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Original Article

Selection of *Lactiplantibacillus plantarum* strains as inoculant of rice straw fermentation and its fermentation characteristics

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Abstract

Objective: This study aimed to select *Lactiplantibacillus plantarum* strains suitable for inoculant of rice straw fermentation and to evaluate its fermentation characteristics.
Methods: The experiment was conducted at the Feed Bioprocess Laboratory, Research Center for Applied Zoology, National Research and Innovation Agency (BRIN). This experiment was designed using a 3×4 factorial arrangement in a completely randomized design (CRD). The first factor was inoculant (1%), and the second was incubation time (7, 14, 28, and 58 days). The first stage was selection of 9 strains of *L. plantarum*, based on the ability to lowering the pH and produce lactic acid. The second stage was observing the growth curve of the selected strains of *L. plantarum* as inoculant for rice straw fermentation. The third stage was monitoring of the rice straw fermentation characteristics, including pH values, concentration of lactic acid, water-soluble carbohydrates (WSC) and NH_3-N, also dry matter, ash and crude protein. Data were analyzed based on analysis of variance, and if there was a significant effect, the data were further analyzed with *Duncan's Multiple Range Test.*
Results: The selected strains *L. plantarum* Ca098 and *L. plantarum* 1A-2 showed significant interaction result ($p<0.05$) compared with treating inoculant and ensiling days. The fermentation time influenced ($p<0.05$) the quality of rice straw fermentation including pH, concentration of lactic acid, WSC, NH3-N, and crude protein.
Conclusions: *L. plantarum* Ca098 showed the best rice straw fermentation characteristics, with fleigh value of 163.86±5.97 at 58 days of fermentation. *L. plantarum* Ca098 showed the highest lactic acid, the lowest pH and improve crude protein content.

Keywords: Fermentation; Inoculant; Rice straw; *Lactiplantibacillus plantarum*

INTRODUCTION

Straw is rice plant residue that is available in relatively large quantities. This waste is the remaining vegetative part after harvesting rice seeds. The components of rice straw include leaf blades, leaf sheaths, and stems [1]. The average grain-to-straw ratio is 1:1.25 [2]. Rice straw contains 7% lignin and 13% silicate, so technology is needed to increase the digestibility of crude fiber for use as ruminant feed ingredients [3]. Fermentation is one method of processing rice straw using microorganisms for preservation, which can guarantee feed availability. The addition of microorganisms to the fermentation process

causes the fiber and lignin content to decrease and utilizes dissolved carbohydrates in sugarcane waste [4]. Fermentation is generally carried out by adding lactic acid bacteria (LAB). As an inoculant, LAB will use dissolved carbohydrates in the coffee bean fermentation process to produce volatile aromatic compounds [5]. Inoculant is the most commonly used biological additive for forage fermentation, which will affect the quality of rice straw. LAB inoculant affects the nutrition of fermented forage produced [6]. *Lactiplantibacillus plantarum*, *Enterococcus faecium*, and Pediococcus spp are often used as inoculants. Most available inoculants were not developed for rice straw but for main feed crops, such as corn, alfalfa, and grass. Therefore, in this study, several strains of L. *plantarum* isolated from fermented corn and fermented tape were selected as inoculants in fermented rice straw to increase nutrient availability [7] through acidification and inhibiting the proteolytic activity of plant enzymes. This study aims to evaluate the suitable *L. plantar*um strain for fermentation of Sintanur Variety rice straw with the right fermentation time to preserve rice straw.

MATERIALS AND METHODS

Research materials

The material used was rice straw of the Sintanur variety obtained from the Muara Experimental Garden, Bogor. *Lactiplantibacillus plantarum* strains Ca042, Ca048, Ca098, Ca099, Ca121, Ca126, Ca129, and Ca144 came from a collection from the Indonesian Culture Collection (InaCC) which was isolated from fermented corn plants, while L. *plantarum* 1A-2, isolated from cassava tape, is L. *plantarum* BTCC570 [8].

Selection of LAB growth ability

LAB's ability to produce acid was tested by growing it in liquid MRS media for 8 hours at 25°C [9]. The parameters measured are the pH value and lactic acid concentration. Measuring pH values using a pH meter (BP3001 Trans Instruments) refers to research [10]. Measurement of lactic acid concentration refers to Borshchevskaya *et al.* [11]; a 50 µL sample was centrifuged at 3000 rpm for 5 minutes to separate the supernatant and pellet. Then, 2 ml of 0.2% $FeCl_3$ solution was added to the supernatant and tested using a spectrophotometer (HP-1100; Hewlett-Packard Co., Palo Alto, CA, USA) at 390 nm. The selected strains were tested again by growing them in liquid MRS media for 18 hours at 25°C, and the parameter measured was the number of colonies using the total plate count (TPC) method. Measurements of pH and lactic acid concentration were carried out as previously described.

Observation of growth curves

Starter preparation for the growth curve was carried out by growing 1 ose of *L. plantarum* colonies into 25 mL of MRS broth and incubating for 24 hours at 30°C. 5% starter (1×10^6 cfu/mL) was transferred to 100 mL MRS broth media and incubated at 30°C. Every 2 hours, samples are taken to observe cell growth spectrophotometrically at a wavelength of 600 nm [12].

Experimental design

This study used a completely randomized design (CRD) with a 3 × 4 factorial pattern 4 repetitions. Treatment consists of 2 factors, namely inoculant and fermentation time. The first factor is the variation of the L. *plantarum* inoculant strain, namely (P0) without inoculant; (P1) L. *plantarum* Ca098 inoculant 1% (v/w); (P2) L. *plantarum* 1A-2 inoculant 1% (v/w). The second factor is the fermentation time, namely 7, 14, 28, and 58 days.

Rice straw fermentation and analysis of fermentation samples

After being aired in an open and shady place for 1 night, fresh rice straw is cut into 3-5 cm pieces. Rice straw fermentation is carried out using a silo in the form of a vacuum plastic bag with a capacity of 200 g. The air in the plastic bag was removed using a vacuum machine (Yuu Zoo®). The treatment applied was the addition of 2 variations of inoculant strains *L. plantarum* Ca098 and *L. plantarum* 1A-2. Addition of 1% *L. plantarum* strain inoculum was done with a population of $1x10^6$ cfu/mL. All rice straw fermentation treatments were added with 10% rice bran. Fermentation

of rice straw was carried out at room temperature in the dark for 58 days, with weekly observations.

Analysis of fermented samples

For pH measurements, lactic acid concentrations, soluble carbohydrates (WSC), and ammonia (NH_3-N) were carried out using liquid samples. Liquid samples from each rice straw fermentation were prepared from 10 g of sample, added to 90 mL of sterile water, ground for 1 minute using a blender, then filtered using sterile gauze. The pH value and lactic acid concentration were measured as previously described. While testing the content of dry matter, ash, and crude protein using a sample of fermented rice straw that has been dried at an oven temperature 60°C for 72 hours and ground to pass through a 1 mm sieve.

Analysis of WSC levels

The WSC content was calculated using the Anthrone method using 3 ml of supernatant produced from the centrifugation process, then 2.5 mL of concentrated sulfuric acid (H_2SO_4) and 5% phenol were added, then measured using a spectrophotometer (Shimadzu UV VIS 1201) at a wavelength of 490 nm. with D-glucose as standard [13].

Analysis of NH3-N levels

A liquid sample of 10 µl was put into a test tube, added 1.5 mL of phenol solution and 1.5 mL of NaOCl solution, and boiled for 15 minutes, then analyzed using a spectrophotometer at a wavelength of 630 nm. The results from the spectrophotometer are then divided by 17 for the concentration of NH3-N [14].

Analysis of dry matter content

Analysis of dry matter content refers to the Association of Official Analytical Chemists (AOAC) method [15]. As much as 1-2 g of sample in a porcelain cup, dried in an oven at 105°C for 2 hours. Before weighing, the samples were cooled in a desiccator for 1 hour. The weighing was carried out three times with an interval of 1 hour, and the dry matter was calculated.

Ash content analysis

Ash content analysis refers to AOAC [15]. A 1-2 g sample in a porcelain dish was ashed using a furnace at 600°C for 8 hours. Before weighing, the samples were cooled in a desiccator for 1 hour. After weighing, the ash content is calculated.

Analysis of crude protein content

Analysis of crude protein content was carried out using the FOSS equipment manufacturer's method using KjeltecTM 8400. At the digestion stage, 0.5 g of the sample was put into a Kjeldahl tube, and 2.5 mL of H2S04 and 1 Kjeltab tablet were added. The ingredients that have been mixed are digested until transparent. The distillation and titration stages are carried out in a Kjeldahl tube containing the sample and tested on a Kjeldahl macro apparatus previously prepared with a 0.1 N HCL solution with 40% NaOH solution and a receiver solution.

Fleigh value calculation

The fleigh value calculation is carried out using the pH value and dry matter content (DM) based on the formula where NF= 220 + (2 × %DM – 15) – (40 × pH). Fleigh values are classified into five categories, namely very good (>85), good (60-80), good enough (55-60), moderate (25-40) and poor (<20) [16].

Data analysis

The data obtained during the research were analyzed statistically with an analysis of variance according to the design used. Treatment means were compared using the Duncan Distance Test [17]. Mean differences were considered significant at $P<0.05$. Standard errors (SE) were calculated from the residual mean squares in the analysis of variance. The mathematical model and design used are:

$$Yijk = + i + \beta j + \alpha\beta k + eijk,$$

Where Yijk is the observation on the combination of treatment ij and j replications, is the overall sample average, i is the effect of factor A, j is the effect of factor B, Y is the effect of interaction AB, and eijk is the error associated with the observation.

Table 1. Lactic acid concentration and pH values of *L. plantarum* strains

Parameter	Control	Ca042	Ca048	Ca098	Ca099	Ca121	Ca126	Ca129	Ca144	1A-2
Lactic acid ($g.L^{-1}$)	0	0.22	0.53	0.62	0.45	0.38	0.58	0.44	0.31	0.79
pH	5.7	4.27	4.42	4.41	4.51	4.31	4.39	4.41	4.31	4.46

Table 2. pH value, number of colonies and lactic acid concentration of selected strains

Parameter	*L. plantarumI* Strain	
	1A-2	Ca098
pH	4.47	4.46
Total colony (cfu/ml)	1.3×10^{8}	1.8×10^{11}
Laktat acid ($g.L^{-1}$)	0.49	0.53

RESULTS

Selection and growth ability of L. *plantarum* strains

Nine isolates grown in MRS medium for 8 hours showed decreased pH and various lactic acid production (Table 1). The *L. plantarum* 1A-2 and *L. plantarum* Ca098 strains showed nearly the same pH values, 4.46 and 4.41, respectively. Other strains showed lower pH values, but the lactic acid concentration produced was also low, so only 2 strains were selected for use as inoculants in rice straw fermentation. L. *plantarum* 1A-2 produced the most lactic acid, followed by L. *plantarum* Ca098.

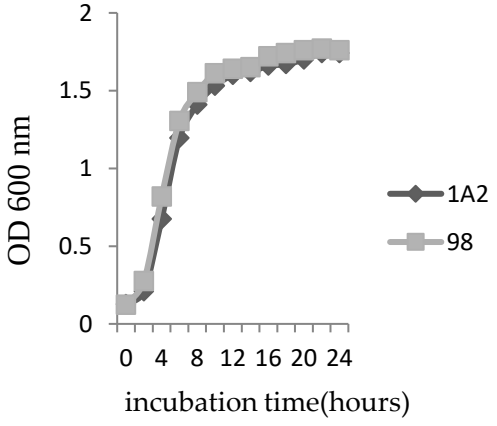

Figure 1. Growth curve of *L. plantarum* 1A-2 and *L. plantarum* Ca098 strains

The selected *L. plantarum* strains, namely *L. plantarum* 1A-2 and *L. plantarum* Ca098, grew well and showed almost the same growth rate in MRS broth at 30ºC for 24 hours (Figure 1). The growth curve of *L. plantarum* Ca098 and *L. plantarum* 1A-2 begins in the lag phase (adaptation), which occurs during the 0th to the 2nd hour. The next phase is the log phase (growth), where the bacteria proliferate quickly from the 2nd to the 10th hour. Furthermore, growth is relatively constant from the 10th to the 22nd hour because this phase is stationary. The strains used as inoculants can be based on observations of growth characteristics such as growth speed, pH decrease, and lactic acid concentration (Table 2).

Chemical composition of rice straw and rice bran

The chemical composition of rice straw and rice bran is presented in Table 3. As ingredients in fermentation, rice straw and rice bran have different nutritional values. Adding rice bran to rice straw fermentation will affect the fermentation process and results. Chemical composition testing is carried out to determine changes that occur during the fermentation process.

Table 3. Chemical composition of rice straw and rice bran

Parameter	Rice straw	Rice bran
Dry material (%)	68.31	89.65
Crude protein (% DW)	6.86	8.13

Effect of inoculant on the characteristics of rice straw fermentation

Adding *L. plantarum* Ca098 and *L. plantarum* 1A-2 inoculants influenced the fermentation characteristics of rice straw, as in Table 4, and Figures 2, 3, and 4. There was a decrease in pH, NH3-N, and WSC values in the treatment with *L. plantarum* Ca098 and *L. plantarum* 1A-2 compared to the control treatment. Fermentation of rice straw with the addition of different L. plantarum inoculants were able to lower the pH to 4.73, and 4.59 at 14 days of fermentation compared to the

Table 4. Characteristics of rice straw fermentation

Parameter	Inoculant	Fermentation time (Days)			
		7	14	28	58
Dry material (%)	K	63.81±6.33^{ab}	64.78±11.41^{ab}	65.20±1.60^{ab}	65.16±4.52^{ab}
	1A-2	71.76±3.40^{bc}	76.75±9.87^{c}	68.80±1.88^{a}	68.51±5.42^{ab}
	Ca098	62.67±1.78^{a}	67.70±2.41^{a}	64.67±2.50^{ab}	64.47±3.03^{ab}
Ash (%DW)	K	20.30±0.71	19.79±1.42	18.43±1.78	20.42±0.84
	1A2	19.30±0.80	19.50±1.15	19.92±0.66	19.09±0.42
	098	19.43±1.09	19.92±1.04	19.82±0.69	18.69±0.72
Crude protein (%DW)	K	6.46±0.24^{bc}	6.51±0.38^{bc}	6.43±0.24^{bc}	6.35±0.46^{bc}
	1A2	6.22±0.21^{abc}	5.72±0.85^{a}	6.38±0.10^{bc}	6.40±0.25^{bc}
	098	6.68±0.20^{c}	6.02±0.24^{ab}	6.62±0.08^{c}	6.62±0.14^{c}
pH	K	5.15±0.09^{g}	4.91±0.16^{f}	4.61±0.04^{c}	4.60±0.05^{c}
	1A-2	4.85±0.09^{e}	4.73±0.10^{d}	4.39±0.09^{b}	4.28±0.05^{a}
	Ca098	4.72±0.05^{d}	4.59±0.05^{c}	4.31±0.05^{a}	4.25±0.01^{a}
Lactic acid (g.L^{-1})	K	3.03±0.44^{a}	3.25±0.46^{ab}	16.52±1.31^{d}	21.70±1.67^{e}
	1A-2	4.03±0.27^{ab}	12.68±0.49^{c}	23.65±1.86^{f}	26.08±1.15^{g}
	Ca098	4.88±0.48^{b}	13.43±1.08^{c}	28.79±2.17^{h}	33.71±2.05^{i}
NH_3-N(mM)	K	0.20±0.03^{ab}	0.38±0.10^{c}	0.34±0.11^{bc}	0.70±0.15^{e}
	1A-2	0.12±0.04^{a}	0.31±0.08^{bc}	0.27±0.05^{abc}	0.52±0.18^{d}
	Ca098	0.12±0.04^{a}	0.29±0.13^{bc}	0.37±0.08^{c}	0.75±0.16^{e}
Fleigh value	K	126.54±12.11^{a}	138.09±25.11^{ab}	150.92±3.40^{bcd}	151.01±8.73^{bcd}
(%)	1A-2	154.36±7.27^{bcde}	168.98±20.48^{de}	166.69±4.74^{de}	170.59±12.29^{e}
	Ca098	141.23±5.79^{abc}	156.65±5.21^{cde}	161.86±3.66^{de}	163.86±5.97^{de}

The means in the same column with different superscripts are significantly different ($p<0.05$). SEM= standard error of the mean; Different a-g values indicate significant differences between fermentation times in the same treatment; dry matter. ash. crude protein. pH. lactic acid. NH3-N and Fleigh value.

control 4.91 and continued to decrease at 28 days of fermentation with a pH of 4.39 and 4.31 compared to the control 4.61, while at 58 days of fermentation, the pH reached 4.28 and 4.25 compared to the control 4,60. An increase in lactic acid concentration followed the decrease in pH during fermentation. NH3-N concentration decreased significantly during fermentation with inoculant treatment, but there was an increase with fermentation time. WSC concentration decreased after 14 days of fermentation with the addition of inoculant.

The concentration of lactic acid increased with the inoculant treatment and the duration of fermentation compared to the control. The highest concentration of lactic acid 33.71 g.L-1 was obtained in treatment with *L. plantarum* Ca098 at 58 days of fermentation. The analysis of variance and further tests on pH values, NH3-N concentration, WSC, and lactic acid in rice straw fermentation showed significant differences ($p<0.05$).

The results of calculating the Fleigh value are presented in Table 4. The addition of *L. plantarum* and fermentation time had a significant effect on the Fleigh value. Adding L. plantarum Ca098 showed a higher Fleigh value when compared to other treatments because it had the lowest pH of 4.25, so it was in a good category. Based on the Fleigh value category, the characteristics of rice straw fermentation with the addition of L. plantarum are included in the good category (Table 4).

Effect of inoculant on nutrient content of fermented rice straw

The effect of adding inoculant on the nutrient content of rice straw fermentation is

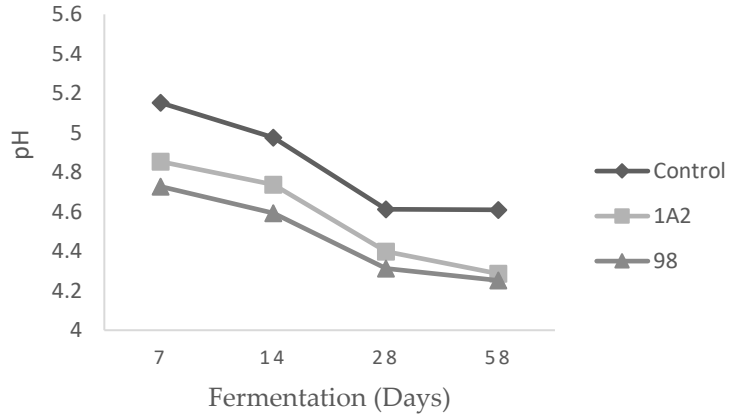

Figure 2. pH value during rice straw fermentation

presented in Table 4. There were differences in the nutrient content of rice straw fermentation between the control and the addition of *L. plantarum* Ca098 and *L. plantarum* 1A2 inoculants.

In all treatments with inoculants, *L. plantarum* Ca098 and *L. plantarum* 1A-2 showed increased dry matter content and decreased ash. *L. plantarum* Ca098 inoculant showed increased levels of crude protein compared to controls.

The interaction between the added inoculant treatments and the fermentation time significantly affected the observed parameters ($p<0.05$). Fermentation of rice straw treated with inoculant showed relatively high dry matter levels on the 14th day of the fermentation process. Then, it decreased at the end of the fermentation process, while dry matter in the control was stable until the end of fermentation (58 days). The effect of inoculants on the ash content in rice straw fermentation was not significantly different (Table 4). Fermented rice straw treated with *L. plantarum* Ca098 inoculant showed higher levels of crude protein ($p<0.05$) than the control.

DISCUSSION

The selection of *L. plantarum* strains to be used as inoculants in rice straw fermentation was done by looking at their ability to lower pH and produce lactic acid. In the growth phase, from the log phase to the initial stationary phase, the *L. plantarum* inoculant produces primary metabolite compounds in lactic acid, acetic acid, and hydrogen peroxide [18]. Therefore, *L. plantarum* inoculant can be used in the fermentation of various fiber sources and produces lactic acid quickly in large quantities. Lactic acid bacteria added to the fermentation process function to ensure fermentation by accelerating the fermentation rate, increasing lactic acid production, lowering the pH value, and reducing proteolysis when ingested by ruminants [19]. In this study, *L. plantarum* significantly increased the concentration of lactic acid and decreased the pH value simultaneously. The low pH value in all fermentation treatments of rice straw inoculated with *L. plantarum* can prevent protein degradation, as shown in Figure 2, increased crude protein levels in Table 4. Adding *L. plantarum* Ca098 reduces the pH quickly and increases the concentration of lactic acid compared to *L. plantarum* 1A-2; the fermented rice straw produced had a higher crude protein content than other treatments. The use of *L. plantarum* 1A-2 inoculant was reported to produce silage with good and stable quality on elephant grass [20], corn crops [21], and rice straw [22].

Whiter and Kung [23] stated that homofermentative LAB reduces proteolysis and deamination in the fermentation process by causing a rapid decrease in pH. The decrease in pH occurs by the acidity increase as a function of fermentation time (Figure 2) [10]. pH stabilization depends on the concentration of lactic acid and possibly the presence of other organic acids. The optimum pH value for good straw fermentation is around 3.8 to 4.2, and there is an increase in lactic acid content by acid hydrolysis of structural carbohydrates [6]. Higher pH

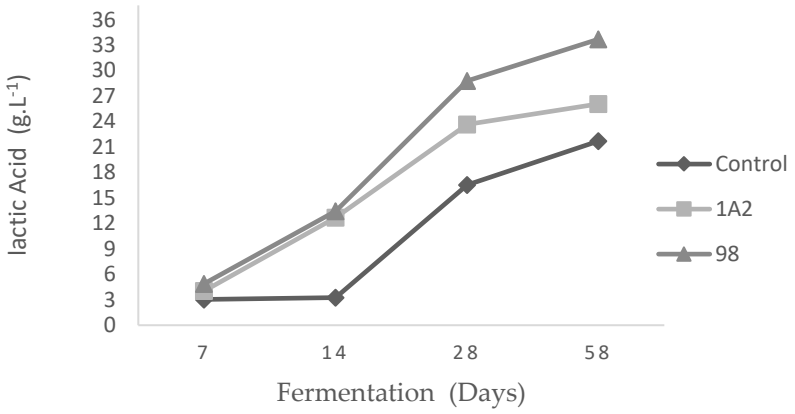

Figure 3. Lactic acid concentration (g.L-1) in rice straw fermentation

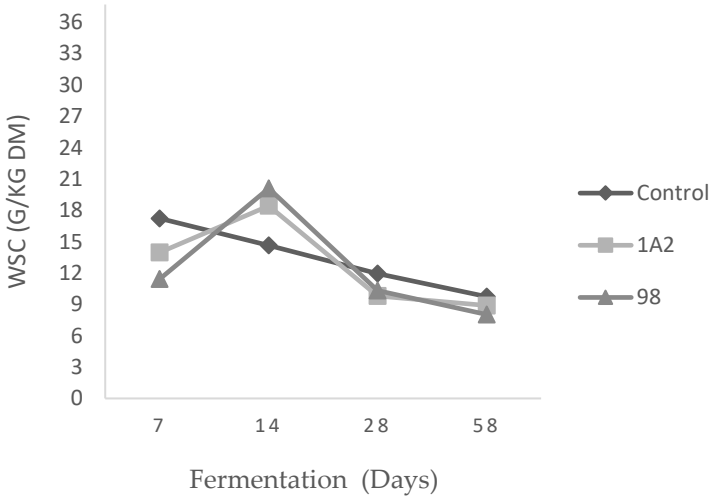

Figure 4. Concentration of dissolved carbohydrates (g.kg-1 DM) in rice straw fermentation

values can be considered by looking at other fermentation characteristics.

Providing inoculant to fermented rice straw increased lactic acid concentration in fermentation for 14, 28, and 58 days (Figure 3). Hopzafel and Wood [24] stated that 1 mol of lactic acid, 1 mol of acetate, and 1 mol of ethanol are converted from 1 mol of glucose by hetero-lactic lactic acid bacteria. In comparison, 2 mol of lactic acid is converted from 1 mol of glucose by homolactic lactic acid bacteria. The initial stage of the fermentation process carried out by LAB is to use dissolved carbohydrates and compete with enterobacteria. As indicated by the amount of lactic acid not increasing sharply or sloping, subsequent fermentation was dominated by LAB until the end of the fermentation process. In the controls, the amount of lactic acid was also significantly different from the inoculant given. The decrease in pH value in the control occurred due to sugar fermentation by microbes that naturally exist in fresh straw as epiphytic microbes [25]. Oliveira *et al*. [26] stated that lactic acid production will be optimal and stable until the end of the fermentation process. If there is a decrease, it is probably caused by the catabolism of amino acids and the production of unwanted secondary metabolite, such as butyric acid, thereby reducing the nutritional value.

The effect of inoculant on WSC in rice straw fermentation is shown in Figure 4, where WSC is used by *L. plantarum* to support its life and produce lactic acid. Rice straw and rice bran have sufficient WSC content, such as glucose and fructose; the amount can reach 30-46% of their dry weight. Besides that, there are small amounts of sucrose, xylose, arabinose, galactose, and mannose [27]. Lactic acid is the final product of dissolved carbohydrates that

is beneficial during fermentation [27]. Adding inoculant reduced WSC starting 14 days of rice straw fermentation and continued to decrease until 58 days of fermentation. The decrease in WSC concentration indicated that the soluble carbohydrates were used as a fermentation substrate for LAB during the fermentation process. The high consumption of WSC in rice straw fermentation is associated with the ability of microbes to grow using acid hydrolysis of structural carbohydrates available in rice straw so that it can lower the pH. The interaction that occurs is the raw material for rice straw fermentation, high WSC concentrations, and the right fermentation time to reduce the pH of rice straw fermentation optimally.

NH_3-N levels showed a decrease with the addition of an inoculant. There is an increase in NH_3-N levels related to the duration of fermentation, which is still relatively low so that it does not damage the crude protein of fermented rice straw [29]. Crude protein levels increased with the addition of *L. plantarum* Ca098 inoculant.

The fleigh value is a parameter that can be used to determine the quality of silage based on the pH value and fermentation dry matter content [16]. The fleigh value of straw fermentation ranges from 133.18 [30]. A high fleigh value when adding inoculant indicates the success rate of rice straw fermentation. The higher the fleigh value, the better the fermentation quality.

CONCLUSION

The *L. plantarum* Ca098 strain as an inoculant in rice straw fermentation produced excellent fermentation quality, with a fleigh value of 163.86±5.97 at 58 days of fermentation. The *L. plantarum* Ca098 strain produced the highest lactic acid concentration, lowest pH and increased crude protein levels.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest with any financial organization regarding the material discussed in this manuscript.

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REFERENCES

1. Rosmiza, M. Z., W. P. Davies, A. C. R. Rosniza, M. Mazdi, and M. Jabil. 2014. Farmers' knowledge on potential uses of rice straw: an assessment in MADA and Sekinchan, Malaysia. Malay. J. Soc. Space. 10(5):3-43.
2. Darsani, R. Y. and Koesrin. 2018. Preferensi petani terhadap karakter beberapa varietas unggul padi lahan rawa pasang surut. Penelitian Pertanian Tanaman Pangan. 2(2):85-94. Doi: 10.21082/jpptp.v2n2.2018.-p85-94
3. Rhofita, E. I. 2015. Study of the utilization of rice straw waste in the upstream. J. Environ. Eng. 1(2):1-6. Doi: 10.1016/j.jobab-.2020.07.001
4. Carvalho, B. F., C. L. S. Avila, M. G. C. P. Miguel, J. C. Pinto, M. C. Santos, and R. F. Schwan. 2015. Aerobic stability of sugar-cane silage inoculated with tropical strains of lactic acid bacteria. Grass Forage Sci. 70:308-323. Doi: 10.1111/gfs.12117
5. Pereira, G. V. M., D. P. C Neto, A. B. P Medeiros, V. T. Soccol, E. Neto, A. L. Woiciechowski, and C. R. Soccol. 2016. Potential of lactic acid bacteria to improve the fermentation and quality of coffee during on-farm processing. Int. J. Food Sci. Tech. 51(7):1689-1695. Doi: 10.1111/ijfs. 13142
6. Yang, F., Y. Wang, S. Zhao and Y. Wang. 2020. *Lactobacillus plantarum* inoculants delay spoilage of high moisture alfalfa silages by regulating bacterial community composition. Front. Microbiol. 11:1989. Doi: 10.3389/fmicb.2020.01989
7. Goodman, B. A. 2020. Utilization of waste straw and husks from rice production: A review. J. Bioresour. Bioprod. 5:143–162. Doi: 10.1016/j.jobab.2020.07.001
8. Ridwan, R., I. Rusmana, Y. Widyastuti, K. G. Wiryawan, B. Prasetya, M. Sakamoto, and M. Ohkuma. 2015. Fermentation characteristics and microbial diversity of

tropical grass-legumes silages. Asian-Australas J. Anim. Sci. 28:511–518. Doi: 10.5713/ajas.14.0622

9. Kim, J. G., J. S. Ham, Y. W. Li, H. S. Park, C-S. Huh, and B-C Park. 2017. Development of a new lactic acid bacterial inoculant for fresh rice straw silage. Asian-Australas J. Anim. Sci. 30(7):950-956. Doi: 10.5713/-ajas.17.0287
10. Carvalhoa, B. F., C. L. S Ávila, J. C. Pinto, J. Neri, and R. F. Schwan. 2014. Microbiological and chemical profile of sugar cane silage fermentation inoculated with wild strains of lactic acid bacteria. Anim. Feed Sci. Technol. 195:1-13. Doi: 10.1016/j.anifeedsci.2014.04.003
11. Borshchevskaya, L. N., T. L. Gerdeeva, A. N Kalinina, and S. P. Sineokii. 2016. Spectrophotometric determination of lactid acid. J. Anal. Chem. 70(8):755-758. Doi: 10.1134/51061934816080037
12. Cappuccino, J. G. and N. Sherman. 2017. Microbiology; a laboratory manual. 11th edn. In: State University of New York (ed) Rockland Community College, NY, USA. p. 121-127.
13. John, A., G. Barnett, and T. B. Miller. 1950. The determination of soluble carbohydrate in dried samples of grass silage by the anthrone method. J. Sci. Food Agric. 1(11): 336-339. Doi 10.1002/jsfa.2740011106
14. Souza N. K. P., E. Detmann, S. C. Valadares Filho, V. A. C. Costa, D. S. Pina, D. I. Gomes, A. C. Queiroz, and H. C. Mantovani. 2013. Accuracy of the estimates of ammonia concentration in rumen fluid using different analytical methods. Arq. Bras. Med. Vet. Zootec. 65:1752–1758. Doi: 10.1590/S0102-09352013000600024
15. AOAC. 2019. Official methods of analysis. 21th ed. Assoc. Off. Anal. Chem., Arlington, VA.
16. Idikut, L., B. A. Arikan, M. Kaplan, I. Guven, A.I. Atalay, and A. Kamalak. 2009. Potential nutritive value of sweet corn as a silage crop with or without corn ear. J. Anim. Vet. Adv. 8(4):734-741.
17. Steel, R. G. and J. H. Torrie. 1980. Analysis of covariance. Principles and procedures of statistics: A Biometrical Approach. McGraw-Hill Book Company, Inc., NY. p. 401-437.
18. Wang, J., Q. Wang, Z. Xu, W. Zhang, and J. Xiang. 2015. Effect of fermentation conditions on lactic acid production from soybean straw hydrolysate. J. Microbiol. Biotechnol. 25(1):26–32. Doi: 10.4014/-jmb.1405. 05025
19. Contreras-Goveaa, F. E., R. E. Muck, G. A. Broderick, and P. J. Weimer. 2013. *Lactobacillus plantarum* effects on silage fermentation and in vitro microbial yield. Anim. Feed Sci. Technol. 179:61–68. Doi: 10.1016/j.anifeedsci.2012.11.008
20. Hapsari, S. S., Suryahadi, and H. A. Sukria. 2016. Improvement on the Nutritive Quality of Napier Grass Silage through Inoculation of *Lactobacillus plantarum* and Formic Acid. Med. Pet. 39(2):125-133. Doi: 10.5398/medpet.2016.39.2.125
21. Sari, N. F., R. Ridwan, and Y. Widyastuti. 2017. The quality of corn silage product from Technopark of Banyumulek Lombok, West Nusa Tenggara. Buletin Peternakan, 41(2):156-162. Doi: 10.21059/buletinpeternak .v41i2.15513
22. Sarwono, K. A., R. Rohmatussolihat, M. Watman, S. Ratnakomala, W. D. Astuti, R. Fidriyanto, R. Ridwan, and Y. Widyastuti. 2022. Characteristics of fresh rice straw silage quality prepared with addition of lactic acid bacteria and crude cellulase. AIMS Agric. Food, 7(3): 481–499. Doi: 10.3934/agrfood.2022030
23. Whiter, A. G. and L. Kung Jr. 2012. The effect of a dry or liquid application of *Lactobacillus plantarum* MTD1 on the fermentation of alfalfa silage. J. Dairy Sci. 2001;84:2195-202. Doi: 10.3168/jds.S0022-0302(01)74666-8
24. Holzapfel, W. H. and B. J. B. Wood. 2014. Lactic acid bacteria in contemporary perspective. Dalam Wood, B. J. B dan W. H. Holzapfel (ed). The Genera of Lactic Acid Bacteria, Blackie Academic & Professional, London. p. 1-6.
25. Heinritz, S. N., S. D. Martens, P. Avila, and S. Hoedtke. 2012. The effect of inoculant and sucrose addition on the silage quality of tropical forage legumes with varying ensilability. Anim. Feed Sci. Tech. 174: 201-210. Doi: 10.1016/j.anifeedsci.2012.03.017
26. Oliveira, A. S., Z. G. Weingberg, I. M. Ogunade, A. A. P. Cervantes, K. G.

Arriola, Y. Jiang, D. Kim, X. Li, M. C. M. Gonçalves, D. Vyas, and A. T. Adesogan. 2017. Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. J. Dairy Sci. 100:4587–4603. Doi: 10.3168/jds.2016-11815

27. Zhao, J., Z. Dong. J. Li, L. Chen, Y. Bai, Y. Jia, and T. Shao. 2019. Effects of lactic acid bacteria and molasses on fermentation dynamics, structural and nonstructural carbohydrate composition and in vitro ruminal fermentation of rice straw silage. Asian-Australas J. Anim. Sci. 32(6):783-791. Doi: 10.5713/ajas.18.0543
28. Hoffmann, F., M. G. B. Pagnoncelli, L. R. R. Mayer, A. C. Fluck, R. Macagnan, P. C. Taraves. L. Zorzi, and W. F. Almeida, 2019. *Lactobacillus plantarum* LPBR01 as inoculant in sugar cane silage. J. Anim. Sci. 41(04):2-7. Doi: 10.4025/actascianimsci.v41i1.45708
29. McDonald, P., R. A. Edwards, J. F. D. Greenhalgh, C. A. Morgan, L. A. Sinclair and R. G. Wilkinson. 2022. The nutritional characteristics of foods: silage. In: Animal nutrition. Eight edition. Pearson. Harlow, England. p. 521-542.
30. Naibaho, T. Despal, and I. G. Permana. 2017. Perbandingan silase ransum komplit berbasis jabon dan jerami untuk meningkatkan ketersediaan pakan sapi perah berkualitas secara berkesinambungan. Buletin Makanan Ternak 104(2):12-20.