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Effect of Fibrolytic Enzymes, Cellulolytic Fungi and Lactic Acid Bacteria on Fermentation Characteristics, Structural Carbohydrate Composition and In Vitro Digestibility of Rice Straw Silage

Hao Ding, Zhe Han, Junfeng Li, Xinbao Li, Zhihao Dong, Jie Zhao, Siran Wang and Tao Shao *

Institute of Ensiling and Processing of Grass, College of Agro-Grassland Science, Nanjing Agricultural University, Nanjing 210095, China

* Correspondence: shaotao@njau.edu.cn

Abstract: This study aimed to investigate the effect of fibrolytic enzymes, cellulolytic fungi and lactic acid bacteria on the fermentation quality, structural carbohydrate composition and in vitro digestibility of rice straw silage. This experiment followed a completely randomised block design; four treatments were designed: (1) distilled water (control, CON); (2) fibrolytic enzymes (2.0 g/kg fresh weight (FW), E); (3) *Trichoderma reesei* (4400 U/kg FW, F); (4) *Enterococcus faecium* Y83 (1×10^6 cfu/g FW, Y83). All additives were diluted with distilled water and sprayed onto the rice straw (20 mL/kg FW). The rice straw was placed into a laboratory silo (10 L) after uniformly mixing and stored at ambient temperature (17–22 °C) ensiling for 3, 7, 14, 30 and 60 days. The fermentation quality in treated silages was improved compared to the CON, as indicated by lower pH, propionic acid, acetic acid and ammonia nitrogen (NH_3-N) contents. Furthermore, Y83 had the lowest ($p < 0.05$) pH and highest ($p < 0.05$) lactic acid content after 60 days of ensiling. Y83 significantly ($p < 0.05$) decreased the neutral detergent fibre content compared with CON, E and F. In addition, E and Y83 had significantly ($p < 0.05$) higher in vitro dry matter digestibility and in vitro neutral detergent fibre digestibility than CON and F. Overall, Y83 can be used as a promising inoculant for improving the fermentation quality of rice straw silage.

Keywords: cellulolytic bacteria; fermentation quality; in vitro digestibility; rice straw

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1. Introduction

A large amount of rice straw is produced after rice harvest, which is one of the main crop residues in the world. Each ton of rice produces approximately 1.35 tons of rice straw; therefore, the rice straw yield of the world has been estimated to be approximately about 1140 million tons annually [1]. However, most rice straw is left unused, improperly disposed or burnt directly in fields, resulting in a waste of resources and environmental pollution. The forage utilization of rice straw might be a promising way forward, whereas rice straw has a low digestibility because it is mainly composed of cellulose, hemicellulose and lignin. Ensiling is an efficient method for improving the palatability of rice straw [2].

The low water soluble carbohydrate (WSC) and less epiphytic lactic acid bacteria (LAB) of rice straw make it hard to obtain quality silage [3]. Previous studies showed that fibrolytic enzymes and inoculants have been used to improve the fermentation quality of silage [4]. The application of fibrolytic enzymes in silage can partially solubilise neutral detergent fibre (NDF) and acid detergent fibre (ADF), release reducing sugars and promote the growth of LAB [5]. Furthermore, Gandra et al. [6] reported that the supplementation of fibrolytic enzymes in Jersey heifers' diets can improve NDF digestibility and nitrogen absorption. However, Vicini et al. [7] found that the application of fibrolytic enzymes in diets had no improvement in animal performance. These inconsistent results may be

related to enzyme types, concentration and activity, as well as differences in the substrate. Cellulolytic fungi have been widely used for commercial cellulase production because of their remarkable ability to secrete cellulase. Among cellulolytic fungi, *Trichoderma reesei* is an excellent cellulase producer, which can produce appreciable levels of endoglucanase and exoglucanase [8]. Ren et al. [9] found that *Trichoderma reesei* had the most active cellulose degradation and lower pH compared with *Aspergillus niger* when used as amendments for corn stover silage. However, β-glucosidase is deficient in the cellulolytic system of *Trichoderma reesei*, which is the key enzyme in the hydrolysis of cellobiose into glucose.

In order to obtain high-quality rice straw silage, a long-lasting and stable microbial additive is necessary for the degradation of lignocellulose. In a previous study, facultative anaerobic cellulolytic LAB (*Enterococcus faecium* Y83) were isolated from the rumens of the Tibetan yaks (*Bos grunniens*), which could enhance fermentation quality and enzymatic conversion efficiency [10]. However, limited information is reported on the effectiveness and application of cellulolytic LAB in rice straw silage. This work aimed to investigate the effect of fibrolytic enzymes, cellulolytic fungi and isolated cellulolytic LAB on the fermentation characteristics, structural carbohydrate composition and in vitro digestibility of rice straw silage.

2. Materials and Methods

2.1. Additives and Silage Preparation

Fibrolytic enzymes, cellulolytic fungi (*Trichoderma reesei*) and a facultative anaerobic cellulase-producing strain (*Enterococcus faecium* Y83) were used as silage additives in this experiment. The fibrolytic enzymes were a mixture of cellulase and hemicellulose (*w*/*w*, 1:1), while cellulase activities were 5600 U/g (Oddfoni Biological Technology Co., Ltd., Nanjing, China). *T. reesei* was obtained from China Centre for Industrial Culture Collection. The fungi were cultured anaerobically on potato dextrose agar at 30 °C for 7 days before inoculation, and the detected cellulase activity was 220 U/mL. The strain Y83 was isolated from yak rumen and identified as *E. faecium* by 16S rDNA sequence analysis (GeneBank Accession Number: MF 678854). According to the method of Zheng et al. [11], the strain Y83 was cultured in deMan, Rogosa and Sharpe (MRS) broth medium for subsequent inoculation.

Rice straw was obtained from Baima Teaching and Research Base of Nanjing Agricultural University (31.61° N, 119.18° E, altitude 25 m above sea level, Jiangsu, China) and chopped into lengths of about 2–3 cm with a forage cutter. All chopped rice straw was thoroughly mixed and divided into four equal piles. Treatments were as follows: controlled with distilled water (CON), fibrolytic enzymes applied at 2.0 g/kg fresh weight (FW, E), *T. reesei* applied at a dose equivalent to a cellulase activity of 4400 U/kg FW (F) and *E. faecium* Y83 inoculated at 1×10^6 cfu/g FW (Y83). All additives were diluted with distilled water and sprayed at an equivalent of 20 mL/kg FW into the corresponding pile of chopped rice straw. Approximately 5.30 kg of rice straw from each pile was packed into 10 L laboratory silos and stored at ambient temperature (17–22 °C) after being sealed with screw tops and plastic tape. Five silo replicates of each treatment were opened after 3, 7, 14, 30 and 60 days of ensiling for subsequent analyses.

2.2. Analysis of Extract and Solid Samples

The fresh rice straw or silage was placed into a clean plastic container and mixed uniformly. Sixty-gram sample was homogenised with 120 mL distilled water and stored in the refrigerator at 4 °C for 24 h. The sample extracts were then filtrated through two layers of cheesecloth and Whatman filter paper (11 μm pore size, Xinhua Co., Beijing, China). The pH of the filtrate was measured immediately with a pH meter (HANNA pH 211; Hanna Instruments Italia Srl, Villafranca Padovana, Italy). The filtrates were stored at −20 °C for subsequent determination of ammonia nitrogen (NH_3-N) and organic acids. The filtrates were centrifuged for 10 min at 4 °C, 10,000× *g* and filtrated through a microfilter (0.22 μm) for organic acids' determination, and then carried out using Agilent 1260 HPLC system (Agilent Technologies, Inc., Waldbronn, Germany) equipped with a

refractive index detector (column: Carbomix®H-NP5; Sepax Technologies, Inc., Newark, DE, USA; eluent: 2.5 mmol/L H_2SO_4, 0.5 mL/min; temperature: 55 °C). The NH_3-N was determined by the phenol–hypochlorite reaction [12].

The dry matter (DM) contents of fresh material and silage were determined by a freeze dryer (Freeze Dryer-1A-50, Boyikang, Beijing, China), and then ground through a 1 mm screen in a laboratory knife mill (FW100, Taisite Instrument Co., Ltd., Tianjin, China). The ground samples were analysed for total nitrogen (TN), WSC, NDF, ADF and ADL. The TN was determined by a Kjeldahl nitrogen analyser (Kjeltec 8200; FOSS, Sweden), and crude protein (CP) content was calculated as TN × 6.25. The WSC content was analysed by colorimetry after reaction with anthrone reagent [13]. The NDF, ADF and ADL contents were measured according to Van Soest et al. [14] using the ANKOM filter bag technique with an ANKOM 200i fibre analyser (ANKOM Technologies, Inc., Fairport, NY, USA). The hemicellulose content was calculated as NDF minus ADF, and the cellulose content was calculated as ADF minus ADL.

2.3. In Vitro Incubation of 60-Day Silage

Silage samples after 60 days of ensiling were conducted to determine in vitro gas production and digestibility following the method of Menke et al. [15]. Rumen fluid was collected before the morning feeding from three fistulated Jinnan beef steers (419 ± 1.9 kg of live weight). The steers were fed with a diet containing 50% corn silage, 47% concentrate and 3% soybean oil on a DM basis and had free access to water. Rumen fluid was filtered through four layers of cheesecloth and mixed with an anaerobic mineral buffer (1:2, *v*/*v*) into a 39 °C thermos bottle flushed with CO_2. Dry silage samples (500 mg) were placed into filter bags (F57; ANKOM Technology, Macedon, NY, USA) that were previously washed with acetone, dried at 55 °C for 24 h and weighed. Then, each bag was placed into each 100 mL serum bottle with 30 mL inoculum under CO_2 at 39 °C. Three serum bottles with only inoculum were as blank. The volume of gas production (GP) was measured at 4, 8, 12, 24, 48 and 72 h of incubation by a calibrated syringe and corrected with blank bottles. Data of GP were fitted to the non-linear equation:

$$y = b\left(1 - e^{-ct}\right) \quad (1)$$

where y is the cumulative volume of GP at incubation time t, b is the potential GP, and c is the rate constant of GP. After 72 h incubation, the filter bags were gently rinsed with distilled water and dried at 65 °C for 48 h to constant weight. In vitro DM digestibility (IVDMD), NDF digestibility (IVNDFD) and ADF digestibility (IVADFD) were determined based on their respective weight differences before and after incubation.

2.4. Statistical Analyses

All analyses were conducted using the general linear model (GLM) procedure of SPSS. 22. The data related to fermentation characteristics and carbohydrate components were subjected to two-way ANOVA. The in vitro parameters were subjected to one-way ANOVA. Different sample means were compared for significance by Tukey's multiple range method, and significance was declared at $p < 0.05$.

3. Results

3.1. Chemical Compositions of Raw Rice Straw

Table 1 shows the chemical composition of rice straw prior to ensiling. The rice straw had a high DM content (411 g/kg FW) and fibre fraction but relatively low WSC content (46.3 g/kg DM).

Table 1. Chemical composition of raw rice straw (g/kg DM or as stated).

Items	Rice Straw
Dry matter (g/kg FW)	411 ± 3.61
Crude protein	61.2 ± 2.74
Water soluble carbohydrate	46.3 ± 1.25
Neutral detergent fibre	715 ± 6.24
Acid detergent fibre	432 ± 5.29
Acid detergent lignin	65.8 ± 0.70
Cellulose	366 ± 5.29
Hemicellulose	283 ± 5.20

FW, fresh weight; DM, dry matter.

3.2. Fermentation Characteristics of Rice Straw Silage

Treatments and ensiling days had significant ($p < 0.05$) effects on the contents of lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA) and NH_3-N (Table 2). However, no difference was detected in DM content among treatments. All silages showed a gradual downward trend in pH during ensiling. The LA contents in all silages increased at the first 30 days of ensiling and then decreased. From day 7 to day 60 of ensiling, the pH of Y83 silages was lower ($p < 0.05$) than that of the control, while the LA content in Y83 silages showed an inverse result. The AA contents in all silages increased gradually during the whole ensiling period, and Y83 and F silages had lower ($p < 0.05$) AA contents than the control at the end of ensiling. Trace amounts of PA and BA were detected (<1.50 g/kg DM) in all silages throughout ensiling. The NH_3-N content in all silages increased with the progress of ensiling. Compared with the control, F and Y83 had lower ($p < 0.05$) NH_3-N contents during ensiling.

Table 2. Effect of additives on fermentation quality of rice straw silage (g/kg DM or as stated).

Items	Treatments [1]	Ensiling Days (d)					SEM	*p*-Value [2]		
		3	7	14	30	60		T	D	T × D
Dry matter (g/kg FW)	CON	397	400	397	391	380	1.60	ns	<0.001	ns
	E	408	405	402	392	384				
	F	409	404	398	396	386				
	Y83	410	406	403	397	391				
pH	CON	4.56	4.52 ^{a}	4.55 ^{a}	4.43 ^{a}	4.41 ^{a}	0.025	<0.001	ns	ns
	E	4.51	4.49 ^{a}	4.46 ^{a}	4.38 ^{ab}	4.36 ^{a}				
	F	4.56	4.49 ^{a}	4.43 ^{a}	4.35 ^{ab}	4.34 ^{a}				
	Y83	4.31	4.20 ^{b}	4.18 ^{b}	4.16 ^{b}	4.15 ^{b}				
Lactic acid	CON	20.3	22.8 ^{b}	27.2 ^{b}	24.3 ^{d}	19.6 ^{c}	1.67	<0.001	<0.001	<0.001
	E	21.0	23.6 ^{b}	25.4 ^{b}	34.1 ^{c}	29.8 ^{bc}				
	F	21.3	25.5 ^{b}	27.1 ^{b}	37.6 ^{b}	32.2 ^{b}				
	Y83	26.0	41.3 ^{a}	56.3 ^{a}	61.6 ^{a}	51.7 ^{a}				
Acetic acid	CON	10.5 ^{a}	11.9	12.6	16.5 ^{a}	21.2 ^{a}	0.502	<0.001	<0.001	ns
	E	8.66 ^{ab}	9.99	11.2	12.5 ^{ab}	17.4 ^{ab}				
	F	7.61 ^{ab}	9.45	11.3	11.5 ^{ab}	13.2 ^{b}				
	Y83	6.43 ^{b}	8.85	9.01	10.1 ^{b}	12.7 ^{b}				
Propionic acid	CON	0.50	0.61	0.72	0.77	1.13	0.054	<0.001	<0.001	<0.001
	E	0.55	0.72	0.90	0.97	1.02				
	F	ND	ND	0.68	0.79	1.02				
	Y83	ND	ND	ND	ND	ND				

Table 2. *Cont.*

Items	Treatments [1]	Ensiling Days (d)					SEM	*p*-Value [2]		
		3	7	14	30	60		T	D	T × D
Butyric acid	CON	0.42	0.55	0.73	0.86	1.21	0.150	<0.001	<0.001	<0.001
	E	0.49	0.78	0.87	0.85	1.16				
	F	ND	ND	ND	0.60	0.91				
	Y83	ND	ND	ND	0.56	0.86				
NH_3-N (g/kg TN)	CON	60.1 ^{a}	74.6 ^{a}	78.3 ^{a}	84.3 ^{a}	93.4 ^{a}	1.93	<0.001	<0.001	ns
	E	57.7 ^{a}	61.1 ^{ab}	64.6 ^{ab}	66.8 ^{b}	77.3 ^{ab}				
	F	39.7 ^{b}	56.3 ^{b}	54.3 ^{b}	60.6 ^{b}	69.9 ^{b}				
	Y83	29.7 ^{c}	39.3 ^{c}	59.3 ^{b}	65.0 ^{b}	69.8 ^{b}				

DM, dry matter; SEM, standard error of means; NH_3-N, ammonia nitrogen; TN, total nitrogen; and ND, not detected. [1] CON, control; E, fibrolytic enzyme; F, *Trichoderma reesei*; and Y83, *Enterococcus faecium* Y83. [2] T, treatments; D, ensiling days; T × D, interaction between treatments and ensiling days; and ns, not significant. $^{a–d}$ Values with different superscripts differ significantly among treatments on the same ensiling day ($p < 0.05$).

3.3. Carbohydrate Compositions of Rice Straw Silage

The change in structural carbohydrate compositions of rice straw silage is shown in Table 3. Treatments significantly ($p < 0.05$) affected the contents of NDF, ADF, ADL, hemicellulose and cellulose. However, there was only a numerical difference in the content of hemicellulose among treatments during ensiling. With the progress of ensiling, NDF, ADF and cellulose contents gradually decreased in all silages. The contents of NDF in silage treated with additives were lower ($p < 0.05$) than the control throughout ensiling, and Y83 further decreased NDF content relative to other additives at the end of ensiling. After 30 days of ensiling, all additives decreased ADF contents as compared to the control. While no difference was detected in the contents of ADL and cellulose among treatments during the first 14 days of ensiling, all additives decreased ($p < 0.05$) these compositions at the end of ensiling.

Table 3. Effect of additives on structural carbohydrate compositions of rice straw silage (g/kg DM).

Items	Treatments [1]	Ensiling Days (d)					SEM	*p*-Value [2]		
		3	7	14	30	60		T	D	T × D
NDF	CON	702 ^{a}	698 ^{a}	693 ^{a}	691 ^{a}	687 ^{a}	1.42	<0.001	<0.001	ns
	E	691 ^{b}	685 ^{b}	678 ^{b}	675 ^{b}	670 ^{b}				
	F	688 ^{b}	683 ^{b}	676 ^{b}	673 ^{b}	669 ^{b}				
	Y83	688 ^{b}	685 ^{b}	682 ^{b}	676 ^{b}	660 ^{c}				
ADF	CON	425	423 ^{a}	421 ^{a}	420 ^{a}	414 ^{a}	1.18	<0.001	<0.001	ns
	E	419	412 ^{b}	415 ^{b}	408 ^{b}	400 ^{b}				
	F	424	417 ^{ab}	412 ^{b}	408 ^{b}	401 ^{b}				
	Y83	419	419 ^{ab}	417 ^{ab}	412 ^{b}	397 ^{b}				
ADL	CON	64.2	63.6	63.1	63.6 ^{a}	64.5 ^{a}	0.29	<0.001	ns	ns
	E	62.5	61.4	61.7	61.7 ^{ab}	61.2 ^{a}				
	F	61.7	61.8	61.6	60.4 ^{b}	60.5 ^{b}				
	Y83	61.0	59.9	59.4	59.8 ^{b}	58.1 ^{b}				
Hemicellulose	CON	277	274	272	271	272	1.04	0.011	ns	ns
	E	272	273	263	266	269				
	F	264	266	264	265	267				
	Y83	268	266	264	264	263				

Table 3. *Cont.*

Items	Treatments [1]	Ensiling Days (d)					SEM	*p*-Value [2]		
		3	7	14	30	60		T	D	T × D
Cellulose	CON	361	360	357	356 [a]	350 [a]	1.12	0.012	<0.001	ns
	E	356	351	353	346 [b]	339 [b]				
	F	362	355	350	347 [b]	341 [b]				
	Y83	358	359	358	352 [ab]	338 [b]				

DM, dry matter; SEM, standard error of means; FW, fresh weight; NDF, neutral detergent fibre; ADF, acid detergent fibre; and ADL, acid detergent lignin. [1] CON, control; E, fibrolytic enzyme; F, *Trichoderma reesei*; and Y83, *Enterococcus faecium* Y83. [2] T, treatments; D, ensiling days; T × D, interaction between treatments and ensiling days; and ns, not significant. [a–c] Values with different superscripts differ significantly among treatments on the same ensiling day ($p < 0.05$).

As shown in Figure 1, the content of WSC was significantly ($p < 0.05$) influenced by treatments, ensiling days and their interactions. There was a trend wherein the content of WSC decreased rapidly in all silages during ensiling. All additives had a higher ($p < 0.05$) WSC content than the control during the first 3 days of ensiling. After 30 days of ensiling, the content of WSC in E silages was higher than the control, but there were no differences between F and the control. Throughout ensiling, Y83 silages had a higher ($p < 0.05$) WSC content than other silages.

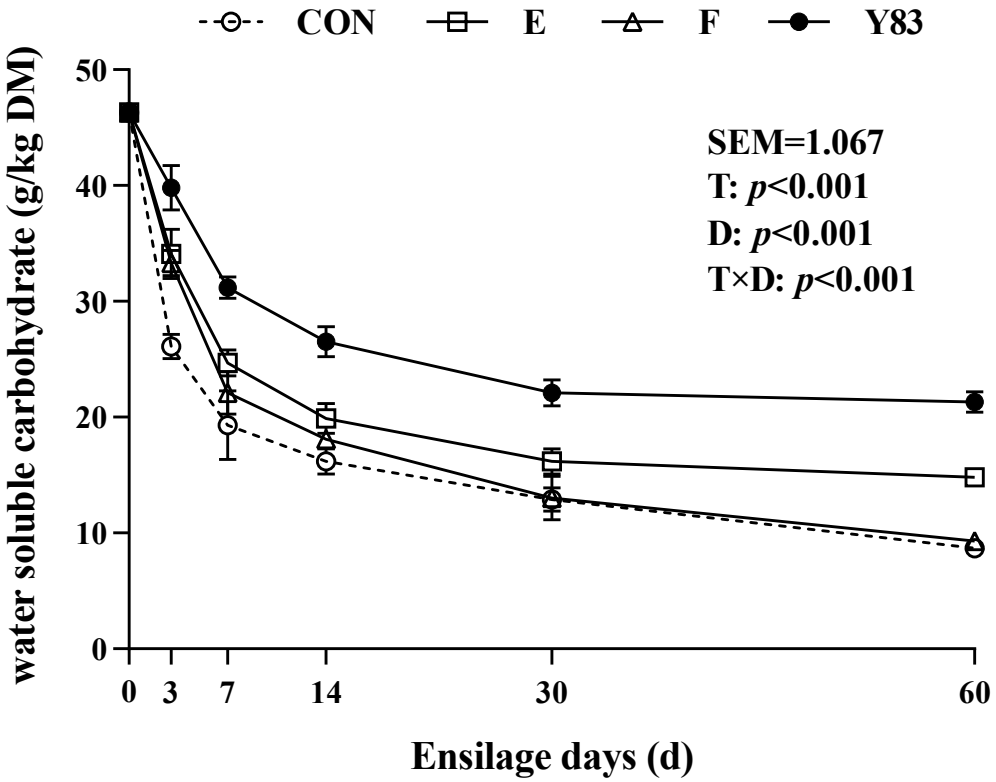

Figure 1. Effect of additives on water soluble carbohydrate (WSC) contents of rice straw silages during ensiling. Abbreviations: CON, control; E, fibrolytic enzyme; F, *Trichoderma reesei*; Y83, *Enterococcus faecium* Y83; T, treatments; D, ensiling days; and T×D, the interaction between treatments and ensiling days ($p < 0.05$).

3.4. In Vitro Gas Production and Digestibility

The gas production and in vitro digestibility of rice straw silage are given in Figure 2 and Table 4. All additives increased ($p < 0.05$) potential gas production (b) relative to the control. However, there was no statistical difference in the detection of gas production rate constant (c) across treatments. The gas production after 24 h incubation in Y83 silages was higher ($p < 0.05$) than that in the control, whereas no effect was observed in silage treated with E and F. Compared with the control, Y83 and E silages had higher ($p < 0.05$) IVDMD, IVNDFD and IVADFD, but there was no difference between F and the control.

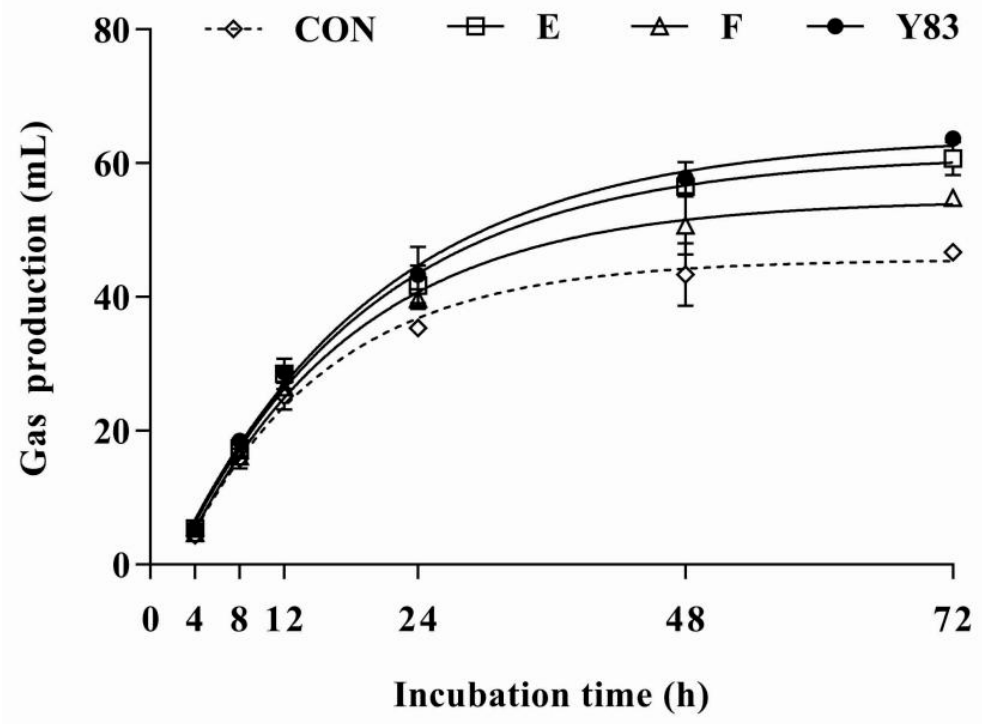

Figure 2. Gas production profiles (mL/g dry matter) from in vitro fermentation of rice straw silage for 72 h. Abbreviations: CON, control; E, fibrolytic enzyme; F, *Trichoderma reesei*; and Y83, *Enterococcus faecium* Y83.

Table 4. Gas production kinetics, in vitro dry matter digestibility, in vitro neutral detergent fibre and in vitro acid detergent fibre of rice straw silages after 60 days of ensiling.

Items	Treatments				SEM	*p*-Value
	CON	E	F	Y83		
Potential gas production, b (mL)	45.6 ^{c}	61.2 ^{a}	54.5 ^{b}	64.0 ^{a}	2.26	<0.001
Gas production rate constant, c (mL/h)	0.076	0.056	0.063	0.054	0.0037	ns
GP_{24} (mL)	35.3 ^{b}	41.7 ^{ab}	39.7 ^{ab}	43.3 ^{a}	1.12	0.034
In vitro dry matter degradability (%)	49.4 ^{b}	58.5 ^{a}	48.4 ^{b}	58.8 ^{a}	1.70	0.007
In vitro neutral detergent fibre (%)	44.6 ^{b}	53.4 ^{a}	45.1 ^{b}	55.5 ^{a}	1.56	<0.001
In vitro acid detergent fibre (%)	38.5 ^{b}	49.3 ^{a}	40.7 ^{b}	50.1 ^{a}	1.87	0.022

DM: dry matter; SEM: standard error of mean; GP_{24}: 24 h net gas production; and ns: not significant. CON: control; E: fibrolytic enzyme; F: *Trichoderma reesei*; and Y83: *Enterococcus faecium* Y83. $^{a–c}$ Values with different superscripts differ significantly ($p < 0.05$).

4. Discussion

4.1. Fermentation Characteristics of Rice Straw Silage

To obtain stable and high-quality silage, the WSC content of raw materials for silage should be 60–80 g/kg DM, and the number of attached LAB should be more than 1×10^5 cfu/g FW [16]. Therefore, it is difficult to obtain stable silage by the natural fermentation of rice straw. In this study, treated silages showed good fermentation characteristics, as indicated by negligible PA and BA contents, as well as low NH_3-N contents (<78.0 g/kg TN). This suggested that the addition of fibrolytic enzyme, *T reesei* and *E faecium* (Y83) could improve the fermentation quality of rice straw silage.

Homofermentative LAB can effectively convert WSC into LA, which affects the rapid decline in the pH value at the early stage of ensiling [17]. *E faecium* (Y83) was identified as homofermentative LAB, which may explain the result that Y83-treated silage had the highest LA content and the lowest pH at the early stage of fermentation. Moreover, our previous study confirmed that Y83 has high Carboxymethyl cellulase (CMCase) and filter paper enzyme (FPase) activity, can sustainably degrade structural carbohydrates and provide substrates for LAB [10]. This could explain why the Y83-treated silage had remarkably higher LA content during ensiling. Although E and F accelerated the production of LA during the early stage of ensiling, there was a reduction in the accumulation of LA at the mid–late stage of ensiling, leading to less LA content than those in the Y83 silage. This result may be due to the accumulation of LA and the decline of pH value, which inhibited the activity of the enzyme. As for F-treated silage, the secretion of enzymes by fungi under anaerobic conditions might be suppressed by the acidic environment in the silo.

The additive-treated silages had a lower AA content than the control, indicating the additive may reduce the production of AA. Wang et al. [18] and Zhao et al. [19] also reported that LAB and fibrolytic enzyme could decrease the AA production of hulless barley straw silage and rice straw. The AA content of E-treated and F-treated silage were higher than that of Y83, which might be ascribed to the degradation of structural carbohydrate and the production of pentose. Pentose can be released by degrading xylan with hemicellulase and then converting it into AA through d-xylos-5-phosphate [20]. However, Y83 has a high CMCase and FPase activity, which degrade cellulose into hexoses (glucose and fructose) and produce bimolecular LA through the 6-phosphogluconate pathway, resulting in that Y83-treated silage had lower AA accumulation than E-treated and F-treated silage [21]. On the other hand, the lowest pH in Y83 might inhibit the metabolism of LAB at the later stage of ensiling, while the activity of heterofermentative LAB might convert LA to AA in E-treated and F-treated silage with higher pH [22]. This was consistent with the decrease of LA content after 90 days of ensiling. Additive-treated silages showed lower BA and NH_3-N contents than the control. This is probably because the fast LA production and pH reduction in treated silage could inhibit clostridia fermentation during the early stage of ensiling [19].

4.2. Carbohydrate Compositions of Rice Straw Silage

The degradation of NDF and ADF in silage is mainly due to acid hydrolysis, enzymatic action and microbial activity [23]. However, the reduction in structural carbohydrate degradation could be attributed to different mechanisms [24]. The NDF contents of the control gradually reduced, which might be related to the acid hydrolysis caused by the accumulation of organic acid. The treated silage had lower NDF and ADF content than the control, which could be explained by the enzymatic action and microbial activity. The NDF contents of treatments were lower than the control during ensiling. This indicated that the fibrolytic enzyme, *T reesei* and Y83 can promote the degradation of lignocellulose, which is similar to the reports of Li et al. [25] and Lee et al. [26]. Anaerobic fungi can decompose structural carbohydrate by the invasion of straw cell walls and cellulolytic activities, which could explain why F had lower NDF and ADF contents than the control [27]. Y83-treated silage had a lower NDF content than the control, reflecting that the Y83 strain also could degrade lignocellulose. This could be attributed to its ability to secrete cellulase.

Actually, the NDF does not reflect the degradation of the specific components of lignocellulose because it consists of cellulose, hemicellulose and lignin [28]. In the present study, all treated silages had lower cellulose than the control after 30 days of ensiling, which indicated that E, F and Y83 performed better on degrading cellulose in rice straw silage. Cellulose is a linear chain of glucose linked by β-1,4 bonds [28], and it can be degraded to hexoses, which can promote the higher LA accumulation of homofermentative LAB [21]. The above finding was consistent with the lowest cellulose and highest LA in the Y83-treated silage. Tao et al. [29] and Zhao et al. [30] concluded that ensilage cannot affect the ADL content by natural fermentation. However, the interaction between lignin and cellulose blocks the effective utilization of cellulose; it is necessary to remove the protective effect of lignin on cellulose [31]. Lignin could be degraded by laccase and lignin peroxidase [32]. Therefore, we speculate that the lower ADL content of F and Y83 than the control may relate to the secretion of laccase and lignin peroxidase by *T reesei* and Y83. Further research is needed to clearly explain this phenomenon.

The magnanimous consumption of WSC was mainly attributed to plant respiration and aerobic microorganisms during the first 3 days of ensiling [18]. The highest WSC content of Y83 may be attributed to the degradation of cellulose and hemicellulose by its enzyme. The WSC produced by lignocellulose degradation supplemented the substrate for LAB growth; otherwise, the low WSC (<50 k/kg) content of fresh rice straw may not benefit LAB fermentation in producing sufficient LA.

4.3. In Vitro Gas Production and Digestibility

In vitro digestibility could reflect the nutritional value and intake of silage, which has been confirmed by a previous study; the higher IVDMD depends on less DM loss and a reduction in the contents of lignocellulose after ensiling [33]. In this experiment, Y83 increased IVDMD compared with the control, which could be explained by the lower NDF content. Cao et al. [34] suggested that the enzyme degradation of lignocellulose resulted in a loosening between cellulose and hemicellulose. Therefore, rumen microorganisms could attach to and colonise on lignocellulose surfaces more easily, which contributes to the improvement in NDF and ADF digestibility. This is consistent with lower IVNDFD and IVADFD in Y83-treated and E-treated silages. Although the F-treated silage had a lower NDF content, it had no improvement in IVNDFD. This could be explained by the fact that *T reesei* biodegraded the most digestible hemicellulose, resulting in NDF being more difficult to degrade for rumen microbes. Similarly, Dehghani et al. [35] reported that more degradation of the cell wall did not always improve in vitro digestibility, which was also related to different kinds of inoculations in silage. Further research on the effect of *T reesei* on the in vitro digestibility of silage is needed to illustrate and explain this phenomenon.

In vitro gas production would reflect true differences in silage fermentability [24]. In this study, treated silages had higher in vitro gas production than the control, which indicated the additives could increase in vitro gas production. The rapidly fermentable component of silage can improve the parameter of GP_{24}, and the potential GP depends on the digestible DM of silages [36]. Y83-treated silages had higher WSC and lower NDF contents after ensiling, which could provide more rapidly fermentable components for rumen microbes. This could explain that Y83-treated silages had the highest in vitro gas production. Furthermore, Wang et al. [37] suggested that the rumen microbial biomass might increase when stimulated by the enzyme and LAB inoculation, which also could improve the in vitro gas production of silage. Overall, inoculation with enzyme and Y83 improved the in vitro digestibility and gas production of rice straw silage compared with the control.

5. Conclusions

This study revealed that additives were required to enhance the fermentation stability of fresh rice straw silage. *E faecium* Y83 was more effective at improving silage quality compared with fibrolytic enzyme and *T reesei*, as indicated by the lower pH and NDF content, as well as higher LA and WSC content in Y83-treated silages. Furthermore, *E faecium* Y83 not only had the highest IVDMD but also significantly increased in vitro gas production. Overall, *E faecium* Y83 was recommended to enhance the fermentation quality, nutritive components and in vitro digestibility of rice straw silage.

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