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FERMENTABILITY OF POST DETOXIFIED BITTER CASSAVA PEEL-LEAF (*Manihot esculenta* (Kruntz) SILAGE INOCULATED WITH *Lactobacillus plantarum* MONOCULTURE

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ABSTRACT

The potent of bitter-variety cassava leaf was importantly great for fulfilling livestock requirement for feed. The handicap of usage of this cassava is the existence of cyanogenic glucocides that have toxic effect. A two-phases study was conducted with the purpose to investigate fermentability of bitter-variety cassava silage made of cassava peel and leaf. The first was defoxification study with *Aspergillus niger*, and the second phase was ensilage phase with *Lactobacillus plantarum.* At the first phase study, the usage of six percent of *Aspergillus niger* was optimal level in decreasing cyanic acid concentration. At the second phase, five kinds of treatments were applied to improve the quality of silage. The results of this study showed that the treatment of *L. plantarum* to post-detoxified cassava peel and leaf silage increased dry matter digestibility, VFA, N-NH_3 and microbial protein, however, the inoculation did not affect organic matter digestibility. Dry matter digestibly (DMD) ranged from 33.01 to 40.06 percent, VFA concentration was 208 to 238 mM/L., N-ammonia ranged from 20.97 to 50.52 mM/L, and microbial protein ranged from 141.81 to 373.45 mg/mL., whereas organic matter digestibility (OMD) ranged from 62.88 to 69.79%. The conclusions of this study is that *L. plantarum* increases silage quality of bitter-variety cassava peel and leaf, in relation with fermentability.

Keywords : Cyanogenic Glucocides, Detoxified, Bitter-Variety Cassava

INTRODUCTION

Feeding of ruminants in the tropic often difficult because of deficiencies in feed supply, in both quality and quantity (Wanapat and Devendra, 1992). The availability of feed and feedstuff in animal farming is one of the key factor that greatly affect the success of the farming. The development of various kinds of cassava processing industries resulted in abundant supply of cassava by-product such as peel and leaves. The data from The Indoesian Departement of Agriculture (2004) indicated that the area of cassava plantation in Indonesia reaches a number of 1,259,125 ha with total production of cassava tuber of 19,507,049 tonnes, extended over 30 provinces with Lampung, Central Java and east Java the main producers of cassava.

The leaves and peels of cassava, until recently have not been used optimally, due to the presence of antinutrient, cyanogenic glucocides (HCN) that has toxic character (effect).

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The efforts to minimize the level (concentration) and the toxic effect of cyanogenic glucocides had been conducted such as wilting, drying, steaming and silage making, however, the results were yet satisfactory.

The treatments, to some exyend, decreased glucoside concentration, however, they denaturized, decreased the quality of protein content in forage. Multy-phase fermentation was applied in this study to overcome the problems mentioned above. The first step was fermentation using Aspergillus niger applied to cassava peels and leaves for 4 days. The second step was inoculation with Lactobacillus plantarum to the product of fermentation of step one. The inoculated forage was then ensiled for 28 days. The first step of fermentation with Aspergillus niger would neutralize cyanogenic glucocide and increae digestibility, due to the activitoes of extracellular enzymes excreted by the yeast such as glucocidase, cellulose, amylase, and catalase. In ensilage process, Lactobacillus plantarum, a homofermentative bacteria, ferments available carbohydrates into lactic acid, in order to the ensilage process, therefore, good quality silage might be produced, with high digestibility as well.

MATERIALS AND METHODS

Materials used were : a mixture of pells and leaves of cassava, harvested at the age of 8 – 12 mpnth, with peel to leaf ratio of 3 : 1, that has been detoxified with *Aspergillus niger* 6 percent. Pure culture of Lactobacillus plantarum from Inter University Center UGM , mollases and rumen liquor.

Rumen liquor was collected by plastic tube through permanent rumen fistula and brought through pre-gasses (CO_2). The flask containing rumen liquor was kept in a thermostatic bucket containing water at 39 °C. Rumen liquor of two steers were pooled and used for in vitro studies.

In in vitro experiment (Tilley and Terry, 1963), 0.5 g substrate with different treatment were incubated with 40 ml McDougals buffer and 10 ml strained rumen liquor in conical flask fitted with rubber bung having Bunsen valve. After passing enough anaerobic CO_2 in to conical flask it was kept for incubation at 39^0C in water bath having stirrer facility for 48 h. After 48 h of incubation 1.0 ml of 25% H_2SO_4 was added to arrest microbial fermentation.

Dry matter, organic matter digestibily and volatile fatty acid was determined in the sample by measuring the difference of DM (AOAC, 1998).

Randomized complete block design was used in this study. The periods of rumen fluid collection were used as blocks. There were five blocks and five treatment in this study as follows :

H1 : the best product of forage (peel and leaves) of step one fermentation with *Aspergillus niger* 6 percent + 1 % mollases + 10^4 cfu *L. plantarum*/g silage DM

H2 : the best product of forage (peel and leaves) of step one fermentation with *Aspergillus niger* 6 percent + 10^4 cfu *L. plantarum*/g silage DM

H3: the best product of forage (peel and leaves) of step one fermentation with *Aspergillus niger* 6 percent + 10^5 cfu *L. plantarum*/g silage DM

H4 : the best product of forage (peel and leaves) of step one fermentation with

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Aspergillus niger 6 percent + 10^6 cfu *L. plantarum*/g silage DM

H5 : the best product of forage (peel and leaves) of step one fermentation with *Aspergillus niger* 6 percent + 10^7 cfu *L. plantarum*/g silage DM

RESULT AND DISCUSSIONS

Silase quality in general

The making of silage took 28-day period, resulting in good-quality silage. The good guality silage was characterized by the non-existence of bulky silage, no growth of mold, no juice, was produced when the silage was squeezed. Pepper (1983) report that in order to make a good-quality silage, a proper management of silage making is needed i.e., forage characteristies (i.e. size of chopping, the quatity of fermented carbohydrates, speed of forage materials entering into silo, and anaerobic condtion).

The colour of the resulted silage was green-chocolatich in general. The colour of good-quality silage was chocolatish or bright, gree-chocolatish (Ensminger and Olentine, 1987). If fermentation proceeds for long period, temperatur may increase, resulted in the damage of chlorophyl colour (McDonald, 1981).

The means of silage pH ranged from 4.63 to 4.86. These pHs showed that acid conditions were achieved in the ensilage process, resulted in good-quality silage production. The principle of ensilage in to create anaerobis and acid conditions, in order to deactivate putrevactive (eq proteolytic) bacteria, but the conditions still promote lactic acid production by lactic acid-producing bacteria, to the level that pH of opproximately a 4.3 was achieved (constant), therefore, the forage can be preserved in fresh condition for long period.

Table 1. Physical characteristics of silage

Treatment	colour	pH	mold	damage (%)	Effluent
H_1	Chocolatish	4,76	-	-	-
H_2	Chocolatish	4,85	-	-	-
H_3	Chocolatish	4,86	trace	0,85	-
H_4	Chocolatish	4,63	-	-	-
H_5	Chocolatish	4,75	-	-	-

Table 2. Parameter result of study

Treatment	Parameters				
	DMD (%)	OMD (%)	VFA (mM/L)	N-NH3 (mM/L)	Microbial protein (mg/ml)
H1	39.36	69.79	212	20.97	373.45
H2	34.87	68.56	208	40.77	223.16
H3	40.06	66.38	232	50.12	141.81
H4	39.08	62.89	238	39.48	177.97
H5	33.21	68.99	218	30.08	280.79

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Dry matter digestibility

Digestibilities of dry matter (DMD) and organic matter (OMD) represent the characteristics of carbohydrates in feedstuffs. The ability of feedstuffs to provide nutrients, quantitatively as well as qualitatively for ruminal microbes and their host can be approached by DMD and OMD as well.

The mean of in vitro DMD (%) of this study was 37.32 ranged from 33.21 to 39.36 as shed in figure 1.

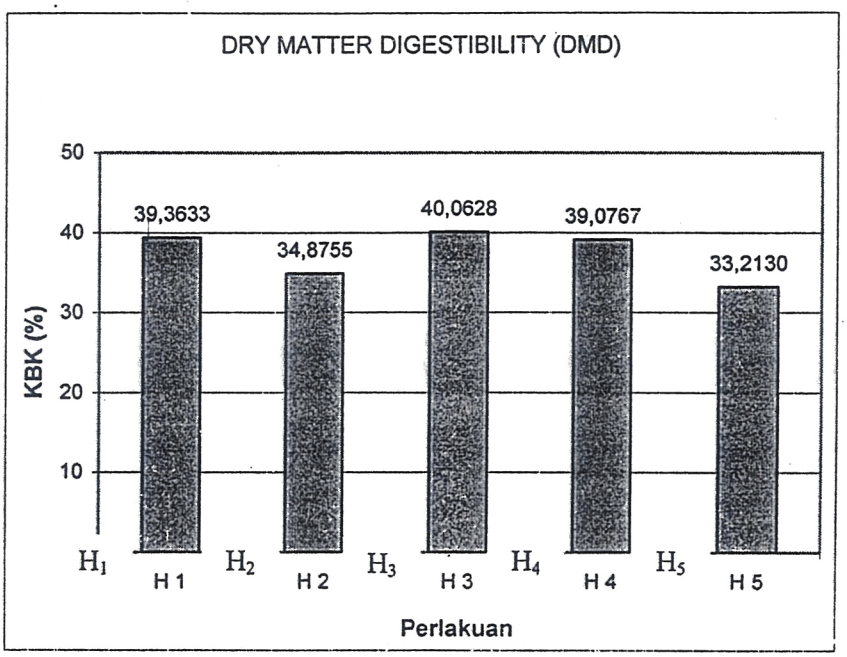

Figure 1. Block curve of means of DMD at some levels of Lactobacillus plantarum inoculations to cassava peel-leaf silage.

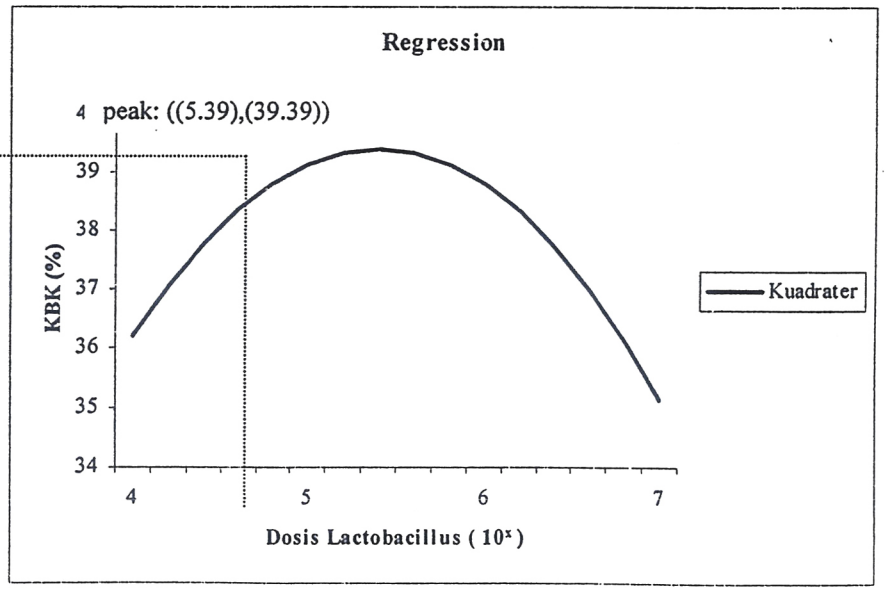

Figure 2. Quadratic regression curve of DMD at various levels of Lactobacillus plantarum

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Analysis of variances showed that the addition of *Lactobacillus plantarum* to the silage cassava peel-leaf (P< 0.5)that had been detoxified had a significant effect on DMD. Crontrast orthogonal test showed that there was no significant differences between DMD of H1 and H2. The addition of molasses to H1 resulted in a tendency that the mean of DMD of H1 was a higher than that of H2. Rationally, *L. plantarum* in H1 might grow better than that in H2 due to the addition of carbohydrate from molasses as well as that from the forage itself.
Ortoghonal polynomial test showed quadratic curve with an equation
$Y = -8.5588 + 17.78301x - 1.648701x^2$; ($R^2 = 38.229\%$). It meant that the DMD in the rumen increased in accordance with the increase of *Lactobacillus plantarum* of 10^4 cfu/g silage DM up to $10^{5,39}$ cfu/g silage DM. The coefficient of determination (R^2) showed that the DMD of silage was 38.23 percent affected by *Lactobacillus plantarum.* The peak of the curve (at $10^{5.39}$ cfu/g silage DM) was 39.39 percent. It meant that at the addition of $10^{5.39}$ cfu of *Lactobacillus plantarum*/g silage DM, the DMD was maximum, at 39.39 percent as showed by curve (figure 2).

Organic matter digestibily (OMD)
The mean (%) of OMD of this study was 67.32, ranged from 62.59 (A4) up to 69.79 (H1), as showed in figure 3.
The H1 and H2 treatments had the same levels of *Lactobacillus plantarum* inoculation, 10^4 cfu/g silage DM, however, the A1 treatment was enriched with mollases that caused the total level of available carbohidrates in H1 to be higher than that in H2, as a result, more energy for the growth of *L. plantarum* during ensilage was available, therefore, ruminal microbes would have more opportunity to grow better in H1 than that in H2.
The decrease in soluble carbohydrates as one of the organic compohents resulted in limited amount of energy source for the growth of rumen bacteria, in consequence, the performances of the bacteria also decreased especially for substrate degradation. The process of digestibilities in the ruminants largerly (greatly) depends upon the activities of rumen bacteria, which depends further to the nutrient composition in a ration (diet) (Stokes, 1991).
The analysis of variances showed that inoculation of *Lactobacillus plantarum* to the detoxified silage did not give any significant differences.

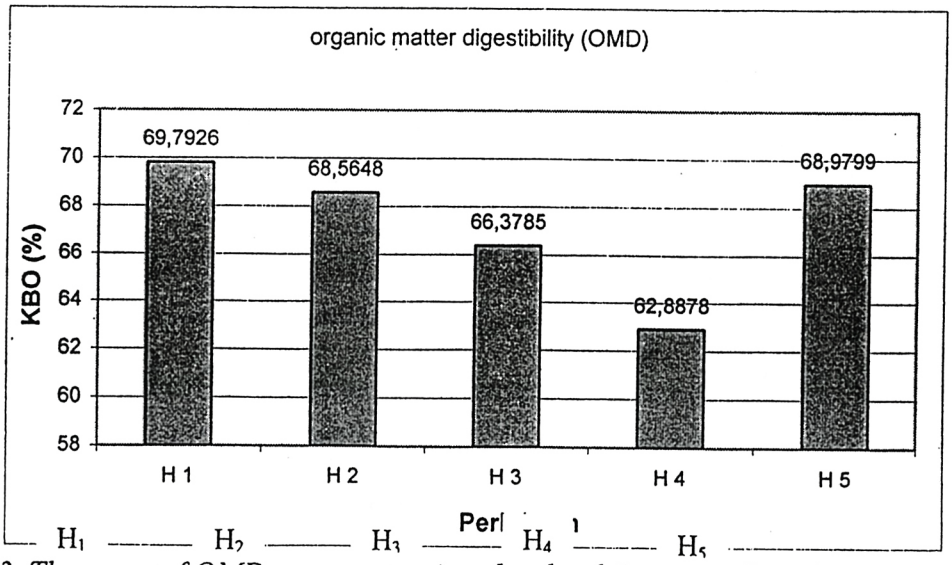

Figure 3. The curve of OMD means at various levels of Lactobacillus plantarum to silage of cassava peel-leaf

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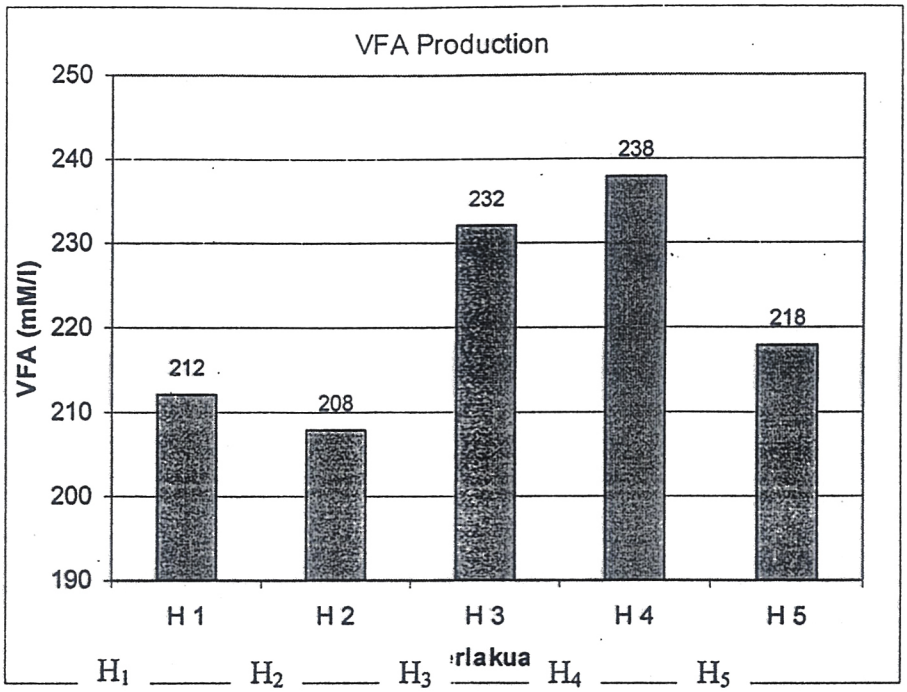

Figure 5. The curve of the means of VFA production at various levels of Lactobacillus plantarum inoculation to cassava peel-leaf silage

Volatile Fafty Acids (VFA) production

Volatile fatty acids (acetic, propionic and butyric acids) represent main sources of energy for host animal, and are the results of the broken down carbohydrates in the rumen. The mean of VFA production (mM/L) in this study was 221.6 ranged from 208 (H2) up to 238 (H4) as showed in figure 5.

The analysis of variance indicated that inoculation of *L. plantarum* to detoxified cassava peel-leaf silage gave significat effect on VFA production. Contrast orthogonal test showed that no differences was detected between H1 and H2. H1 had greater level of fermentable carbohydrates than that of H2 due to mollases addition in H1, therefore, H1 tended to have higher level of VFA than that of H2 (figure 5).

Orthogonal polynomial showed a quadratic curve of VFA production, with an equation $Y= -114.80 + 124.0x - 11.00\ x^2$; (R^2 = 30.22%). It meant that VFA production increased in accordance with the increase of *Lactobacillus plantarum* inoculation of 10^4 cfu/g to $10^{5,66}$ cfu/g sialge DM, that decreased again up to the inoculation of 10^7 cfu *Lactobacillus plantarum* /g silage DM. Determinant coefficient (R^2) of 30.22% showed that production of VFA was affected by *Lactobacillus plantarum* inoculation as much as 30.22 %. The peak of curve (238.04 mM) was achieved at the inoculation of $10^{5.66}$ cfu *Lactobacillus plantarum* /g DM silage, which meant that at the inoculation of $10^{5.66}$ cfu *of Lactobacillus plantarum* /g silage DM, the production of VFA was maximum (figure 6).

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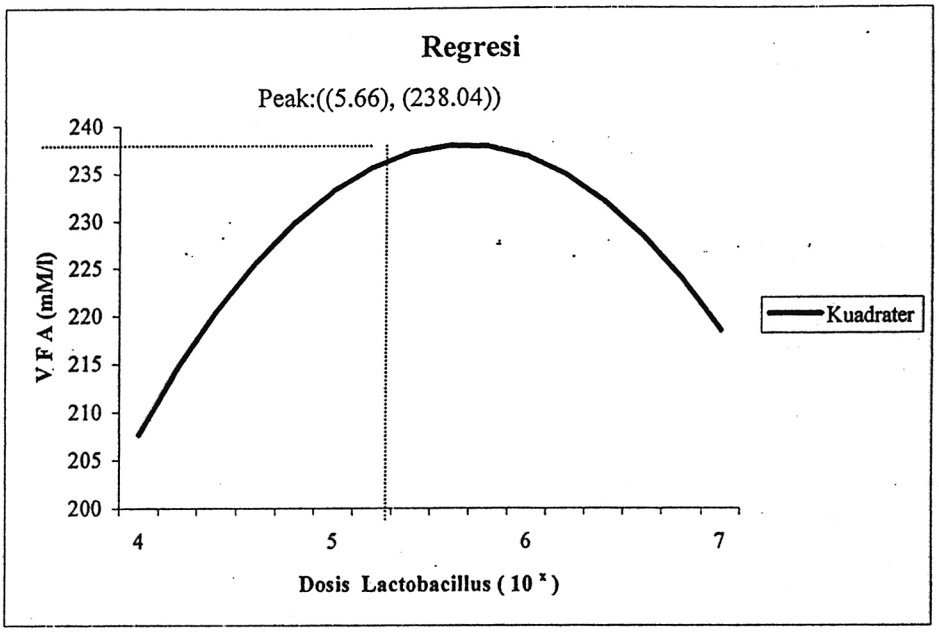

Figure 6. The curve of quadratic regression of VFA productions at various levels of Lactobacillus plantarum inoculation to the cassava peel leaf silage.

N-ammonia

Ammnia is the main source of nitrogen, and is very importat for synthesis of ruminal microbes. Preston and Leng (1987) reported that the optimal rage for microbial protein synthesis is 150 to 250 mg/L (10.7 to 14.3 mM). There are two condition for the resulted ammonia to be efficient for microbial protein synthesis :

1. The initial concentration of ammonia should be under optimum condition
2. Rumen microbes should have available energy source for microbial protein synthesis.

CONCLUSIONS

1. The addition of mollases into cassava Pell-Leaf silage inoculated with *Lactobacillus plantarum* is able to improve digestibilities and microbial protein synthesis
2. *L. plantarum* inoculated to cassava peel-leaf silage affects VFA and ammonia production.

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