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Chemical Evaluation Of Lignocellulolytic Microbes, Yeast and *Lactobacilli* Addition to Rice Straw at Silage Preservation

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ABSTRACT: Lignocellulolytic microbial isolate, *Trichoderma reseei* and rumen fluid, *Lactobacilli plantarum* and *Rhizopus sp* were used as starter for rice straw silage fermentation. The fermentation have an objective to improve the nutritive value of rice straw. There were four different fermented rice straw in respect to various starter used. F1, using *T. reseei - L. plantarum*; F2, rumen fluid isolate - *L. plantarum*; F3 rumen fluid and added by *L. plantarum* as probiotic; F4, *T. reseei - Rhizopus sp, L. plantarum*. After three weeks fermentation, sample were taken out to the analyzed their nutritive value which were include moisture, dry matter, fat, crude protein, true protein, crude fibre, Neutral Detergen Fibre (NDF), Acid Detergen Fibre (ADF), ash, and pH. The result of the experiment showed that the fermentation improved of nutritive value ($P<0.05$). The pH decreased significantly, and the crude protein, true protein, crude fat as well as ash increased significantly. However, the crude fibre, NDF, and ADF tended to decrease, eventhough those value did not show significantly. *Rhizopus sp*, could improve true protein content of rice straw. From this study could be concluded that mixed isolate *T. reseei - L. plantarum* as starter were given the best result.

Key Words: Fermentation, Microbe, Rice Straw, Probiotic.

Introduction

One of important factors which influence sucessful animal production system is the continuity of feed availability. Agriculture waste as a feedstuff can be found in unlimited amount during harvesting season. Proper system needed to be applied in order to obtain continuous animal feed availability. One of the systems is feed conservation by a fermentation.

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

The Cellulolase activities experiment have often been done using culture filtrate of fungi *Trichoderma sp.* (e.g. Montenecourt and Eveleigh, 1977; Royer and Nakas, 1990; Kanpp and Legg, 1985). Akin and Benner (1988) reported that degradation of polysaccharides and lignin could be done by ruminal bacteria and fungi.

Lacto bacillus, one of lactic acid bacteria could be given at the temperature of 15 - 45° C (Brock, 1979). Forster and Wase (1987) said that lactic acid bacteria have an effort to produce lactic acid and so they are tolerant with lower pH medium.

Base on the statement above, this experiment was conducted to find the best method that produce a good quality of feed, having high digestibility and high nutritional value, by addition cellulolitic microorganism, *Lacto bacillus* plantarum and yeast during the rice straw fermentation.

It was expected that cellulolitic microorganism such as *Trichoderma reseei* and rumen fluid isolate could improve rice straw quality by fermentation process. The lowering pH of the fermentation due to the lactic acid accumulation would be achieved if the fermentation is added *Lactobacilli plantarum*.

Materials and Methods

Microorganism. Trichoderma reseei one of the aerobic cellulolytic mircobe, anaerobic cellulolytic isolate which be cultivated from rumen fluid; *Lactobacillus plantarum*, which capable of producing lactic acid and *Rhizopus sp.* were used as inoculums.

Culture media. The basal medium for the isolates contained the following formula per 100 ml of distilated water : KH_2PO_4 0.020 g, K_2HPO_4

0.015 g, NaH_2PO_4 $2H_2O$ 0.230 g, Na_2HPO_4 $12H_2O$ 0.150 g, NH_4NO_2 0.060 g, $MgSO_4$ $7H_2O$ 0.030 g $NaNO_3$ 0.380 g and yeast extract 0.200 g. The pH was adjusted to 6.6 prior to autoclaving. Mineral solution I contained : $ZnSO_4$ $7H_2O$ 0.050 g, $Fe(SO_4)_3$ $6H_2O$ 0.054 g, $MnSO_4$ H_2O 0.060 g in 100 ml distilated water and 0.80 ml NH_4Cl (11/3 ion). Mineral solution II contained $CaSO_4$ $5H_2O$ 0.0025 g and H_3BO_3 0.0570 g per 100 ml distilated water. Liquid culture media made by adding 0.01 ml mineral solution I and 0.01 ml mineral solution II to 100 ml basal media. Solid culture media made by adding 2 grams agar into 100 ml liquid culture media. Substrat. Cellulose were used to cultivate cellulolytic microbe and glucose were use to cultivate microbe that produce lactic acid and *Rhizopus sp.* Substrat consentration was 1%.

Preparation of Inoculum. The inoculum was prepared by growing the organism on slant agar. Biomass were produced by enrichment culture and were grown on liquid media with Erlemeyer flas as fermentor. Nitrogen gas were used to get anaerobic condition.

Straw Fermentation. Fermentation was conducted in laboratory scale using plastic bag silo with total volume of 5 kg. Four types of silage fermentations were discribed bellow.

1. Rice straw was inoculated by *T. reseei* aerobically (2.5% V/W) and incubated for one week. *L. plantarum* was added (2.5% V/W), incubated for 2 weeks, anaerobic fermentation. (F_1)
2. Rice straw was inoculated by rumen fluid isolate, incubated for one week anaerobically. *L. plantarum* was added (2.5% V/W) and incubated for 2 weeks anaerobic fermentation (F_2).
3. Rice straw was inoculated by rumen fluid isolate and incubated for 3 weeks anaerobically. Finally L. plantarum was added (2.5% V/W) (F_3).
4. Rice straw was inoculated by *T. reseei* (1.25% V/W) and *Rhizopus sp.* (1.25% V/W) aerobically and incubated for one week and then *L. plantarum* was added, finally it was fermented anaerobically for 2 weeks (F4).

After 3 weeks fermentation, the sample of every fermented rice straw were taken to be analyzed to determine their nutritional values, including true protein, crude protein, crude fat, crude fibre, moisture content, acid detergent fibre (ADF), neutral detergent fibre (NDF), ash and pH. For the comparison unfermented rice straw were also analyzed (F5).

Analyzes

Microorganism activity. Cellulolytic micro-organism were showed by their ability to degrade carboxy methyl cellulose (CMC). CMC-ase activities were measured using CMC as substrat with enzyme solution from culture supernatant. The amount of reducing sugar released from CMC were measured spectrofotometrically using Nelson-Somogyii method. The activities of *L. plantarum* and *Rhizopus* sp. were showed by their potency in sugar degradation, so in this experiment D-glucose were used as substrat. Reducing sugar left in media were determined by Kalium ferrycyanide as oxidator.

Nutritional value. Moisture and ash content were determined gravimetrically. Crude protein and crude fat were determined by Keydahl methode and Soxhlet extraction. Acid Detergent Fibre and Neutral Detergent Fibre were determined gravimetrically based on their solubilities and pH was determined by pH meter.

Results and Discussion

Enzymatic activities. Cellulolytic activities of *T. reseei* and rumen fluid isolate were shown in Table 1 and 2. The abilities of organism to degrade cellulose were presented by reducing sugar being released from CMC. Table 1 presents them as absorbance unit, and Table 2 shows the concentration of reducing sugar being produced.

Activities of *L. plantarum* and *Rhizopus* were shown in Table 3. Which present the concentration of intact reducing sugar.

T. reseei had cellulolytic potency higher than rumen fluid isolate. *L. plantarum* and *Rhizopus sp.* could be grown using glucose as substrate. It seemed that all of organism grown, could be used as starter.

Nutritional Value of Rice Straw. Table 4 presents the nutritional value of rice straw which were taken from every silos.

Table 1. Reducing sugar being release from carboxy methyl cellulose by *T. reseei* and cellulolytic isolate of rumen fluid (mg/100 mg CMC)

Periode	Type of microbe	
	T. reseei	Isolated rumen fluid
I	0.037	0.042
II	0.007	Undetected
III	-	Undetected
IV	0.007	Undetected
Mean	0.013	0.010

Table 2. Cellulolytic activity of *T. reseei* and isolate rumen fluid (AU/ml)

Periode	Type of mircobe	
	T. reseei	Isolated rumen fluid
I	0.3682	0.4057
II	0.1555	0.0737
III	0.0756	0.0567
IV	0.1543	0.0463
Mean	0.1884	0.1456

Table 3. Intact glucose which was not hydrolyzed by *L. plantarum* and *Rhizopus sp.* (mg/ml)

Periode	Type of microbe	
	Rhizopus	*L. plantarum*
I	0.00343	0.00331
II	0.00073	0.07556
III	0.00030	0.00087
IV	0.00030	0.00174
Mean	0.00119	0.02037

Table 4. Chemical composition of fermented rice straw using various inoculum

Composition (%)	Fermented				
	F1	F2	F3	F4	F5
Moisture	30.99 ab	26.24 b	35.26 a	31.21 ab	10.53 c
Dry matter	69.01 b	73.76 b	64.74 c	68.79 b	89.47 a
Fat	1.73 ab	1.90 a	1.42 b	1.89 a	0.91 c
Crude protein	4.08	3.68 ab	3.44 b	3.47 b	3.01 c
True protein	0.49 b	0.34 c	0.32 d	0.56 a	0.09 a
Crude Fibre (ns)	28.93	29.99	29.91	30.13	31.38
ADF (ns)	38.71	38.98	40.34	39.41	40.10
NDF (ns)	63.43	64.94	64.99	64.61	66.85
Ash	26.80 a	23.08 b	24.37 b	24.24 b	22.35 c
pH	4.43 b	4.60 b	4.40 c	4.57 b	7.33 a

Nutritional value of rice straw which were fermented using various starters were significantly different, especially, in moisture/dry matter, crude protein, crude fat, true protein, ash and pH. True protein content were highest on fermented rice straw using *Rhizopus sp.* as inoculum (F4). It showed that *Rhizopus sp.* may have ability to convert urea become true protein. Moisture, crude fat, crude protein, true protein and ash content were increased by fermentation. The increasing could be an effect of urea, $CaCO_3$, zeolit and water which were added during preparation processes. Crude fibre, NDF and ADF content were not different, however they tended to decrease. Base on the measurement of enzyme activities (Table 1 and 2), *Trichoderma reseei* and rumen fluid as inoculum have cellulolityc activities 0.1884 and 0.1456 A4/ml. Therefore, crude fiber, acid detergent fibre and neutral detergent fibre of fermented rice straw tended to decrease.

Conclusion

From the result of the experiment, it can be concluded that fermentation process using mixed microbe *T. reseei*, *L. plantarum* and *Rhizopus* produced the best silage which were shown by highest nutritional value.

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