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CHEMICAL EVALUATION OF SELULOLITIC MICROBE AND LACTIC ACID BACTERIA NWD 015 ADDITION to *Pennisetum purpu*reum AT SILAGE PROCESS

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ABSTRACT

Celulolytic microbes isolate, lactic acid bacteria (LAB) NWD 015 were used as starter for *Pennisetum purpureum* (PP) silage fermentation. The fermentation have an objective to improve the nutritive value of PP. There were three different fermented PP. In respect to various starter used, F1 using selulolitic microbes, F2 using LAB NWD 015, and F3 using selulolitic microbe and F4 using LAB NWD 015. After three weeks fermentation sample were taken out to the analysis their nutritive value which were include moisture, dry matter, fat, crude protein, crude fiber, NDF, ADF, ash and pH. The result of the experiment showed that the fermentation improved of nutrition value ($P<0.05$). The pH decresed significantly and the crude protein, crude fat as well as ash increased significantly. However, the crude fiber, NDF and ADF decrease, eventhough those value did not show significantly. From this study could be concluded that mixed isolate selulolitic microbe and LAB NWD 015 as starter were given the best result.

Key words: Selulolitic microbe, Lactic acid bacteria, Pennisetum purpureum,, Silage.

INTRODUCTION

Silage is the feedstuff produced by fermentation of forage crops of variable but often high moisture content. Ensilaging means to preserve forage (when abundant) for feeding livestock when fresh material is less available. In general, silage fermentation is a natural process whereby epiphytic lactic acid bacteria ferment water soluble carbohydrates in the crop to a number of products, primarily lactic acid, thereby reducing the pH as rapidly as possible, inhibiting spoilage microbes and preserving the maximum amount of nutrients in the product (Merry and Davies 1999).

The ensilage process is divided into the initial aerobic phase, the fermentation phase and second aerobic phase when the silo is opened. The last phase has consequences for the quality of the product fed by livestock (Merry and Davies 1999). When the silo is opened and or sealed inadequately, the silage is exposed to air. This may lead to aerobic deterioration. Aerobic microorganisms in silage degrade lactic acid and residual water-soluble carbohydrate to CO_2 and protein and amino acids to amines, amides, and ammonia (Seale 1986). Aerobic deterioration generates considerable heat, increases pH, and decreases digestibility (Woolford 1990). The main contaminants associated with aerobic spoilage activity in silage are yeasts, moulds, *Bacillus* spp. and *Listeria* spp. (McDonald et al. 1991). When lactic acid bacteria fail to produce sufficient lactic acid during fermentation to reduce pH and inhibit the growth of butyric acid-

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producing bacteria, i.e. clostridia, the resulting silage will be of poor quality (Mc Donald et al. 1991).

The quality of fermentation will be improved by adding microorganism, enzymes, antibiotic, nitrogen source, as well as energy source. The objective of this addition is to increase nutritive value digestibility, lactate produced and feed palatability (Peppler, 1983).

The cellulose activities experiment have often been done using cellulose filtrate of fungi Trichoderma sp (Royer and Nakas,1990) reported that degradation of polysacharides and lignin could be done by ruminal bacteria and fungi. Lactobacillus one of lactic acid bacteria (LAB) could be given at the temperature of 15 – 45^{0}C. (Brock, 1979). Foster and Wase (1987) said that LAB have an effort to produce lactic acid and so they are tolerant with lower pH medium. LAB NWD 015 was isolate from the young calves feces (Umami,2005).

Base on the statement above, this experiment was conducted to find the best methode that produce a good quality of silage. Having high digestibility and high nutrirional value, by additive cellulolitic microorganism and LAB during the Pennisetum purpureum (PP) fermentation. It was expected that cellulolitic microorganism and LAB isolate could improve PP quality fermentation process. The lowering pH of fermentation due to the lactic acid accumulation would be achieved if the fermentation is added LAB NWD 015.

MATERIALS AND METHODS

Microorganism. Cellulolytic microbe one of the an aerobic cellulolytic isolate which be cultivated from rumen fluid. LAB NWD 015 which capable of producing lactic acid were used as inoculum. Feces of Young calves as microbe source were used to get LAB NWD 015 usinbg MRS medium. LAB NWD 015 produced 41.61% lactic acid, pH value of 4.07 and total microbe count of 4,78 X 10 ^{8} in medium.

Culture media. The basal medium for the cellulolytic isolates contained the following formula per 100 ml of distilated water, KH_2PO_4, 0.020 g, K_2HPO_4; 0.015 g, Na $H_2PO_4 12H_2O$ 0.230 g, $NaH_2PO_4 12H_2O$ 0.15g, NH_4NO_2 0.060g,$MgSO_4$ $7H_2O$ 0.030 g, $NaNO_3$ 0.380 g and yeast extract 0.200 g the pH was adjusted to 6.6 prior to autoclave. Mineral solution I contained ZnSO47H2O 0.050 gram, Fe (SO4)3 6H2O 0.054 gram MnSO4H2O 0.060 gram in 100 ml destiled water and 0.8 ml NH4Cl. Mineral solution II contained CaSO4 5 H2O 0.0025 gram and H3BO3 0.057 gram in 100 ml destiled water. Liquid culture medium made by adding 0.01 ml mineral I and 0.01 ml mineral solution II to 100ml basal medium. Solid culture medium made by adding 2 gram agar into 100 ml liquid culture medium. Substact cellulose were used to cultivate cellulolytic microbe and glucose were used to cultivate microbe that produce lactid acid. Substrat concentration was 1 %.

Preparation of Inoculum. The inoculum was prepared by growing the organism on liquid medium. Biomass were produced by enrichment culture and were grown on liquid medium with erlenmeyer flash as fermentor. CO_2 were used to get an aerobic condition.

***Pennisetum purpureum* (PP) fermentation**. Fermentation was conducted in laboratory scale using jar glass as silo with total volume of 2 kg. Three types of silage fermentation were dicribed bellow:

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1. PP was inoculated by LAB 2.5 % v/w and incubated for 2 weeks (F1)
2. PP was inoculated by cellulolytic microbe isolate in incubated for 2 weeks was added 2.5 % v/w (F2)
3. PP was inoculated by LAB and cellulytic microbe isolate (1.25 % and 1.25%) finally it was fermented an aerobically for 2 weeks (F3)

The sample of every fermented PP were taken to be analysed to determine their nutritional values including crude protein, crude fat, crude fiber, moisture contain, ADF, NDF, ash and pH for comparation for unfermented PP were also analysed (F4).

Analysis

Microbe activity. Cellulolytic microbe were showed by their ability to degrade CMC. CMC ase activity were measured using CMC as substrat with enzime solution from culture supernatan. the amount of reducing sugar reliased from CMC were measure spectrofotomaticaly used Nelsonsamogy methode. The activitiesof cllulolytic isolate were showed by their potency in sugar degradation.
In this experiment D-glucose were used as substrat. Reducing sugar left in medium were determined by KFCN as oxidator.

Nutritional value. Moisture and ash contain were determined by gravimatically. Crude protein and crude fat were determined by kjeldahl methode and soxhlet extraction. ADF and NDF were determined gravimatically based on their solubilities and pH was determined by pH meter.

RESULTS AND DISCUSSION

Enzymatic Activities

Characteristic of microbes that use for starter in *Pennisetum purpureum* silage shown in Table 1 and 2.

The abilities of microbe to degrade cellulose were presented by reducing sugar being released from CMC. Table 3 shows the concentration of reducing sugar being produced activities of LAB and cellulolytic were shown in Table 4 which presents the concentration of reducing sugar.

Table 1. Characteristic of LAB NWD 015 isolated from young calves feces

Characteristic	
Morphology	Coccus
Optimum temperatur	38°C
Incubation Time	12 hour
Gram Stanning	positive
Growth Medium	MRS
First Growth pH	5.8
Catalase Type	negative
Amount of Colony cell	4.78 X 10^{8} CFU/ml

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Table 2. Characteristic of isolate cellulolytic of rumen fluid

Characteristic	
CMC-ase activity	0.1566 AU/ml
Growth temperature	38 °C
Amount of colony cell	3 x 10^9 CFU/ml
Incubation time	12 hour
Growth medium	Cellulolytic medium
First growth pH	6.6

Table 3. Cellulolytic activity of isolate cellulolytic of rumen fluid (AU/ml)

Periode	Microbe1
1	0.1682
2	0.1555
3	0.0756
4	0.2271
mean	0.1566

Table 4. Intact glucose which was not hydrolyzed by LAB (mg/ml)

Periode	LAB
1	0.01331
2	0.14556
3	0.12087
4	0.00174
mean	0.07037

Table 5. Chemical composition of fermented PP using various inoculum

Composition (%)	F1	F2	F3	F4
DM	20.99^{ab}	16.24^{b}	21.21^{a}	18.53^{b}
Fat	69.01	73.76	67.79	89.47
CP	4.09	3.78^{ab}	3.57^{b}	3.03^{c}
CF^{ns}	28.93	29.99	30.31	31.38
ADF^{ns}	38.71	38.98	39.41	40.10
NDF^{ns}	63.43	64.94	64.91	66.85
Ash	27.70^{a}	24.06^{b}	25.22^{b}	23.34^{c}
pH	4.32^{b}	4.61^{b}	4.59^{b}	6.45^{a}

LAB and cellulolytic microbe isolate could be grown using glucose as substrat. It shown that all of organism grown could be used as starter.

Nutritional Value of PP. Table 5 presents the nutritional value of PP which were taken from every silos.

Nutritional value of PP which were ferrnented using various starter were significantly different, especially in DM, CP, fat, ash and pH. The increasing could be an effect of use medium on starter. Crude fiber, NDF and ADF content were not different, however they tended to decrease. Base on the measurement of enzyme activities (Table 3) Cellulolytic from rumen fluid as inoculum have cellulolytic activities 0.1566 AU/ml.

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CONCLUSION

From the result of the experiment it can be conclude that fermented process using mixed microbe produced the best silage which were shown by highest nutritional value.

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