Agriculture and Food

AIMS Agriculture and Food, 7(3): 481–499.
DOI: 10.3934/agrfood.2022030
Received: 19 December 2021
Revised: 08 May 2022
Accepted: 24 May 2022
Published: 01 July 2022

http://www.aimspress.com/journal/agriculture

Research article

Characteristics of fresh rice straw silage quality prepared with addition of lactic acid bacteria and crude cellulase

Ki A. Sarwono[1], Rohmatussolihat Rohmatussolihat[1], Muh Watman[2], Shanti Ratnakomala[3], Wulansih D. Astuti[1], Rusli Fidriyanto[1], Roni Ridwan[1] and Yantyati Widyastuti[1,*]

[1] Research Center for Applied Zoology, National Research and Innovation Agency (BRIN), Cibinong, West Java, 16911, Indonesia
[2] Directorate of Laboratory Management, Research Facilities and Science and Technology Park, National Research and Innovation Agency (BRIN), Cibinong, West Java, 16911, Indonesia
[3] Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN), Cibinong, West Java, 16911, Indonesia

* **Correspondence:** Email: yantyati.widyastuti@brin.go.id, yantyatiwidyastuti@yahoo.com; Tel: +62218754587.

Abstract: The objective of this study was to determine the characteristics of fresh rice straw silage quality prepared with addition of *Lactiplantibacillus plantarum* 1A-2 and crude cellulase alone or in combination. Quality of the silage was observed through the chemical composition, chemical structure and *in vitro* digestibility. Six treatments were used in this study, i.e., 1) rice straw without any treatment as control, 2) rice straw with addition of 0.1% *L. plantarum* 1A-2 (LAB1), 3) rice straw with addition of 1% crude cellulase (E1), 4) rice straw with addition of 0.1% *L. plantarum* 1A-2 and 1% cellulase enzyme. (LAB1 E1), 5) rice straw with addition of 2% crude cellulase (E2), 6) rice straw with addition of 0.2% *L. plantarum* 1A-2 and 2% crude cellulase (LAB2E2). Each treatment was replicated by four times (n = 24). Ensilage was carried out for 60 days. Data obtained were analyzed by using one-way analysis of variance (ANOVA) according to complete randomized design. The result indicated that the treatments increased dry matter (DM) ($p = 0.001$), crude protein ($p < 0.001$) and lactic acid ($p < 0.001$). Meanwhile, reduced pH ($p < 0.001$) and organic acids (acetic, propionic and butyric ($p < 0.001$)). Total crystallinity index (TCI) of rice straw silage varied among treatments and decreased in crystallinity (%) except for LAB2E2, which showed the lowest crystalline size. The treatment increased DM digestibility ($p = 0.397$) with the highest in LAB2E2. There is significant effect ($p < 0.001$) on increasing the main SCFA products from *in vitro* rumen fermentation. This study

suggests that addition of *L. plantarum* 1A-2 inoculant alone or with crude cellulase improved fresh rice straw silage quality.

Keywords: fresh rice straw; silage; lactic acid bacteria; crude cellulase; X-Ray diffraction; Fourier-transform infrared spectroscopy

1. Introduction

Rice straw represents as a highly available plant structural polysaccharide that can be utilized as feedstuff for ruminants. Every year, the global production of rice straw is 8×10^{11} kg as waste from the rice production process [1,2]. The utilization of rice straw as feedstuff is limited due to the nutritional content of rice straw. Rice straw contains a high level of plant cell wall components such as cellulose, hemicellulose, and lignin [3]. The high content of plant cell wall in rice straw leads to low digestibility when fed to ruminant. Efforts have been made to use rice straw as feed as optimum as possible through several approaches, such as use it while still fresh [4] and understand its degradation pattern in the rumen. Degradation of rice straw in the rumen of dairy cattle took place by a strong contribution of tightly attached bacteria as observed by Illumina sequencing [5]. Another important approach was to observe the chemical structure of feed by spectroscopy-based analysis such as Fourier transform infrared (FTIR) [6,7].

Ensiling or fermentation process of silage has been widely used to preserve the nutrient value of rice straw, which can increase the feeding value of rice straw, increase dry matter (DM), organic matter (OM) digestibility, and dry matter intake (DMI) [8,9]. Ensiling depends on the activity of microorganisms and many works had been conducted to investigate the effect of adding lactic acid bacteria (LAB) inoculant to silage, to ensure that LAB dominates and enhance lactic acid concentration in the fermentation process of silage [10,11]. It is widely accepted that LAB inoculants improved silage quality by increasing lactic acid to preserve nutrients in the silage quickly and inhibit spoilage silage microorganisms such as fungi and clostridial bacteria. At the same time, there is a decrease in pH, ammonia, and organic acids concentration [11,12].

Apart from LAB inoculant, hydrolytic enzymes are also used as additives to increase fermentation quality of silage. The enzyme has two major mode of actions in improving silage quality by 1) releasing of sugar from materials in silage to be used by LAB to produce lactic acid and acetate acid and 2) degrading plant cell walls to reduce fiber content of the silage material [13]. Actinomycetes are widely known as enzymes producers. They are known to contribute in the degradation of soil organic matter by producing enzymes capable of degrading cellulose, hemicellulose, and lignin. Important enzymes produced by *Streptomyces* species such as cellulase, xylanase, amylase, mannanase, have commercial applications in a variety of industries [14]. The addition of cellulase to rice straw is expected to break down fiber to release simple sugar for LAB to produce lactic acid. Enzyme additives might enhance the quality of low-quality forage and increase rumen digestion of fiber *in vitro* [15]. Addition of LAB inoculant and enzyme offers promising result to improve fermentation of the silage and rice straw digestibility. However, there are some inconsistent results from previous experiments. This discrepancy is mainly related to the differences of materials used in the study [12]. Therefore, the purpose of this study was to evaluate the effect of addition of LAB inoculant and crude cellulase alone or in combination on the quality of fresh rice straw silage. Evaluation based on the structure of rice

straw fiber still rarely done using advanced analytical techniques such as XRD and FTIR. This work is the first stage of an attempt to use rice straw for feed which will be made into a complete feed.

2. Materials and methods

2.1. Ethics statement

The experimental protocol used in this study was approved by Animal Ethics Committee of the Indonesian Institute of Sciences (approval number 39/klirens/III/2021).

2.2. Animals and sampling procedure

The animals used for rumen fluid donor were consisted of 4 male fistulated Ongole cross breed cattle and were placed in individual cages. The rumen fluid was collected through the fistula just before morning feeding at around 8 a.m. local time. The collected rumen fluid was strained through four layers of cheesecloth and kept at 39 °C in a thermos flask during transfer to the laboratory.

2.3. Silage additives

Lactiplantibacillus plantarum 1A-2, previously reported as *L. plantarum* BTCC570 [16] was used as silage inoculant meanwhile *Streptomyces* sp. 292 was used to produce crude cellulase. Both *L. plantarum* 1A-2 and *Streptomyces* sp. 292 were from the collection of Indonesian Culture Collection, under the number of InaCC B253 and InaCC A1140, for *L. plantarum* 1A-2 and *Streptomyces* sp. 292, respectively.

Crude cellulase was prepared under following condition, *Streptomyces* sp. 292 was inoculated into 50 mL of preculture of ISP-2 modification medium (4 $g \cdot L^{-1}$ yeast extract, 10 $g \cdot L^{-1}$ malt extract, 1 $g \cdot L^{-1}$ glucose and 10 $g \cdot L^{-1}$ rice straw) and was then aerobically incubated at 37 °C for 6 days with shaking at 200 rpm. A 10% of preculture was then transferred to 500 mL fresh medium for crude cellulase production, incubated at 37 °C for 6 days in shaking incubator. Supernatant was harvested by centrifugation at 8000 rpm for 10 minutes at 4 °C. The activity of crude cellulase was measured by using the method of Miller [17] with modifications. Carboxymethylcellulose (CMC) was used as the substrate [18]. Glucose standard was used to plot the standard curve. Measurement of glucose released from CMC was expressed as Unit (U), in which, U = mg of glucose equivalents released $min^{-1} \cdot mL^{-1}$ crude enzyme. The activity of crude cellulase was 0.248 $U \cdot mL^{-1}$.

2.4. Silage materials

Rice variety used in this study was Inpari 32, which represent as one of new high yielding rice varieties. Fresh rice straw was collected from Indonesian Center for Rice Research, Sukamandi, West Java. Rice was harvested and threshed traditionally by hand. Rice straw then collected for ensiling. After 1-day wilting at room temperature, collected rice straw was cut into 2–3 cm long manually and mixed to ensure parts of rice straw were distributed equally.

2.5. Silage preparation

Inoculant of *L. plantarum* 1A-2 was diluted on sterile distilled water to achieve desired application rate at 10^9 CFU·mL^{-1}. Chopped rice straw (2.5 kg) and rice bran (125 g) represent as basal materials of silage, which then added by silage additives alone or in combination according to the treatment, homogenized and divided into four replicates. The control was sprayed with the same volume of sterile distilled water. Six treatments were used in this study, i.e., 1) rice straw without any treatment as control, 2) rice straw with addition of 0.1% *L. plantarum* 1A-2 (LAB1), 3) rice straw with addition of 1% crude cellulase (E1), 4) rice straw with addition of 0.1% *L. plantarum* 1A-2 and 1% crude cellulase (LAB1E1), 5) rice straw with addition of 2% crude cellulase (E2), 6) rice straw with addition of 0.2% *L. plantarum* 1A-2 and 2% crude cellulase (LAB2E2). Treatment 1–4 and 5–6 with addition of 1% (25 mL) and 2% (50 mL) additives, respectively, according to proportion of *L. plantarum* 1A-2 and cellulase.

After mixing, the rice straw mixture was put into a vacuum emboss food grade plastic container (30 × 20 cm) as silo in ensiling process which contain 500 g of mixed silage materials. The air in the plastic bag was sucked using a vacuum machine (Yuu Zoo®), then was stored at room temperature for 60 days.

After 60 days of ensiling, silage was opened and measured for temperature, mold percentage, and moisture content. A representative of sample, 10 grams of silage was mixed with 90 mL of sterile distilled water and then mixed in a blender machine for 1 min. The mixture was then strained by using two layers of cheesecloth. Fresh juice was used for pH measurement and the rest was kept in a freezer at −20 °C until analysis of NH_3-N, lactic acid and short-chain fatty acid (SCFA). The remaining silage from each silo was divided into 2 parts. First part was dried in drying oven at 60 °C and was ground to pass through 1 mm sieve for chemical analysis, while the second part was kept in freezer.

2.6. Chemical analysis of silage

Ground dry silage sample was used for analysis of dry matter (DM) and organic matter (OM) which were conducted according to AOAC [19] procedure. Crude protein (CP) was determined by using Kjeltec 8400 following FOSS manufacturer's procedure. Crude fiber (CF), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were analyzed according to the FOSS manufacturer's procedure using a Fibertec 2010. Hemicellulose content (%) was calculated using following formulae

$$Hemicellulose\ (\%) = NDF\ (\%) - ADF\ (\%) \quad (1)$$

The ground dry sample was also used for X-ray diffraction (XRD) and Fourier Transform Infrared (FTIR) analyses. X-ray diffraction (XRD) analysis was conducted by using an XRD diffractometer (X'Pert PRO, Panalytical, The Netherlands). The sample was prepared by grounding to sieve through 100 mesh. XRD patterns were collected with Cu radiation at absorbance wave at 0.154 nm, voltage 40.0 kV, current 30 mA. Data were collected from 10–80° 2 Ø with step size 0.02, and speed at 2°/min. Crystallinity (%) and amorphous (%) were calculated by using the following formula

$$Crystalinity\ (\%) = \frac{lcrystaline}{(lcrystalline + lamorphous)} \quad (2)$$

$$Amorphous\ (\%) = \frac{lamorphous}{(lcrystalline + lamorphous)} \tag{3}$$

Scherrer's equation [20] was used for estimating crystallite size:

$$\beta = \frac{k\lambda}{\tau} \tag{4}$$

where λ is the wavelength of the incident X-ray (1.5418 Å), θ the Bragg angle corresponding to the (002) plane, β the full-width at half maximum (FWHM) of the X-ray peak corresponding to the (002) plane, τ is the X-ray crystallite size, and k is a constant with a value of 0.89 [21].

Fourier Transform Infrared (FTIR) Spectrometry was used to analyze functional groups of the control and treated rice straw silage samples. The FTIR spectra of all samples were run on a Perkin-Elmer UATR Two (Waltham, MA, USA) spectrometer and were recorded in the absorption band mode in the range of 4000–400 cm^{-1}, number of scanning 16, resolution 4 cm^{-1}, and data interval 1 cm^{-1}. Total crystalline index (TCI), hydrogen bond index (HBI), lignin-cellulose (L/C) ratio were calculated as the ratio of intensities at particular wave numbers (cm^{-1}) as: 1371/2918; 3333/1320; and 1516/899 respectively [22].

NH_3-N was analyzed by adding 10 μL of sample to test tube and added with 1.5 mL of phenol solution and 1.5 mL of NaOCl solution and incubated in a water bath at 100 °C for 15 min. The absorbance was measured by using a spectrophotometer at 390 nm and was divided by 17 to determine NH_3-N concentration [23]. Lactic acid concentration was analyzed by using a spectrophotometric according to the method by Borshchevskaya et al. [24].

Short chain fatty acids (SCFA) analysis was performed using GC-MS Simadzu-QP2010 SE comprising of AOC-20i+s autosampler and MEGA-WAX MS column (length, 30 m; i.d., 0.25mm; and film thickness, 0.25 μm). Ultrahigh purity helium (99.99%) was the carrier gas with 3 mL/min flow rate. The sample, 1.25 mL, was prepared by mixing with 30 mg 5-Sulfosalicylic acid dehydrate in 1.5 mL tube. Sample was centrifuged at 13000 rpm, 4 °C for 10 minutes. The particle-free of sample (1 μL) was taken using a syringe and injected into the injector with a split ratio of 50:1 with the solvent cut time was set at 3 min. The injection, transfer line, and ion source temperatures were all at 250 °C. The column temperature was at 100 °C and held for 9 min followed by an increase to 200 °C at a rate of 10 °C/min for 10 min. The total run time was approximately 29 min. The identities and quantification of SCFA were confirmed with Volatile Free Acid Mix (Supelco, CRM46975) standard.

2.7. In vitro digestibility of rice straw silage

In vitro rumen fermentation to determine digestibility was conducted by the method of Theodorou et al. [25] with modification. Rumen fermentation media for rumen *in vitro* incubation was a mixture of strained rumen fluid and McDougall buffer [26] in a ratio of 1:2 (rumen fluid: buffer). The buffer was pre-heated to 39 °C and flushed with CO_2 gas before mixing. Rumen fermentation media was added to 100 mL serum bottle containing 0.5 g ground silage as substrate. The bottle was flushed with CO_2 gas for 30 s before sealed in order to get an anaerobic condition. Incubation was carried out in a water bath at 39 °C for 48 h. Gas production was recorded at 2, 4, 8, 10, 12, 24 and 48 h incubation using a syringe. After 48 h incubation, samples were centrifuged at 378 × g for 10 min to separate supernatant and residue. Supernatant was analyzed for pH using a pH meter (BP3001 Trans Instruments) and partial SCFA followed the method as described above. The residue was further

incubated with 50 mL pepsin-HCl solution (containing 2 $g \cdot L^{-1}$ pepsin and 17.8 $mL \cdot L^{-1}$ HCl) at 39 °C for 48 h. After 48 h, the residue was separated from the solution by vacuum filtration using Whatman™ papers no 41 (CAT No.1441-125). Dry matter digestibility (DMD) and organic matter digestibility (OMD) were determined according to Tilley and Terry [27].

2.8. Statistical analysis

The data of silage quality were analyzed according to complete randomized design by using the following mathematical model

$$Y_{ij} = \mu + T_i + \varepsilon_{ij} \quad (5)$$

where Y_{ij} = the predicted output for predictor variable Y; μ = overall mean of the treatment; T_i = i-th treatment effect; and ε_{ij} = error. The data of the rumen fermentation profile *in vitro* were analyzed by using ANOVA according to complete block randomized design. Duncan test was used to differentiate between treatment means when the ANOVA test showed a significance difference. The level of significance of an effect was set at $p < 0.05$. Significant differences were accepted if $p < 0.05$. Data of gas production were fitted with the Gompertz model [28] which were defined as

$$P = be^{-Le^{-ct}} \quad (6)$$

where 'P' is volume of gas production (mL) at time t, 't' is incubation period (h), 'b' is asymptotic gas production (mL), 'e' is Euler's number, 'L' is lag time (h), and 'c' is gas production rate constant ($mL \cdot h^{-1}$). The data were analyzed using IBM SPSS Statistic 23 (SPSS Inc., IL, USA).

3. Results

3.1. Characteristics of rice straw silage

The temperature of silage during harvest was in the range of 28–30 °C. Addition of lactic acid bacteria and crude cellulase did not have any effect on the moisture content and mold content of silage during harvest (moisture content $p = 0.081$; mold $p = 0.290$). Moisture content of Control, LAB1, E1, LAB1E1, E2, and LAB2E2 were 26.678%, 29.650%, 28.478%, 28.023%, 32.115% and 31.270%, respectively. Meanwhile, mold content of Control, LAB1, E1, LAB1E1, E2 and LAB2E2 were 7.290%, 6.865%, 4.645%, 6.510%, 7.995% and 8.425%, respectively (moisture content $p = 0.081$; mold $p = 0.290$). DM and OM content of silage significantly increased ($p = 0.02$) in LAB1 and LAB1E1, respectively, but not with other treatments. A highly significant effect on silage CP content was observed ($p < 0.01$), in LAB1 increased in CP content by 9% compared to that of control. Silage CF content significantly decreased by treatment with the highest decrease in LAB1E1. NDF content of rice straw silage was significantly decreased by E1 and LAB1E1 treatments. Meanwhile, ADF content of all treatment were significantly lower than control ($p < 0.001$). Hemicellulose content of silage was significantly increased by treatment ($p = 0.02$) with the highest hemicellulose content was LAB2E2 treatment. There was a highly significant reduction of silage pH ($p < 0.01$) in all treatments except for E1 with the highest reduction in LAB2E2. NH_3-N concentration of silage significantly reduced in E2 and LAB2E2 ($p < 0.01$). Lactic acid content significantly increased in all treatments except for E1

($p < 0.01$). The highest lactic acid was observed in LAB2E2 where lactic acid content increased by 300% than control.

Table 1. Chemical composition of rice straw silage.

Item	Treatments						SEM	p-value
	Control	LAB1	E1	LAB1E1	E2	LAB2E2		
Dry Matter (%)	94.265^{a}	94.295^{a}	95.097^{a}	96.612^{b}	95.217^{a}	94.505^{a}	0.215	0.002
Organic Matter (%DM)	71.265^{a}	73.668^{b}	72.138^{ab}	75.678^{c}	73.038^{b}	73.245^{b}	0.348	0.001
Crude Protein (%DM)	8.135^{b}	8.870^{c}	7.605^{a}	8.218^{b}	8.363^{b}	8.138^{b}	0.091	< 0.001
Crude Fiber (%DM)	32.558^{b}	25.755^{a}	27.273^{a}	25.838^{a}	26.345^{a}	27.495^{a}	0.637	0.005
Neutral Detergent Fiber (%)	66.932^{b}	59.893^{a}	66.866^{b}	60.826^{a}	64.582^{ab}	67.206^{b}	0.841	0.009
Acid Detergent Fiber (%)	60.355^{d}	47.259^{ab}	52.127^{c}	44.456^{a}	49.228^{bc}	47.496^{ab}	1.137	< 0.001
Hemicellulose (%)	6.577^{a}	12.633^{b}	14.739^{bc}	16.370^{bc}	15.354b^{c}	19.710^{c}	1.059	0.002
pH	5.395^{c}	4.600^{b}	5.325^{c}	4.548^{b}	4.440^{ab}	4.248^{a}	0.095	< 0.001
Ammonia-N ($mmol \cdot dL^{-1}$)	2.060^{c}	1.965^{c}	1.900^{bc}	1.528^{ab}	1.135^{a}	1.168^{a}	0.091	< 0.001
Lactic acid ($g \cdot L^{-1}$)	1.133^{a}	3.888^{b}	1.270^{a}	3.345^{b}	3.940^{b}	4.640^{c}	0.293	< 0.001
Acetic acid ($mmol \cdot dL^{-1}$)	9.340^{c}	5.455^{b}	8.975^{c}	4.258^{ab}	3.888^{a}	3.755^{a}	0.518	< 0.001
Propionic acid ($mmol \cdot dL^{-1}$)	2.160^{c}	1.602^{b}	1.980^{c}	0.000^{a}	1.580^{b}	0.000^{a}	0.186	< 0.001
Butyric acid ($mmol \cdot dL^{-1}$)	2.925^{c}	1.645^{b}	2.658^{c}	1.315^{ab}	0.870^{a}	0.758^{a}	0.179	< 0.001

Note: SEM: standard error of mean. $^{a–d}$ Means with different superscripts within a row significantly differed ($p < 0.05$). Control: without additive; LAB1: 0.1% *L. plantarum* 1A-2; E1: 1% cellulase; LAB1E1: 0.1% *L. plantarum* 1A-2 and 0.9% cellulase; E2: 2% cellulase; LAB2E2: of 0.2% *L. plantarum* 1A-2 and 1.8% cellulase.

The acetic acid content of silage significantly reduced in all treatments ($p < 0.01$) except for E1. The highest reduction was found in LAB2E2 with a 60% reduction followed by E2 with a 59% reduction than the control. There was also a significant reduction in the propionic acid content of silage ($p < 0.01$). Propionic acid in LAB1E1 and LAB2E2 was not detected meanwhile there was a 26% and 41% reduction than the control on LAB1 and E2, respectively. There was a highly significant effect of treatment on butanoic acid content of silage. The highest reduction was observed in LAB2E2 with a 75% reduction followed by E2 with a 70% reduction.

The results showed that there is a change in crystallinity of samples (Figure 1). According to the XRD result (Table 2), the crystalline decreased in LAB1, E1, LAB1E1, and E2 but increased in LAB2E2 compared to the control. The lowest crystallite size was observed in LAB2E2 by 10.00 nm. The absorbance ratio A_{3333}/A_{1320} which is considered as the hydrogen bond Index (HBI), the ratio of A_{1371}/A_{2918} which is the indication of total crystallinity index (TCI), and the ratio of A_{1516}/A_{899} which is the indication of lignin per cellulose (L/C) for rice straw silage were calculated and reported in Table 2. Compared to the control, the TCI value decreased in LAB1 and increased in the other treatments with the highest TCI was observed in LAB2E2. The result of this study showed that L/C increased in LAB1 (0.77) but decreased in E1 (0.70), LAB1E1 (0.69), E2 (0.68) and LAB2E2 (0.71) compared to the control (0.74).

Table 2. Crystallinity properties of rice straw silage.

Variables	Treatments					
	Control	LAB1	E1	LAB1E1	E2	LAB2E2
TCI (A1371/A2918)	0.74	0.73	0.92	0.78	0.85	0.98
HBI (A3333/A1320)	0.81	0.81	0.86	0.87	0.79	0.91
L/C (A1516/A899)	0.74	0.77	0.70	0.69	0.68	0.71
Crystallinity (%)	21.84	20.7	20.91	21.22	21.11	23.89
Amorphous (%)	78.16	79.3	79.09	78.78	78.89	76.11
Crystallite Size (nm)	10.16	10.75	10.80	11.45	11.38	10.00

Note: TCI: Total crystallinity index; HBI: Hydrogen bond index; L/C: lignin per cellulose. Control: without additive; LAB1: 0.1% *L. plantarum* 1A-2; E1: 1% cellulase; LAB1E1: 0.1% *L. plantarum* 1A-2 and 0.9% cellulase; E2: 2% cellulase; LAB2E2: of 0.2% *L. plantarum* 1A-2 and 1.8% cellulase.

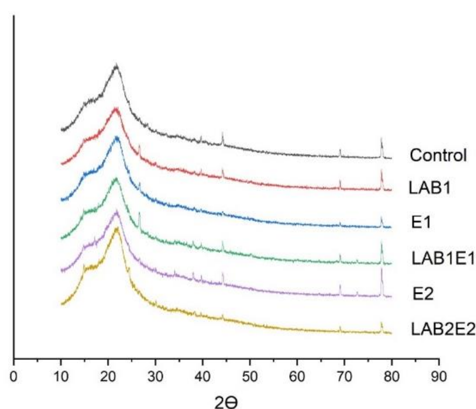

Figure 1. XRD Spectra of rice straw silage.

Note: Control: without additive; LAB1: 0.1% *L. plantarum* 1A-2; E1: 1% cellulase; LAB1E1: 0.1% *L. plantarum* 1A-2 and 0.9% cellulase; E2: 2% cellulase; LAB2E2: of 0.2% *L. plantarum* 1A-2 and 1.8% cellulase.

From the observation of chemical structure of rice straw silage by FTIR, (Figure 2) it can be seen that a strong band in 3700–3000 cm^{-1} corresponds to the O–H stretching band and the peak in the region of 3000–2800 cm^{-1} corresponds to the C–H stretching [29]. The peak of the band at around 1731 cm^{-1} was related to the C = O stretching of hemicelluloses [30]. The increase in the intensity of these peaks in all treatments compared to control showed the increasing trend of hemicellulose. The bands at around 1640 cm^{-1} were present in all samples that corresponds to the conjugated C = O group in the alkyl groups of lignin [31].

Table 3. Gas production, digestibility and rumen fermentation profiles.

Parameters	Treatments						SEM	p-value
	Control	LAB1	E1	LAB1E1	E2	LAB2E2		
Gas Production 4 h (mL)	0.000^{a}	0.000^{a}	4.000^{b}	3.625^{b}	4.250^{b}	2.500^{ab}	0.480	0.004
Gas Production 6 h (mL)	0.500^{a}	0.750^{a}	6.000^{b}	4.375^{b}	5.000^{b}	4.250^{b}	0.556	0.002
Gas Production 8 h (mL)	0.500^{a}	0.750^{a}	7.750^{b}	5.875^{b}	6.875^{b}	5.000^{b}	0.706	< 0.001
Gas Production 10 h (mL)	2.375^{a}	3.375^{a}	18.500^{b}	8.500^{ab}	9.750^{ab}	8.875^{ab}	1.644	< 0.011
Gas Production 12 h (mL)	4.500^{a}	5.625^{a}	22.250^{b}	11.875^{ab}	12.000^{ab}	11.375^{ab}	1.735	0.027
Gas Production 24 h (mL)	14.000^{a}	15.500^{a}	32.125^{b}	22.625^{ab}	23.125^{ab}	22.375^{ab}	1.807	0.033
Gas Production 48 h (mL)	25.875^{a}	28.375^{a}	45.750^{b}	35.125^{ab}	35.750^{ab}	35.000^{ab}	1.879	0.022
L (h)	6.695^{b}	4.000^{a}	5.963^{b}	3.158^{a}	3.618^{a}	3.420^{a}	1.59	< 0.001
B (mL)	27.640^{a}	34.510^{b}	30.375^{a}	38.960^{c}	36.933^{bc}	37.568^{bc}	0.94	< 0.001
C ($mL \cdot h^{-1}$)	0.095	0.091	0.092	0.083	0.087	0.085	0.008	0.243
DMD (%)	47.295	49.673	47.020	48.773	51.600	50.748	0.725	0.397
OMD (%)	38.055	40.918	38.465	40.380	44.978	41.843	1.107	0.545
NH_3-N (%)	2.410^{a}	3.008^{ab}	2.480^{a}	3.325^{b}	3.103^{ab}	2.603^{ab}	0.113	0.078
pH	6.943	6.930	6.950	6.885	6.898	6.918	0.010	0.422
Acetic acid ($mmol \cdot dL^{-1}$)	9.633^{a}	20.183^{c}	15.758^{b}	22.583^{d}	25.288^{e}	32.735^{f}	1.530	< 0.001
Propionic acid ($mmol \cdot dL^{-1}$)	4.315^{a}	6.953^{b}	6.268^{b}	9.725^{c}	9.110^{c}	12.500^{d}	0.588	< 0.001
Iso butyric acid ($mmol \cdot dL^{-1}$)	0.383^{a}	0.548^{abc}	0.478^{ab}	0.668^{bcd}	0.740^{cd}	0.808^{d}	0.041	0.006
Butyric acid ($mmol \cdot dL^{-1}$)	1.288^{a}	2.075^{bc}	1.853^{ab}	2.970^{d}	2.690^{cd}	3.708^{e}	0.182	< 0.001
Iso-valeric acid ($mmol \cdot dL^{-1}$)	0.420^{a}	0.665^{bc}	0.560^{ab}	0.853^{cd}	0.918^{d}	1.230^{e}	0.061	< 0.001
Valeric acid ($mmol \cdot dL^{-1}$)	0.425^{a}	0.503^{ab}	0.518^{ab}	0.590^{bc}	0.570^{bc}	0.675^{c}	0.020	0.001
Total SCFA ($mmol \cdot dL^{-1}$)	16.472^{a}	30.928^{c}	25.432^{b}	37.386^{d}	39.311^{d}	51.657^{e}	2.353	< 0.001
A/P ratio	2.258	2.910	2.512	2.398	2.774	2.665	0.061	0.117

Note: SEM: standard error of mean. $^{a–d}$ Means with different superscripts within a row significantly differed ($p < 0.05$). Control: without additive; LAB1: 0.1% *L. plantarum* 1A-2; E1: 1% cellulase; LAB1E1: 0.1% *L. plantarum* 1A-2 and 0.9% cellulase; E2: 2% cellulase; LAB2E2: of 0.2% *L. plantarum* 1A-2 and 1.8% cellulase.

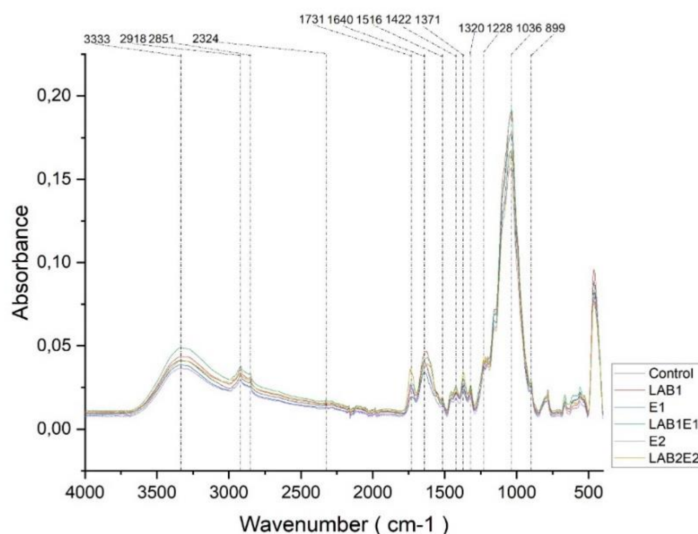

Figure 2. FT-IR absorbance for IR wavenumber from 400 to 4000 cm^{-1} of rice straw silage.

Note: Control: without additive; LAB1: 0.1% *L. plantarum* 1A-2; E1: 1% cellulase; LAB1E1: 0.1% *L. plantarum* 1A-2 and 0.9% cellulase; E2: 2% cellulase; LAB2E2: of 0.2% *L. plantarum* 1A-2 and 1.8% cellulase.

Guaiacyl ring-related IR spectra (Aromatic C–O stretching mode for lignin: guaiacyl ring of lignin) were present in all samples at around 1,516 cm^{-1} [32] and had a lower peak in E1 and E2. Peak around 1422 cm^{-1} and 1320 cm^{-1} in all samples were related to the symmetric CH_2 bending and wagging [33]. A comparison to the control sample showed that LAB2E2 increased the intensities of the bands at 1371 cm^{-1} that related to the C-H bending in cellulose [34]. Peak at 1228 cm^{-1} related to C-O stretching band of ether linkage [35]. The bands at around 1036 cm^{-1} corresponds to the stretching C-O in cellulose [36,37]. The peak observed in 899 cm^{-1} corresponds to the β-1,4 glycosidic bond linkages [38].

3.2. In vitro digestibility of rice straw silage

The effect of silage additives on *in vitro* rumen fermentation is shown in Table 3. Gas production in the first 4 to 8 h showed a significant difference ($p < 0.05$) higher than the control and LAB1, except for LAB2E2, which was not significantly different in the first 4 h. E1 showed significantly different results ($p < 0.05$) with higher gas production from 10 h until the end of fermentation (48 h) compared to the control and LAB1, but not significantly different from other treatments. Kinetics of gas production was obtained from gas production data and fitted using Gompertz equation [28,39]. Lag time (L) of gas production in the control was significantly higher ($p < 0.05$) than other treatments. Treatment LAB1E1 showed significantly ($p < 0.05$) the highest total potential gas production (B). Data showed that all treatments increased total potential gas production (B) from the lowest (27.640 mL) in control, to the highest (38.960 mL) resulted from treatment LAB1E1. Different result showed from gas production rate (C) which is not significant among all treatments.

DMD and OMD did not show a significant difference ($p > 0.05$) from all treatments, but there was a tendency to increase digestibility compared to control. LAB1E1 showed a significantly higher difference ($p < 0.05$) in the NH_3-N content compared to the control and E1, but not significantly

different between the other treatments. The pH value of the rumen fermentation was in the normal range of 6.89–6.95 and was not significantly different in all treatments. *In vitro* rumen fermentation profiles after 24 h incubation is presented in Table 3. Treatments were significant on each SCFA production, in which control produced significantly the lowest ($p < 0.05$) compared with other treatments. But the rumen A/P ratio was not significantly affected by treatments.

4. Discussion

4.1. Characteristics of rice straw silage

Ensiling of fresh rice straw with additives is an effective way to preserve its nutrients as well as to improve its digestibility. In this present study, *L. plantarum* 1-A2, a heterofermentative LAB, and crude cellulase were used as additives to improve the fermentation process of rice straw. Work on addition of LAB inoculant and enzyme have been conducted to improve the digestibility of rice straw. Combination of *L. plantarum* and cellulase showed the highest lactic acid concentration among all silages over the 7 d of ensiling and also the lowest abundance of *Enterobacteriaceae* over 30 d of ensiling. The combination of *L. plantarum* and cellulase further improved fermentation quality compared to silage treated with *L. plantarum* or cellulase alone, since the combination had a synergistic effect on mixed silage fermentation [40]. Changes on quality, gas kinetics and *in vitro* digestibility of rice straw silage with *Lactobacillus casei* TH14, cellulase and molasses additives were also reported [41].

The addition of LAB inoculant and crude cellulase alone or in combination did not have any effect on the mold content of the silage. Addition of heterofermentative LAB inoculant could lead to a significant reduction of mold content in the silage [12]. Ensiling process in tropical region with high humidity, high temperature, and high oxygen availability is vulnerable to fungal contamination [42]. Heterofermentative LAB such as *L. plantarum* is known to produce antifungal substances such as acetic acid and propionic acid [43].

An increment of DM content by the addition of combination of LAB inoculant and crude cellulase at 1% showed that it can increase preservation ability from DM loss of silage. DM of silage should have a minimal loss which indicates the efficiency of the ensiling process [44]. CF significantly reduced by LAB inoculant with or without crude cellulase relative to control. The addition of LAB inoculant and crude cellulase alone or in combination might change the composition of fiber of rice straw silage. This is in agreement with previous studies that LAB inoculant and enzyme addition might decrease the fiber content of silage [45–47]. Dewar et al. [48] suggested that ensiling process could change plant structural carbohydrate content by three mechanisms such as enzymes, acidolysis, and microbial degradation. Enzymes hydrolyze structural carbohydrates at the initial stage of ensiling, which then allows microorganisms, especially LAB, to use the carbohydrate and converted to lactic acid. Thus, this process would reduce the pH of silage. In the present study, CP content significantly increased by the addition of LAB inoculant alone at the rate of 0.1%. The addition of LAB inoculant inhibits CP loss from the ensiling process. An increase in CP content with the addition of LAB inoculant to silage was reported by Li et al. and Marbun et al. [10,49]. NH_3-N concentration was suppressed by the addition of crude cellulase at a level of 2% alone or in combination with LAB inoculant. An increase in NH_3-N concentration by the addition of heterofermentative LAB inoculant was reported by Muck et al. [50]. A decrease in pH was observed by the addition of LAB inoculant

alone or in combination with crude cellulase, which is related to an increase in lactic acid production. Addition of crude cellulase alone at a level of 1% (E1) might not sufficiently to provide fermentable substrate for LAB to produce lactic acid thus did not have a significant effect on the pH of silage. However, crude cellulase at 2% (E2) support the growth of LAB inoculant to produce lactic acid by 300% and effectively reduced the pH of silage. Crude cellulase used here is low in its activity because it is produced from a wild culture of actinobacteria, however this *Streptomyces* sp. 292 produced cellulase activity only with very small activity of hemicellulose and mannanase (unpublished results). Heterofermentative LAB is known for its capability to produce lactic acid and acetic acid from the fermentation of pentoses [50]. Lactic acid has the lowest degree of acidity in comparison with other organic acids, such as acetic acid, propionic acid and butyric acid [51]. Thus, higher production of lactic acid might have a greater decrease in the pH of silage [12,52,53]. The addition of LAB inoculant and or without enzyme suppressed acetic, propionic, and butyric acids. E1 and E2 showed a different way in reducing organic acids concentration. Propionic acid was not detected when a combination of LAB inoculant and crude cellulase was added to the silage. This finding confirms that the addition of heterofermentative LAB inoculant reduced pH, acetic acid, butyric acid, and NH_3-N [50].

XRD, FTIR, and NMR are the most widely used methods to study the crystalline structure of lignocellulose material [54]. XRD and FTIR were used here to determine the crystalline structure of rice straw. The crystallinity of rice straw silage was studied using XRD. The result of this study showed that the addition of LAB inoculant and crude cellulase alone or in combination decreased the crystallinity of rice straw silage except in LAB2E2 treatment. During the ensiling process, the amorphous cellulose and hemicellulose were hydrolyzed by water and fermentation microorganisms, resulting in little damage to the crystal region of the fibers. This result was also supported by the crystallinity index that was estimated based on FTIR spectra. Total crystalline index (TCI), Hydrogen bond index (HBI), and lignin per cellulose (L/C) were used to study the crystalline of cellulose. HBI is closely related to the crystal system and the degree of intermolecular regularity [22]. HBI values were determined as the ratio between peak at 3333 (H-bonded absorption) and 1320 (CH2 rocking vibration). LAB1 did not change the HBI value in rice straw silage but increased when the LAB was combined with crude cellulase. The transformation of rice straw from high crystallinity to low crystallinity was supported by the HBI data. The HBI value increased compared to control, which means that fewer available hydroxyl groups in the cellulose chain can interact by inter- and/or intramolecular hydrogen bonding. Lignin to cellulose (L/C) ratio is a parameter obtained by calculating the ratio of absorption intensities at 1516 and 899 cm^{-1}. The decreasing of L/C in E1 and E2 (crude cellulase alone), LAB1E1, and LAB2E2 (combination with LAB) showed the conversion of crystalline to amorphous cellulose (increase in absorption at 899 cm^{-1}). In this study, the decrease in crystallinity was followed by an increase in hemicellulose. Hemicelluloses are generally easier to degrade enzymatically than cellulose [55]. The total crystalline index (TCI) in all treatments was higher than control with the highest TCI was observed in LAB2E2 treatment. TCI corresponds to the degree of crystallinity of cellulose [56]. The results of XRD and TCI calculations from FTIR spectra showed that the crystallinity of the LAB2E2 treatment increased compared to control. The increase of the crystalline of rice straw in LAB2E2 might be due to the changes in structural properties of cellulose and the release of fermentable sugar from biomass. The ensiled treatment had impacts on increasing the mechanical properties such as internal bond strength and modulus of elasticity [57]. Moreover, the addition of crude cellulase in lignocellulosic biomasses could release the fermentable sugars by altering the cell wall structure.

The released fermentable sugars could be used by LAB as indicated by the lowest pH of this treatment among the other treatments.

4.2. In vitro digestibility of rice straw silage

Total gas production is one indicator of the degradation of easily fermentable materials. The addition of additives to rice straw silage resulted in a decrease in total fiber, which is supported by the hemicellulose fiber fraction value, higher amorphous percentage than control, and lower L/C index value. The first 4–8 h of rumen fermentation is the initial process of utilizing the easily digestible parts of feed materials by rumen microbes. Gas production correlated with fiber degradation where LAB and enzyme treatments release simple and more fermentable carbohydrates, available for rumen microorganisms during *in vitro* incubation [58]. It helps rumen microorganisms to degrade feed substrate and produce gas. Complex carbohydrates in control treatment caused rumen microorganisms need more time to degrade feed substrate which resulted the highest lag time (L) and the lowest total potential gas production (B) compared to the other treatments. Increased potential gas production was reported by Zhao et al. [59] which resulted from enzyme treatments in rice straw silage.

The addition of crude cellulase to silage improves the accessibility of rice straw silage fibers by the rumen microbial community. The addition of LAB inoculant combined with crude cellulase provided an additional role in the utilization of WCS after the enzyme was active at the beginning of the silage process. This activity can be seen from the gas production, which is higher than the control and only in LAB without being given a combination with crude cellulase. The addition of silage inoculant, especially *L. plantarum* can affect the decrease in total gas production during *in vitro* fermentation [60]. The crude cellulase treatment affects changes in the composition of the cell wall of rice straw into organic matter that is easily utilized by rumen microbes. The aim of addition of fibrolytic enzymes was to break down the fiber and releasing soluble sugars, to provide a substrate that could be ready to use by microbes [59]. Rice straw silage is the main source of fiber in rumen fermentation, providing opportunities for the role of fibrolytic bacteria in their metabolism such as *Bacteroidetes* and *Fibrobacters* [61]. The main degradation products of fibrolytic bacteria are acetic acid, CO_2, and hydrogen sinks. The digestibility value *in vitro* has a positive relationship with VFAs production [62]. These metabolic products tend to increase total SCFA production, especially acetic acid. The combination of silage additives can potentially increase digestibility, gas production, and SCFA during rumen fermentation [59]. This is in line with the results of the study which showed a high trend of DMD and OMD giving results for total VFA and SCFA (Table 3). This phenomenon can be used as the basis for complete feed making with straw, silage as a source of fiber that has a fairly good digestibility value. The pH at the end of rumen fermentation (48 h) from all treatments was not affected by the additives used. These results are in line with the straw silage experiment with the addition of additives at the end of fermentation in the rumen which did not significantly affect the final pH [10].

The profile of products formed during rumen fermentation has implications for animal productivity and the environment. The most important products of the anaerobic microbial fermentation of carbohydrates in the alimentary tract of ruminants are SCFA, which cover 80% of the animals' demand for gross energy [63,64]. In this study, total SCFA significantly increased in all treatments. It is well known that total SCFA production is positively correlated with digestibility. Although DMD in this study was not significantly increased, higher gas production can be assumed as

higher digestibility. Interestingly, treatments of LAB inoculant and crude cellulase significantly increased acetic, propionic, iso-butyric, butyric, iso-valeric, and valeric acids from rumen fermentation. Guo et al. [65] reported only significantly higher acetic and propionic acids with the addition of *L. plantarum* as silage inoculum compared with non-inoculated silage. The addition of *L. plantarum* was also reported by Zhao et al. [59] to increased acetic acid, but in contrast, decreased propionic and butyric acids. The increase of fermentation products by the addition of LAB was caused by a decrease of structural carbohydrate contents, which provides more soluble components retained in additive-treated silages.

The addition of crude cellulase produced a lower fermentation product compared with LAB1, but still higher than control. However, LAB1E1 produced a higher fermentation product compared to control or LAB1. In contrast, Dönmez et al. [66] reported a significant decrease of total SCFA, acetic acid, propionic acid, and butyric acid by enzyme addition in silage. The effect of enzyme additives on the silage rumen fermentation profile has not been widely studied. Cell wall degrading enzymes, such as cellulases, has been used to increase WSC availability to LAB [67] presumably due to degradation of NDF. Colombatto et al. [68] found that additions of enzymes reduced pH, NDF, and ADF contents of maize silage. A different result was reported by Sun et al. [69] which showed that the addition of cellulase reduced losses of NDF, but increased gas production on maize stover silage. Khota et al. [70] used LAB and cellulase as additives on sorghum silage. The addition of LAB, enzyme and both LAB and enzyme did not show significant changed in *in vitro* rumen digestibility. The effect of fibrolytic enzyme activity on silage fermentation depending on both the temperature and pH conditions [71]. The highest fermentation product from all treatments was found from LAB2E2 which applied double dosage for LAB and enzyme addition. It seems that there is an interaction of LAB and enzyme, to provide higher WSC due to mechanisms of cellulase treatment that can be used by LAB during ensiling. More compounds are available for rumen bacteria to ferment in the rumen. Although LAB and enzyme addition increased SCFA production, in this study A/P ratio was not significantly affected by treatments. It means that the rumen fermentation pattern was not shifted from acetic acid production to propionic acid production.

5. Conclusions

Addition of *L. plantarum* 1A-2 inoculant alone or in combination with crude cellulase improved silage quality. Individual crude enzyme addition was effective to improve silage quality at a level of 2%. Combination of LAB2E2 gave the highest effect on silage quality and produced the highest fermentation products. The increase of the crystalline of LAB2E2 might be due to the use of fermentable sugars released from rice straw biomass by the additives used. There is significant effect on increasing the main SCFA products from *in vitro* rumen fermentation, which provide potential energy source for the animal. This report described new information on the use of rice straw of new high yielding rice variety for silage. Further study on the population of microorganisms involved during ensilage is needed.

Acknowledgements

This study was supported by National Innovation System Research Incentive (INSINAS) program (Contract No. 9/INS/PPK/E4/2021), Ministry of Research and Technology, Republic

Indonesia. The authors express gratitude to the Indonesian Research Center for Rice for providing the rice straw materials, and to the Advanced Characterization Laboratory Cibinong–Integrated Laboratory of Bioproduct, and Characterization Biotechnology Laboratory, through E-Layanan Sains, National Research and Innovation Agency, for facilities, scientific and technical support.

Conflict of interest

All authors declare there is no conflict of interest.

References

1. Domínguez-Escribá L, Porcar M (2010) Rice straw management: The big waste. *Biofuels Bioprod Bioref* 4: 154–159. https://doi.org/https://doi.org/10.1002/bbb.196
2. Gummert M, Hung NV, Chivenge P, et al. (2020) *Sustainable rice straw management*, Cham: Springer Nature.
3. Goodman BA (2020) Utilization of waste straw and husks from rice production: A review. *J Bioresour Bioprod* 5: 143–162. https://doi.org/10.1016/j.jobab.2020.07.001
4. Nguyen DV, Dang LH (2020) Fresh rice straw silage affected by ensiling additives and durations and its utilisation in beef cattle diets. *Asian J Anim Sci* 14: 16–24. https://doi.org/10.3923/ajas.2020.16.24
5. Cheng YF, Wang Y, Li YF, et al. (2017) Progressive colonization of bacteria and degradation of rice straw in the rumen by Illumina sequencing. *Front Microbiol* 8: 1–10. https://doi.org/10.3389/fmicb.2017.02165
6. Kingston-Smith AH, Davies TE, Rees Stevens P, et al. (2013) Comparative metabolite fingerprinting of the rumen system during colonisation of three forage grass (*Lolium perenne* L.) varieties. *PLoS One* 8: e82801. https://doi.org/10.1371/journal.pone.0082801
7. Belanche A, Weisbjerg MR, Allison GG, et al. (2014) Measurement of rumen dry matter and neutral detergent fiber degradability of feeds by Fourier-transform infrared spectroscopy. *J Dairy Sci* 97: 2361–2375. https://doi.org/10.3168/jds.2013-7491
8. Kim JG, Ham JS, Li YW, et al. (2017) Development of a new lactic acid bacterial inoculant for fresh rice straw silage. *AJAS* 30: 950–956. https://doi.org/10.5713/ajas.17.0287
9. Oladosu Y, Rafii MY, Abdullah N, et al. (2016) Fermentation quality and additives: A case of rice straw silage. *Biomed Res Int* 2016: 7985167. https://doi.org/10.1155/2016/7985167
10. Marbun TD, Lee K, Song J, et al. (2020) Effect of lactic acid bacteria on the nutritive value and *in vitro* ruminal digestibility of maize and rice straw silage. *Appl Sci* 10: 7801. https://doi.org/10.3390/app10217801
11. Zhang YG, Xin HS, Hua JL (2010) Effects of treating whole-plant or chopped rice straw silage with different levels of lactic acid bacteria on silage fermentation and nutritive value for lactating holsteins. *Asian-Australas J Anim Sci* 23: 1601–1607. https://doi.org/10.5713/ajas.2010.10082
12. Irawan A, Sofyan A, Ridwan R, et al. (2021) Effects of different lactic acid bacteria groups and fibrolytic enzymes as additives on silage quality: A meta-analysis. *Bioresour Technol Rep* 14: 100654. https://doi.org/10.1016/j.biteb.2021.100654
13. Kung L, Shaver R (2001) Interpretation and use of silage fermentation analysis reports. *Focus Forage* 3: 1–5.

14. Selim MSM, Abdelhamid SA, Mohamed SS (2021) Secondary metabolites and biodiversity of actinomycetes. *J Genet Eng Biotechnol* 19: 72. https://doi.org/10.1186/s43141-021-00156-9
15. Eun JS, Beauchemin KA, Schulze H (2007) Use of exogenous fibrolytic enzymes to enhance *in vitro* fermentation of alfalfa hay and corn silage. *J Dairy Sci* 90: 1440–1451. https://doi.org/10.3168/jds.S0022-0302(07)71629-6
16. Ridwan R, Rusmana I, Widyastuti Y, et al. (2015) Fermentation characteristics and microbial diversity of tropical grass-legumes silages. *Asian-Australas J Anim Sci* 28: 511–518. https://doi.org/10.5713/ajas.14.0622
17. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31: 426–428. https://doi.org/10.1021/ac60147a030
18. Daquioag JEL, Penuliar GM (2021) Isolation of actinomycetes with cellulolytic and antimicrobial activities from aoils collected from an urban green space in the Philippines. *Int J Microbiol* 2021: 6699430. https://doi.org/10.1155/2021/6699430
19. Helrich K, (1990) *Official methods of analysis of the Association of Official Analytical Chemists*. Arlington: The Association.
20. Garvey CJ, Parker IH, Simon GP (2005) On the interpretation of X-ray diffraction powder patterns in terms of the nanostructure of cellulose I fibres. *Macromol Chem Phys* 206: 1568–1575. https://doi.org/10.1002/macp.200500008
21. Cao Y, Tan HM (2005) Study on crystal structures of enzyme-hydrolyzed cellulosic materials by X-ray diffraction. *Enzyme Microb Technol* 36: 314–317. https://doi.org/https://doi.org/10.1016/j.enzmictec.2004.09.002
22. Gaur R, Agrawal R, Kumar R, et al. (2015) Evaluation of recalcitrant features impacting enzymatic saccharification of diverse agricultural residues treated by steam explosion and dilute acid. *RSC Adv* 5: 60754–60762. https://doi.org/10.1039/c5ra12475a
23. Souza NKP, Detmann E, Valadares Filho SC, et al. (2013) Accuracy of the estimates of ammonia concentration in rumen fluid using different analytical methods. *Arq Bras Med Vet Zootec* 65: 1752–1758. https://doi.org/10.1590/S0102-09352013000600024
24. Borshchevskaya LN, Gordeeva TL, Kalinina AN, et al. (2016) Spectrophotometric determination of lactic acid. *J Anal Chem* 71: 755–758. https://doi.org/10.1134/S1061934816080037
25. Theodorou MK, Williams BA, Dhanoa MS, et al. (1994) A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim Feed Sci Technol* 48: 185–197. https://doi.org/https://doi.org/10.1016/0377-8401(94)90171-6
26. McDougall EI (1948) Studies on ruminant saliva. 1. The composition and output of sheep's saliva. *Biochem J* 43: 99–109. https://doi.org/10.1042/bj0430099
27. Tilley JMA, Terry RA (1963) A two-stage technique for the *in vitro* digestion of forage crops. *Grass Forage Sci* 18: 104–111. https://doi.org/https://doi.org/10.1111/j.1365-2494.1963.tb00335.x
28. Bidlack JE, Buxton DR (1992) Content and deposition rates of cellulose, hemicellulose, and lignin during regrowth of forage grasses and legumes. *Can J Plant Sci* 72: 809–818. https://doi.org/10.4141/cjps92-097
29. Adel AM, Abd El-Wahab ZH, Ibrahim AA, et al. (2011) Characterization of microcrystalline cellulose prepared from lignocellulosic materials. Part II: Physicochemical properties. *Carbohydr Polym* 83: 676–687. https://doi.org/10.1016/j.carbpol.2010.08.039

30. Senthamaraikannan P, Kathiresan M (2018) Characterization of raw and alkali treated new natural cellulosic fiber from *Coccinia grandis. L. Carbohyd Polym* 186: 332–343. https://doi.org/10.1016/j.carbpol.2018.01.072
31. Hsu TC, Guo GL, Chen WH, et al. (2010) Effect of dilute acid pretreatment of rice straw on structural properties and enzymatic hydrolysis. *Bioresour Technol* 101: 4907–4913. https://doi.org/10.1016/j.biortech.2009.10.009
32. Liu RG, Yu H, Huang Y (2005) Structure and morphology of cellulose in wheat straw. *Cellulose* 12: 25–34. https://doi.org/10.1007/s10570-004-0955-8
33. Kshirsagar SD, Waghmare PR, Chandrakant Loni P, et al. (2015) Dilute acid pretreatment of rice straw, structural characterization and optimization of enzymatic hydrolysis conditions by response surface methodology. *RSC Adv* 5: 46525–46533. https://doi.org/10.1039/C5RA04430H
34. Binod P, Satyanagalakshmi K, Sindhu R, et al. (2012) Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. *Renew Energy* 37: 109–116. https://doi.org/10.1016/j.renene.2011.06.007
35. Remli NAM, Md Shah UK, Mohamad R, et al. (2014) Effects of chemical and thermal pretreatments on the enzymatic saccharification of rice straw for sugars production. *BioResources* 9: 510–522. https://doi.org/10.15376/biores.9.1.510-522
36. Jangong OS, Heryanto H, Rahmat R, et al. (2021) Effect of sugar palm fiber (SPF) to the structural and optical properties of bioplastics (SPF/starch/chitosan/polypropylene) in supporting mechanical properties and degradation performance. *J Polym Environ* 29: 1694–1705. https://doi.org/10.1007/s10924-020-02019-9
37. Zhuang JS, Li M, Pu YQ, et al. (2020) Observation of potential contaminants in processed biomass using fourier transform infrared spectroscopy. *Appl Sci* 10: 4345. https://doi.org/10.3390/app10124345
38. Liang YG, Cheng BJ, Si YB, et al. (2014) Physicochemical changes of rice straw after lime pretreatment and mesophilic dry digestion. *Biomass Bioenerg* 71: 106–112. https://doi.org/10.1016/j.biombioe.2014.10.020
39. Lavrenčič A, Stefanon B, Susmel P (1997) An evaluation of the Gompertz model in degradability studies of forage chemical components. *Anim Sci* 64: 423–431. https://doi.org/10.1017/S1357729800016027
40. Mu L, Xie Z, Hu LX, et al. (2020) Cellulase interacts with *Lactobacillus plantarum* to affect chemical composition, bacterial communities, and aerobic stability in mixed silage of high-moisture amaranth and rice straw. *Bioresour Technol* 315: 123772. https://doi.org/10.1016/j.biortech.2020.123772
41. Cherdthong A, Suntara C, Khota W (2020) *Lactobacillus casei* TH14 and additives could modulate the quality, gas kinetics and the *in vitro* digestibility of ensilaged rice straw. *J Anim Physiol Anim Nutr* 104: 1690–1703. https://doi.org/10.1111/jpn.13426
42. Ogunade IM, Martinez-Tuppia C, Queiroz OCM, et al. (2018) Silage review: Mycotoxins in silage: Occurrence, effects, prevention, and mitigation. *J Dairy Sci* 101: 4034–4059. https://doi.org/10.3168/jds.2017-13788
43. Wang TW, Teng KL, Cao YH, et al. (2020) Effects of *Lactobacillus hilgardii* 60TS-2, with or without homofermentative *Lactobacillus plantarum* B90, on the aerobic stability, fermentation quality and microbial community dynamics in sugarcane top silage. *Bioresour Technol* 312: 123600. https://doi.org/10.1016/j.biortech.2020.123600

44. Borreani G, Tabacco E, Schmidt RJ, et al. (2018) Silage review : Factors affecting dry matter and quality losses in silages . *J Dairy Sci* 101: 3952–3979. https://doi.org/10.3168/jds.2017-13837
45. Dehghani MR, Weisbjerg MR, Hvelplund T, et al. (2012) Effect of enzyme addition to forage at ensiling on silage chemical composition and NDF degradation characteristics. *Livest Sci* 150: 51–58. https://doi.org/https://doi.org/10.1016/j.livsci.2012.07.031
46. Ebrahimi M, Rajion MA, Goh YM, et al. (2014) The effects of adding lactic acid bacteria and cellulase in oil palm (*Elais guineensis* Jacq.) frond silages on fermentation quality, chemical composition and *in vitro* digestibility. *Ital J Anim Sci* 13: 557–562. https://doi.org/10.4081/ijas.2014.3358
47. Zhao J, Dong ZH, Li JF, et al. (2018) Ensiling as pretreatment of rice straw: The effect of hemicellulase and *Lactobacillus plantarum* on hemicellulose degradation and cellulose conversion. *Bioresour Technol* 266: 158–165. https://doi.org/10.1016/j.biortech.2018.06.058
48. Dewar WA, McDonald P, Whittenbury R (1963) The hydrolysis of grass hemicelluloses during ensilage. *J Sci Food Agric* 14: 411–417. https://doi.org/https://doi.org/10.1002/jsfa.2740140610
49. Li J, Shen YX, Cai YM (2010) Improvement of fermentation quality of rice straw silage by application of a bacterial inoculant and glucose. *Asian-Australas J Anim Sci* 23: 901–906. https://doi.org/10.5713/ajas.2010.90403
50. Muck RE, Nadeau EMG, McAllister TA, et al. (2018) Silage review: Recent advances and future uses of silage additives. *J Dairy Sci* 101: 3980–4000. https://doi.org/10.3168/jds.2017-13839
51. Moon NJ (1983) Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. *J Appl Bacteriol* 55: 453–460. https://doi.org/10.1111/j.1365-2672.1983.tb01685.x
52. Oliveira AS, Weinberg ZG, Ogunade IM, et al. (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. https://doi.org/10.3168/jds.2016-11815
53. Ratnakomala S, Ridwan R, Kartina G, et al. (2006) The effect of *Lactobacillus plantarum* 1A-2 and 1BL-2 inoculant on the quality of napier grass silage. *Biodiversitas J Biol Divers* 7: 131–134. https://doi.org/10.13057/biodiv/d070208
54. Ju XH, Bowden M, Brown EE, et al. (2015) An improved X-ray diffraction method for cellulose crystallinity measurement. *Carbohydr Polym* 123: 476–481. https://doi.org/10.1016/j.carbpol.2014.12.071
55. Agger J, Viksø-Nielsen A, Meyer AS (2010) Enzymatic xylose release from pretreated corn bran arabinoxylan: Differential effects of deacetylation and deferuloylation on insoluble and soluble substrate fractions. *J Agric Food Chem* 58: 6141–6148. https://doi.org/10.1021/jf100633f
56. Spiridon I, Anghel N, Dinu MV, et al. (2020) Development and performance of bioactive compounds-loaded cellulose/collagen/polyurethane materials. *Polymers* 12: 1191. https://doi.org/10.3390/POLYM12051191
57. Ren H, Richard TL, Chen ZL, et al. (2006) Ensiling corn stover: Effect of feedstock preservation on particleboard performance. *Biotechnol Prog* 22: 78–85. https://doi.org/10.1021/bp050174q
58. Bayatkouhsar J, Tahmasbi AM, Naserian AA (2012) Effects of microbial inoculant on composition, aerobic stability, in situ ruminal degradability and *in vitro* gas production of corn silage. *Int J AgriSci* 2: 774–786.

59. Zhao J, Dong ZH, Li JF, et al. (2019) Effects of sugar sources and doses on fermentation dynamics, carbohydrates changes, *in vitro* digestibility and gas production of rice straw silage. *Ital J Anim Sci* 18: 1345–1355. https://doi.org/10.1080/1828051X.2019.1659106
60. Oskoueian E, Jahromi MF, Jafari S, et al. (2021) Manipulation of rice straw silage fermentation with different types of lactic acid bacteria inoculant affects rumen microbial fermentation characteristics and methane production. *Vet Sci* 8: 100. https://doi.org/10.3390/vetsci8060100
61. Hu YQ, He YY, Gao S, et al. (2020) The effect of a diet based on rice straw co-fermented with probiotics and enzymes versus a fresh corn Stover-based diet on the rumen bacterial community and metabolites of beef cattle. *Sci Rep* 10: 10721. https://doi.org/10.1038/s41598-020-67716-w
62. Wang MS, Zhang FJ, Zhang XX, et al. (2021) Nutritional quality and *in vitro* rumen fermentation characteristics of silage prepared with lucerne, sweet maize stalk, and their mixtures. *Agric* 11: 1205. https://doi.org/10.3390/agriculture11121205
63. Ungerfeld EM (2020) Metabolic hydrogen flows in rumen fermentation: Principles and possibilities of interventions. *Front Microbiol* 11: 589. https://doi.org/10.3389/fmicb.2020.00589
64. Bergman EN (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev* 70: 567–590. https://doi.org/10.1152/physrev.1990.70.2.567
65. Guo G, Shen C, Liu Q, et al. (2020) The effect of lactic acid bacteria inoculums on *in vitro* rumen fermentation, methane production, ruminal cellulolytic bacteria populations and cellulase activities of corn stover silage. *J Integr Agric* 19: 838–847. https://doi.org/10.1016/S2095-3119(19)62707-3
66. Dönmez N, Karsli MA, Çinar A, et al. (2003) The effects of different silage additives on rumen protozoan number and volatile fatty acid concentration in sheep fed corn silage. *Small Rumin Res* 48: 227–231. https://doi.org/10.1016/S0921-4488(03)00017-8
67. Weinberg ZG, Ashbell G, Hen Y, et al. (1995) The effect of cellulase and hemicellulase plus pectinase on the aerobic stability and fibre analysis of peas and wheat silages. *Anim Feed Sci Technol* 55: 287–293. https://doi.org/https://doi.org/10.1016/0377-8401(95)00785-L
68. Colombatto D, Morgavi DP, Furtado AF, et al. (2003) Screening of exogenous enzymes for ruminant diets: Relationship between biochemical characteristics and *in vitro* ruminal degradation1. *J Anim Sci* 81: 2628–2638. https://doi.org/10.2527/2003.81102628x
69. Sun ZH, Liu SM, Tayo GO, et al. (2009) Effects of cellulase or lactic acid bacteria on silage fermentation and *in vitro* gas production of several morphological fractions of maize stover. *Anim Feed Sci Technol* 152: 219–231. https://doi.org/10.1016/j.anifeedsci.2009.04.013
70. Khota W, Pholsen S, Higgs D, et al. (2017) Fermentation quality and *in vitro* methane production of sorghum silage prepared with cellulase and lactic acid bacteria. *Asian-Australas J Anim Sci* 30: 1568–1574. https://doi.org/10.5713/ajas.16.0502
71. Colombatto D, Mould FL, Bhat MK, et al. (2004) *In vitro* evaluation of fibrolytic enzymes as additives for maize (*Zea mays* L.) silage: I. Effects of ensiling temperature, enzyme source and addition level. *Anim Feed Sci Technol* 111: 111–128. https://doi.org/10.1016/j.anifeedsci.2003.08.010

AIMS Press

© 2022 the Author(s), licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0)