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SOLID STATE FERMENTATION CHARACTERISTIC OF RICE STRAW USING HERBIVORE'S CECUM MICROBES

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ABSTRACT: This study was aimed at identifying highly capable lignolytic microbes from nature for use in Solid State Fermentation (SSF) of rice straw. The SSF silage was prepared in laboratory scale, as the following treatments: uninoculated (control), *Lactobacillus plantarum* FCC 123 (LP), fiber-degrading fungi (*Aspergillus* sp.) from horse cecum (FF), and fiber-degrading bacteria (*Enterococcus casseliflavus*) from buffalo cecum (FB). Incubation was carried out for a month at room temperature. The observed parameters were: organic acids, water-soluble carbohydrate (WSC), microorganism and nutrient composition. Rice straw SSF that was inoculated with LP showed the highest quality of fermentation, indicated by significant highest lactic acid bacteria (LAB) population, and has the lowest of poor bacteria indicators (coliform, aerobic bacteria, and bacilli). The LP treatment also has the highest LAB content and lowest WSC. Among treatments, FB treatment seems to have given a similar result with LP followed by FF. While the chemical composition seems unaffected by treatments. Compared with the fresh material, all fermentation with and without inoculants has reduced neutral detergent fiber (NDF) and increased acid detergent fiber (ADF), but there were no differences among all treatments. Inoculation of both LP and FB could improve rice straw SSF silage quality, but this system could not improve fiber degradation as well as in liquid state fermentation (LSF).

Keywords: Microorganism, Organic acid, Silage, Straw, Water soluble carbohydrate

INTRODUCTION

Fluctuating forage production as feed fiber sources in tropical could give a negative impact to forage availability for livestock. On the other hand, there are large quantities of rice straw that have challenges for disposal (Rathod et al., 2017) which can provide bulk animal feed, and is one of the major feed sources for the livestock production in some rice-producing countries in Southeast Asia (Tang, 2018). Moreover, feeding straw to livestock could be an alternative forage scarcity in the dry season (Ansah and Issaka, 2018) that leads to unoptimized livestock productivity in arid, semi-arid, and tropical areas (Sheikh et al., 2017) and an environmentally friendly way of utilizing straw as a by-product (Aquino et al., 2020). The use of by-products of agro-industry as SSF raw materials is very useful, they play an important role in the production of high-value animal feed because; 1) the quantity is abundant, 2) less competition between humans and livestock, 3) it is available at minimal cost for further processing, 4) it has an appropriate nutrient composition, and 5) it can aid microbial development during SSF (Oladapo et al., 2019). Meanwhile, SSF has the potential to be a candidate for antibiotic replacement strategies in animal feed (Yang et al., 2021). Using rice straw as livestock feed has been reported by some previous studies (Asmare and Yayeh, 2018; Shahryari et al., 2018), both in raw bulk (Rathod et al., 2017) or processed through various physical, chemical, or biological processes (Shrinivasa and Maski, 2017).

In the past, expansive studies have been done to improve nutritional value of rice straw by chemical and enzymatic pre-treatments. Chemical treatment of rice straw has been studied and documented very adequately, but this pretreatment was corrosive and poison potential to workers, meanwhile enzymatic treatment was expensive (Nurjana et al., 2016; Mutmainna et al., 2021). Inoculants can be applied to increase the nutritional value of rice straw, but they are considered impractical and the process has not been optimized especially under field conditions (Mahesh and Mohini, 2013). Implementation of inoculant from various rice paddy (Oryzae sativa) to total mixed ration silage microbial composition have also been studied (Wahyudi et al., 2022). Fresh rice straw with a DM content of 250 g/kg has a high concentration of water-soluble carbohydrates (WSC; 9.79% DM), which is suitable for silage (Lia et al., 2016). Because rice straw contains high fiber, the inoculant needed must be able to degrade complex carbohydrate bonds. Therefore, one of the possible future technologies for rice straw fermentation should focus on isolating and identifying highly capable lignolytic microbes from nature, and multiplying them for the production of lignolytic enzymes.

In previous study, both lignolytics fungi and bacteria have been isolated from Herbivore's lower gut. The study on rumen liquid state fermentation (LSF) showed that lignolytic fungi has improved 6.69% rice straw crude fiber digestion

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(Wahyudi et al., 2010a), and the lignolytic bacteria has increased 53.77% of feed crude fiber digestibility (Wahyudi et al., 2012), but their role in solid state fermentation (SSF) silage using Herbivore's Cecum Microbes has not been determined. Due to the minimal humidity required, only a limited number of microorganisms such as yeast and filamentous fungi can grow well under SSF conditions (Oladapo, et al., 2019). Among others, starter cultures that are widely used for Solid state fermented-feed are namely; Lactobacillus, yeast, bacillus, and mold (Yang, et al., 2021). This study was addressed to confirm the effect of fiber-degrading fungi and bacteria particularly in SSF silage to identify rice straw fermentation characteristics. Nutritional studies are encouraged to study plant by-products, especially rice straw as feed.

This study focuses to develop a simple technology that may partially solve the livestock's forage problem, particularly in small and mixed farming systems in developing countries.

MATERIALS AND METHODS

Ethical regulations

This study was accepted by health research ethics committee at the University of Muhammadiyah Malang according to seven WHO 2011 standard that was referred to by the guidance of CIOMS 2016.

Study area

Rice straw (*Oryzae sativa*) was obtained from rice field. The condition contained no seed, cut about 10cm from the soil surface and withered, chopped, then put in a plastic bag as soon as possible. The inoculants used in this study were *Lactobacillus Plantarum* (LP) FCC 123; FF, fiber-degrading fungi (*Aspergillus* sp.); and FB, fiber-degrading bacteria (*Enterococcus casseliflavus*). The *L. plantarum* (LP) FCC 123 was a typical strain that isolated from plant materials, a commercial LAB the strain has been known for silage making for decades years. The FF was *Aspergillus* sp., fiber-degrading fungi that has been isolated from horse cecum (Wahyudi et al., 2010a). *Aspergillus* sp. has also been used by Sidauruk et al., (2019) to ferment fiber in banana peel. The FB was *E. casseliflavus*, a fiber-degrading bacteria has been isolated from buffalo cecum (Wahyudi et al., 2010b).

Solid state fermentation silage preparation

The SSF silages were prepared on laboratory scale fermentation system treated with 50 µL LP, FF, and FB inoculants and un-treated control (F0). Approximately 100g of wilted rice chopped on the approximate length of 20mm, put on a plastic bag (KRIS BR 2205 type, 22cm × 500cm) and then the bags sealed with a vacuum sealer machine (KRIS VS200). The bag SSF were left for 30 days incubation at room temperature (average 25°C).

Microbial analysis

A-10g sample was merged into 90ml of sterilized water and diluted serially until 10-9. Agar of deMan Rogosa Sharpe (MRS; Difco) was used for total lactic acid bacillus (LAB) incubated under anaerobic conditions at 30°C for 48h (Pazla et al., 2021). Meanwhile, blue light broth (BLB; Nissui Ltd) and Potato Dextrose Agar (PDA; P2182 Sigma-Aldrich) were used for counting Coliform bacteria and mold respectively. Both were incubated at 30°C for 24h. For distinguishing yeast from molds, the appearance of the morphological colony and cell forming were observed through microscopic observation. Incubating at 75°C for 15 minutes before cultivating in nutrient agar (NA; Nissui Ltd.) would differentiate Bacilli from aerobic bacteria. Incubation would last at 30°C for 24h under aerobic conditions. Viable numbers of microorganisms in colony-forming units per gram of fresh matter (FM) were then counted.

Water soluble carbohydrate analysis

The HPLC was used to measure the WSC content, which included glucose, fructose, and sucrose. The analysis was set up: column, shodex sugar SC1011 (8.0mm x 30cm, Shoko); oven temperature 80°C; mobile phase, water; detector, 1,0ml/min; detector, (Jasco RI-1530).

Organic acid analysis

Cold water extract was used to determine fermentation products of SSF by homogenizing a 10g wet sample in 90ml sterile distilled water. Organic acid contents then were measured by HPLC (Jasco) as described method. The pH meter (Echem E-512 ex GR Scientific) was used to find out the sample pH. Meanwhile, the determination of ammonia-N was measured by steam distillation of filtrates.

Nutrient composition

Rice straw was dried in forced-air oven at 65°C for 48 h and ground to pass a 1 mm screen with a Willey mill (ZM200, Retsch GmbH and Co.). Contents of DM, OM, CP, and EE were analyzed according to methods 934.01, 942.05, 976.05, and 920.39 respectively, of AOAC by reference (William, and George, 2005). NDF was analyzed with a thermo stable amylase and sodium sulfite and ADF was analyzed none sequentially; results were expressed without residual ash (Van Soest et al., 2018).

Data analysis

Data obtained were analyzed by one-way analysis of variance (ANOVA). The differences between pairs of means were measured using Duncan's Multiple Range test (DMRT) ($P < 0.05$). All statistical analyses were performed in SPSS version 16 software.

RESULTS AND DISCUSSION

Microorganism and nutrient composition

The treatment was effect on the microorganism composition (Table 1). Lactic acid bacteria were higher in LP and FB compared to FF and F0 ($P<0.05$) otherwise, coliform was higher in FF and F0 compared to LP and FB ($P<0.05$). Aerobic bacteria were highest in F0, FF, and FB compared to LP ($P<0.05$). Bacilli was highest in FF compared to F0, LP ($P<0.05$) however FB was similar with both. Yeast was highest in FF, followed by LP and the lowest was F0 ($P<0.05$) while FB was similar with LP, FF, and F0. Otherwise, the result of mold was on the contrary with yeast ($P<0.05$). The nutrient composition is shown in Table 2. The result showed that the nutrients composition was not significantly different among treatments.

Generally, in this study ensilage with or without inoculants has increased LAB, meanwhile inoculant reduce aerobic bacteria and mold (Ridwan et al. 2019). LAB showed a significant role in silage fermentation. Species, population, and characteristics of LAB could be suggested as a factor to predict the sufficiency of silage fermentation. Silage can be well-preserved when LAB, especially lactobacilli, reaches at least 105 CFU/g of FM. In all treatments, the LAB population counts 107 (Table 1), suggesting high quality silage may be fermented well by all inoculant. Even though the number of coli-form unexpectedly still high, inoculant addition only cause a slight decreasing.

It has stated that addition of *Aspergillus oryzae* or *Saccharomyces cerevisiae* in SSF influence bacterial composition, but not affected to fungi population. Inoculation of *L. plantarum* FCC 123 (LP) in this study has anaerobic bacteria, coliform, bacilli, and mold compared with control (Yuan et al., 2015). It has ensured that LP could well prepare to rice straw ensilage. Furthermore, inoculation of FF and FB also increased LAB and yeast population, decrease aerobic bacteria, coliform, and mold, but slightly increased bacilli. Meanwhile FB was able to inhibit the pathogen bacteria as well as LP (Mutmainna et al., 2021).

In contrast to Zayed (2018), almost all nutrient parameters in this study were not significantly affected by the addition of inoculant. ADF content of silages were higher than raw material content, the increasing ADF content was caused by reducing of NDF by microorganisms. Yanti et al. (2014) and Zhang et al. (2015) reported that increasing of ADF content in fiber fermentation, is caused hemicellulolytic fraction (NDF) has been hydrolyzed by *Aspergillus niger*. Comparing with fresh material, all fermentation with and without inoculants has reduced NDF (Nurjana et al., 2016) and increased ADF, but there were not differences between all treatments. It could be stated the using of fiber degrading bacteria and fungi in SSF could not improve hydrolyzed NDF and ADF fraction, so in this case the inoculants have not needed.

Table 1 - Microorganism's composition (log cfu/g FM)

Parameters \ Treatments	Raw	F0	LP	FF	FB	P Value
Lactic acid bacteria	nd	7.647^{a}	7.929^{b}	7.668^{a}	7.929^{b}	<0.0001
Coliform	6.699	7.398^{c}	5.407^{a}	7.204^{c}	6.301^{b}	<0.0001
Aerobic bacteria	8.398	8.000^{b}	6.146^{a}	7.954^{b}	7.903^{b}	<0.0001
Bacilli	5.531	5.477^{a}	5.342^{a}	5.740^{b}	5.580^{ab}	<0.002
Clostridia	nd	nd	nd	nd	nd	–
Yeast	7.653	6.699^{a}	7.398^{b}	7.602^{c}	7.477^{bc}	<0.0001
Mold	6.699	7.699^{c}	3.544^{b}	3.000^{a}	3.394^{b}	<0.0001

CFU, colony forming unit; FM, fresh matter; F0, Control; LP, *L. plantarum* FCC 123; FF, fiber-degrading fungi/ *Aspergillus* sp; FB, fiber-degrading bacteria/ *E. casseliflavus*; nd, not detected.

Table 2 - Nutrient composition

Parameters		Raw	F0	LP	FF	FB	P Value
DM (%)		68.92	60.33	59.53	60.04	59.73	0.24
NC (%DM)	OM	83.49	83.41	83.25	83.52	83.51	0.94
	CP	4.49	7.70	7.82	7.56	7.48	0.24
	EE	0.03	0.04	0.04	0.04	0.04	0.06
	ADF	37.78	40.25	39.69	39.94	40.33	0.85
	NDF	62.22	59.75	60.31	60.06	59.67	0.85
	OM	83.49	83.41	83.25	83.52	83.51	0.94

DM, dry matter; NC, nutrient composition; OM, organic matter; CP, crude protein; EE, ether extract; ADF, acid detergent fiber; NDF, neutral detergent fiber; F0, Control; LP, *L. plantarum* FCC 123; FF, fiber-degrading fungi (*Aspergillus sp.*); FB, fiber-degrading bacteria (*E. Casseliflavus*)

Table 3 - Water soluble carbohydrate content

Parameters \ Treatments		Raw	F0	LP	FF	FB	P Value
DM (%)		68.92	60.33	59.53	60.04	59.73	0.24
WSC	Glucose	0.1910	0.0512^{a}	0.0630^{b}	0.0600^{b}	0.0630^{b}	<0.0001
	Sucrose	1.1298	1.0912^{d}	0.5206^{a}	0.7144^{c}	0.6337^{b}	<0.0001
	Fructose	0.1990	0.1380^{a}	0.1610^{b}	0.1410^{a}	0.1440^{a}	<0.0001
Total		1.5198	1.2804^{d}	0.7446^{a}	0.9114^{c}	0.8347^{b}	<0.0001

WSC, water soluble carbohydrate; F0, Control; LP, *L. plantarum* FCC 123; FF, fiber-degrading fungi (*Aspergillus* sp.); FB, fiber-degrading bacteria (*E. Casseliflavus*).

Table 4 - pH value and organic acid content

Parameters \ Treatments	Raw	F0	LP	FF	FB	P Value
Moisture (%)	31.08	39.70	40.53.00	39.96	40.97	0.24
pH	6.39	6.06	5.77	6.05	5.97	0.38
Lactic acid (%FM)	Nd	0.31^{a}	1.22^{c}	1.04^{b}	1.31^{c}	<0.0001
Acetic acid (%FM)	1.53	1.53^{b}	0.73^{a}	0.71^{a}	0.67^{a}	<0.0001
Propionic acid (%FM)	ND	ND	ND	ND	ND	–
n-butyric acid (%FM)	ND	ND	ND	ND	ND	–
VBN (g/kg FM)	0.10	0.15	0.14	0.14	0.14	0.84

F0, Control; LP, *L. plantarum* FCC 123; FF, fiber-degrading fungi (*Aspergillus* sp.); FB, fiber-degrading bacteria (*E. Casseliflavus*); nd, not detected; VBN, volatile inoculantse nitrogen.

Water soluble carbohydrate

Water soluble carbohydrate total was significantly different among treatments (Table 3), the highest was in F0 followed by FB and FF, and the lowest was LP ($P<0.05$). The glucose was similar among LP, FF, and FB but it was higher than F0 ($P<0.05$). The sucrose was highest in F0 followed by FB, and FF, and the lowest was in LP ($P<0.05$). The fructose was highest in LP than those of F0, FF, and FB ($P<0.05$). Water soluble carbohydrate content of materials is crucial factors for good silage preparation. Rice straw in this study contain less than 2% sugar and more than 65% dry matter, so with this condition may FF can grow better than LP or FB. Reported that low content of WSC would result a poor fermentation quality of silage. So, actually rice straw need more WSC and water for silage making, but in this study inoculation of FF has been expected to solve the problem. FF would hydrolyze crude fiber for producing sugar as energy source for LAB, and then will be converted into lactic acid or another organic acid such as acetic acid, propionic acid and butyric acid, but in this case, they could not work well same as previous study silage quality of sorghum that mixed legumes (Ardiansyah et al., 2016).

Organic acid content

The characteristic fermentation and the quality of rice straw during silage fermentation in 30 days are shown in Table 4. The result showed moisture, pH, and VBN were not significantly different among treatments, meanwhile, propionic acid and n-butyric acid were not detected. Lactic acid was highest in LP and FB than those of FF, and F0 was the lowest ($P<0.05$). Acetic acid was highest in F0 compared to LP, FF, and FB ($P<0.05$).

Inoculation of with and without inoculant could reduce pH value lower than raw material, but the pH value of the silages was not low enough. LAB presented on surface of plant residue, they will responsible for silage fermentation and influence the fermentation quality. The data proved that inoculant was important to improve a fermentation process. The reducing pH value was followed by increasing of lactic acid and acetic acid production, on the other hand volatile base nitrogen (VBN) content was decreased. But, if the concentration of acetic acid in the SSF is too high, the palatability of the feed will be greatly reduced (Yang et al., 2021). In this case, LP, FF, and FB have similar pattern for producing all organic acids and VBN in silages. The fermentation quality could be evaluated from lactic acid, organic acid, and VBN content in silage. The low level of VBN indicated the silage was well preserved and has a good quality. Several studies that has been reported (Wahyudi et al., 2017) inoculation of LAB could increase silage quality. Base on Table 4, the pH value is relatively high (5.77 – 6.06). But the control had higher pH value compared to the inoculant as well as previous study (Santoso et al., 2014), indicated that the rice straw contains less WSC, so the lactic acids production not enough reducing pH value. Material should have to contain more than 2% WSC to make good quality silage. Different with LP, FB was hetero-fermentative, cocci form, isolated from buffalo's digestive tract, also has ability to produce lactic acid. Lactococcus generally work in early of silage fermentation process before lactobacilli take over their role in lactic acid production (Santoso et al., 2014). Acetic acid and VBN content tend to decrease on this rice straw silage, propionic acid and butyric

acid even not detected. Rice straw silage in this study only contain 31,08% moisture showed that raw material needed 60-65% moisture in material for good silage making, so rice straw actually need water to make a good silage.

CONCLUSION

The present study demonstrated that the use of different inoculant affected the characteristics of rice straw silage of Solid State Fermentation (SSF). Inoculation of both *Lactobacillus plantarum* FCC 123 and *Enterococcus casseliflavus* could improve rice straw solid state fermentation silage quality, but this system could not improve fiber degradation as well as in liquid state fermentation. Making solid state fermentations on a big scale is required for further investigation.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

Wahyudi and Hendraningsih contribute on design the study. Mahmud, Mulatmi and Prima contribute on data collection and data analysis. All authors were contributing to write the manuscript.

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Conflict of interests

The authors declare that there are no conflicts of interest in this study.

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