applying the fermentation for 14 days is recommended. The conclusion of this study indicates that *Trichoderma sp*. is the suitable starter for fermentation of dry teak leaves.

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