Nutritive Values of Rice Straw Fermentation Used Carbon Sources on Different Level with Various of Inoculant Levels *Aspergillus niger* and *Lactobacillus plantarum*

R. Agus Tri Widodo Saputro[1], Nono Ngadiyono[2], Lies Mira Yusiati[3], I Gede Suparta Budisatria[4]

[1]Agricultural High School in Magelang
[2]Faculty of Animal Science, Gadjah Mada University Yogyakarta
Corresponding author: lorenzarockduth@yahoo.co.id

ABSTRACT: Utilization of agricultural waste products (rice straw) as the basic feed into the strategic thing to be developed to meet the needs of fibrous feed for cattle. Cellulolytic microbes and lactic acid bacteria are sources of inoculum that can improve the quality of rice straw as feed fibrous base. The study was conducted with the aim to obtain the appropriate carbon source for the growth of *Aspergillus niger* and *Lactobacillus plantarum*. The microbial are grown with solid and semi-solid method. The sources of carbon treatment were given two kinds of substrates, namely molasses and rice bran. The treatment of *Aspergillus niger* with a level of 0.5, 10, and 15%. Administration of *Lactobacillus plantarum* was 10% in each treatment. Fermentation is carried out for 21 days. The variables were observed in this study were pH, lactic acid, DM, OM, CP, CF, NDF, ADF, and TDN. Data were analyzed using analysis of variance completely randomized design (CRD) unidirectional pattern and factorial pattern (2 x 4), if there is a real effect, then it was followed by DMRT (Duncan's Multiple Range Test). The results showed that the best substrate was molasses and *Aspergillus niger* at best level of 15%. It was based on the value of the lowest crude fiber and lactic acid produced the highest ($P<0.05$). It was concluded that the use of *Aspergillus niger* and *Lactobacillus plantarum* were best for fermented rice straw; the dry matter of *Aspergillus niger* was 15% and *Lactobacillus plantarum* was 10%.

Keywords: Rice straw, fermentation, digestibility

INTRODUCTION

Forage production in the rainy season is abundant, whereas in the dry season forage production, especially those from low grass even if the long drought reduced production. As for how to overcome the shortage of forage grasses can be done for example by way of utilizing the results of agricultural plant waste, such as rice straw. In line with the increasing intensification of food crop cultivation efforts, the results of agricultural crop residues, especially rice straw will increase. As for how to overcome the shortage of forage grasses, among others, by way of utilizing the results of agricultural crop residues, one of which rice straw.

Low levels of digestibility of rice straw, because the bonding that occurs in rice straw (cellulose and hemicellulose) is difficult to be broken down by rumen microbes. Consumed rice straw is also difficult to digest and many are not utilized by the ruminant digestion. Indeed, the improvement of nutritional value can be done through the processing of agricultural waste through physical, chemical, and microbiology. One of them, to improve the quality of rice straw with innovative technology in the form of rice straw fermentation using cellulolytic microbes and lactic acid bacteria (LAB).

Rice straw fermentation using cellulolytic microbial inoculum and LAB, with secrete enzymes cellulose and xylanase by the cellulolytic microbes, cellulose and hemicelluloses is hydrolyzed into simple sugars that subsequently by LAB is converted to lactic acid so that the pH drops and the process defaunation. Thus there will be an increase in the digestibility of dry matter and total digestible nutrients (TDN). This indicates that the cellulolytic microbes can produce cellulose and xylanase enzymes capable of breaking down lignocelluloses bonds so as to hold the penetration to break down and degrade the cell walls to further convert into simple carbohydrate compound which is used as a substrate by *Lactobacillus plantarum* to produce lactic acid to lower the pH. Cellulolytic microbial isolates and *Lactobacillus plantarum* can be used as treatment fermented rice straw which gives results in improving the quality of the feed substances by lowering the coarse fiber content and improve digestibility of feed, so that the rice straw can be improved nutritional value by using multiple levels of *Aspergillus niger* and *Lactobacillus plantarum*.

MATERIALS AND METHODS

Microbial source used was *Aspergillus niger*, collection of the University Centre for Biotechnology Universitas of Gadjah Mada and *Lactobacillus plantarum*, collection of Nutritional Biochemistry Laboratory of the Faculty of Animal Science, Universitas Gadjah Mada. The fermented material is rice straw and bran IR64 obtained from farmers in the district of Magelang, and molasses. It also used reagents for microbial growth, CMC-ase activity determination, determination of lactic acid levels by Baker and Summerson method, and determination of the chemical composition of fermented rice straw with proximate method.

Aspergillus niger was grown in the sterile Potato Dextrose Broth (PDB) medium, then incubated at room temperature for 4 days. *Aspergillus niger* was then tested to determine its cellulolytic ability and CMC-ase activity. *Lactobacillus plantarum* was grown in sterile liquid Man Rogosa Sharpe (MRS) medium then incubated for 24 hours. This study aims to increase microbial isolates of *Aspergillus niger* and LAB (*Lactobacillus plantarum*) and studying it. The study begins from enrichment (enrichment culture) isolates and optimizes isolates with different temperature and time. *Aspergillus niger* was grown in liquid PDB medium sterile, then reproduced in the semi-solid fermentation. Semi-solid medium is a medium GDP plus 10% rice straw substrate. Fermentation is done for 4 days. At the end of fermentation is determined CMC-ase activity of his. Rice straw Fermentation were using two kinds of treatment, the levels of *Aspergillus niger* (0, 5, 10, and 15%) and the kinds of carbon sources (molasses and rice bran) that in studies using analysis of variance completely randomized factorial 2x4 pattern. Implementation begins with the fermentation of rice straw chopping fresh rice straw with a size of 3 to 5 cm. Furthermore, rice straw is weighed as much as 100 g was mixed with a carbon source is molasses or rice bran each 2% of the total as feed then coupled with *Aspergillus niger* with a level of 0, 5, 10, and 15% of the total as feed and 10% *Lactobacillus plantarum* in all levels. Once thoroughly mixed and then put into a glass fermenter, then pressed so dense that the air out and become anaerobic atmosphere tube to be incubated for 3 weeks at room temperature. After incubation for 3 weeks, fermentation is terminated and the weighing is done to determine the loss of dry matter (DM). After the sample was taken to determine the level of acidity of the fermentation is done by using a pH meter pH measurement and analysis of lactic acid in spektrofotometris using Baker and Summerson (Hawk et al., 1976). In addition to sample preparation was carried out to determine the chemical composition of rice straw fermentation using proximate analysis.

Data weight loss, pH, lactic acid and chemical composition of fermented rice straw were analyzed using analysis of variance completely randomized design (CRD) 2x4 factorial design. Further tests followed by Duncan's Multiple RangeTest (DMRT) to find out the difference between the mean (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

Cellulolytic activity *Aspergillus niger*

The test results CMC-ase activity of *Aspergillus niger* showed enrichment in semisolid condition produces CMC-ase activity which is better than the enrichment in liquid form. Medium in the form of a liquid medium consisting of the GDP, while the semisolid medium is a medium comprising a substrate, namely GDP and rice straw. Many rice straw contain cellulose that is capable hydrolysed by *Aspergillus niger*. Cellulose can be hydrolyzed by the enzyme is acid-swollen cellulose, carboxymethyl cellulose (CMC), cellulose azure, and trinitrophenyl Cm-cellulose hydrolyzed by endoglucanases (Coral *et al.,* 2002). The addition of rice straw in semisolid medium causes the activity of *Aspergillus niger* is better than that without given (liquid medium) because susbtrat sources used will be complete. CMC-ase activity on *Aspergillus niger* using PDA medium with cellulose substrate is added 0.542 U / ml (Narasimha *et al.,* 2005). Medium according to research results Kasmiran and Tarmizi (2012) that the enzyme activity of *Aspergillus niger* on sustrat coconut pulp with long incubation four (4) days showed large hasilse 2.39 U/ml. By looking at the increase in enzyme activity when used in semisolid medium showed fermentation method can be used to multiply the inoculum in the fermentation process (Table 1).

Table 1. Summary enzime CMC-ase and the fungus *Aspergillus niger*

Enrichment	CMC-ase (U/ml)
Liquid	4.644
Semisolid	5.492

Nutritional Value of Rice Straw Fermentation

Level *Aspergillus niger* and the kinds of carbon sources do not provide significant effect on dry matter (DM) rice straw fermentation. This is consistent with the results of research Dradjat *et al.* (2013) who did the fermentation of rice straw to feed cattle Bali basis. *Aspergillus niger* inoculum levels give real effect to the content of organic matter rice straw fermentation ($P<0.05$). Provision of 5% led to significantly increase the levels of organic matter, however, the increase in *Aspergillus niger* from 5 to 10% does not lead to an increase in organic matter. This is consistent with the results Kasmiran (2011) using local microorganisms to ferment rice straw. Provision of *Aspergillus niger* inoculum with different levels of influence on the levels of crude protein ($P<0.05$). Provision of *Aspergillus niger* as much as 5 and 15% led to significantly increase levels of crude protein with the highest levels resulting from *Aspergillus niger* inoculum level was 5%. Giving molasses as the carbon source resulted in CP levels were higher than rice bran ($P<0.05$). Provision of *Aspergillus niger* inoculum levels give real effect to changes in crude fat (CF) ($P<0.05$). The higher the level of inoculum administration increases levels of crude fat. The highest crude fat produced from *Aspergillus niger* inoculum levels are respectively 15, 10, 5 and the lowest This is consistent with the results of research Irawan (2012) with 10% giving buffalo rumen contents for the fermentation of rice straw.

Provision of *Aspergillus niger* inoculum levels give real effect to changes in crude fiber (CF) ($P<0.05$). Provision of *Aspergillus niger* inoculum will hydrolyze crude fiber thereby increasing the digestibility. Lowest crude fiber produced from *Aspergillus niger* inoculum levels are respectively 15, 5, 10, and the highest 0%. Giving inoculum of 5 and 10% did not make a difference, but the granting of 0 and 15% make a difference. Provision of *Aspergillus niger* inoculum level does not give real effect to changes hay NDF fermentation. Provision of *Aspergillus niger* inoculum levels seem to significantly affect change in the ADF (P<0.05). The highest ADF produced from *Aspergillus niger* inoculum level row was 0, 15, 5, and the lowest 10%. Giving inoculum of 0, 5, and 15% did not make a difference, but it makes a difference to the level of the provision of 10%. Generally *Aspergillus niger* are capable of producing cellulolytic enzymes and amylolytic enzymes such as amylase and gluco-amylase. Cellulose is a component commonly found in plants. *Aspergillus niger* is able to break down cellulose into simple sugars. Crude fiber is able to be hydrolyzed by *Aspergillus niger* using the synergy of three types of enzymes, namely cellobiohydrolase, endoglucanase and β-glucosidase (Bath, 2000 cit. Narasimha *et al.*, 2005). This ability causes the crude fiber content decreased. This is consistent with the results of research Lamid (2006) fermentation of rice straw by adding rumen bacteria xylanolytic origin. Decline in crude fiber in fermented rice straw by giving some cedar *Aspergillus niger* lower levels of crude fiber according to the results of research Kusumaningrum *et al.* (2012).

Provision of *Aspergillus niger* inoculum levels give real effect to the change NFE (P<0.05). The highest NFE produced from *Aspergillus niger* inoculum levels are respectively 5, 0, 10, and the lowest 15% of 42.53 ± 0.80. Giving inoculum of 0 and 5% did not make a difference, but it makes a difference in the level of provision of inoculant 15%. This is consistent with the results of research Kusumaningrum *et al.* (2012), with the provision of some cedar *Aspergillus niger* on rice straw fermentation extract material without increasing the levels of nitrogen.

Table 2. The nutrient content of rice straw fermentation using *Aspergillus niger* various levels and kinds of different carbon sources

Additive Type		Level inoculum				Mean Total
		0%	5%	10%	15%	
DM	Molasses	40.99±2.37	42.83±2.13	43.54±1.53	43.30±0.56	42.66±1.85
	Rice Bran	44.51±0.37	45.31±1.75	45.02±0.35	46.07±5.87	45.23±2.69
	Average[ns]	42.75±2.45	44.07±2.21	44.28±1.28	44.68±4.03	43.94±2.61
OM	Molasses	85.70±0.56	86.40±0.33	86.29±0.21	85.01±0.16	85.85±0.65[a]
	Rice Bran	86.42±0.28	88.22±1.09	86.27±1.67[a]	85.29±0.20	86.55±1.40[b]
	Average	86.06±0.56[a]	87.31±1.23[b]	86.27±1.06[b]	85.15±0.22[a]	86.19±1.13
CP	Molasses	5.34±0,17	6.46±0,49	5.06±0.35	5.80±0.31	5.66±0.63[a]
	Rice Bran	5.05±0.36	6.23±0.20	5.04±0.30	6.23±0.50	5.63±0.69[b]
	Average	5.20±0.30[a]	6.34±0.36[b]	5.05±0.29[a]	6.01±0.44[b]	5.65±0.64
EE	Molasses	1.82±1.20	1.91±0.75	2.40±0.20	3.55±0.17	2.42±0.95[a]
	Rice Bran	1.50±0.27	1.97±0.64	3.30±0.46	3.00±0.36	2.44±0.86[b]
	Average	1.66±0.80[a]	1.94±0.62[a]	2.84±0.59[b]	3.27±0.40b	2.43±0.88
CF	Molasses	34.84±0.48	34.02±0.73	36.08±0.65	33.05±0.21	34.50±1.26[a]
	Rice Bran	34.66±0.35	34.82±1.58	32.99±0.72	33.62±1.68	34.03±1.30[b]
	Average	34.75±0.38[b]	34.41±1.18[ab]	34.54±1.79[ab]	32.33±1.12[a]	34.26±1.27
NFE	Molasses	43.71±1.65	44.02±0.43	42.74±.68	42.62±0.12	43.27±1.01[a]
	Rice Bran	45.21±0.48	45.20±0.79	44.93±2.45	42.44±1.25	44.45±1.73[b]
	Average	44,46±1.37[b]	44.61±0.86[b]	43.84±2.01[ab]	42.53±0.80[a]	43.85±1.51

	Molasses	81.32±1.37	78.39±0.34	80.40±1.40	77.91±2.46	79.50±1.99
NDF	Rice Bran	79.33±2.84	78.20±2.18	80.27±1.87	79.10±1.21	79.22±1.95
	Average[ns]	80.33±2.27	78.29±1.40	80.33±1.48	78.50±1.85	79.36±1.94
	Molasses	63.58±1.37	65.61±3.53	40.55±8.04	61.78±2.17	57.88±11.25[a]
ADF	Rice Bran	63.40±2.09	59.12±4.21	58.64±6.08	59.70±3.92	60.21±4.17[b]
	Average	63.49±1.59[b]	62.36±4.98[b]	49.9±11.78[a]	60.74±3.05[b]	59.05±8.38

CONCLUSIONS

The use of *Aspergillus niger* and *Lactobacillus plantarum* for the best fermented rice straw *Aspergillus niger* was 15 and 10% *Lactobacillus plantarum* of dry matter. The use of molasses substrate better when compared to rice bran, it is seen from the results of the analysis of crude proteins, crude fiber, crude fat, ADF, and the results of physical testing rice straw fermentation.

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