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Fermentation Quality and Aerobic Stability Evaluation of Rice Straw Silage with Different Ensiling Densities

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Abstract: Ensiling density has significant importance for the quality and preservation of silage. Appropriate ensiling density could improve the nutritional value, extend the storage time of silage and reduce the risk of mold and spoilage. This work aimed to evaluate the effect of ensiling densities on the fermentation quality and aerobic stability of rice straw. The rice straw was obtained after threshing, then chopped and ensiled into a 10 L laboratory silo with three ensiling densities (high density at 700 g/L, medium density at 600 g/L and low density at 500 g/L). Five silos per density were opened after 3, 5, 7, 14, 30 and 60 days of ensiling, and then, the fermentation quality and aerobic stability were analyzed. During ensiling, high density had the highest lactic acid content, and the lowest pH and ammonia nitrogen. There was no difference ($p > 0.05$) in the propionic acid, butyric acid and ethanol contents among all silage, and the contents of propionic acid and butyric acid were trace amounts. On day 60 of ensiling, the Flieg's point of high density and medium density were higher than the low density. During aerobic exposure, the continuous lactic acid decrease and pH increase were observed in all silage. The aerobic bacteria and yeasts count in the high density and medium density were lower than that in the low density. The aerobic stability of the high density (26 h) and the medium density (24 h) were higher than that of the low density (13 h). It was suggested that if the ensiling density is higher than 600 g/L, it could effectively improve the fermentation quality and aerobic stability of rice straw.

Keywords: rice straw; ensiling density; fermentation quality; aerobic stability

Citation: Liu, H.; Li, X.; Hu, J.; Zhao, J.; Xu, G.; Dong, D.; Jia, Y.; Shao, T. Fermentation Quality and Aerobic Stability Evaluation of Rice Straw Silage with Different Ensiling Densities. *Fermentation* **2024**, *10*, 20. https://doi.org/10.3390/fermentation10010020

Academic Editor: Qing Zhang

Received: 27 November 2023
Revised: 22 December 2023
Accepted: 26 December 2023
Published: 27 December 2023

1. Introduction

Rice (*Oryza sativa* L.) is one of the main crops in China. The cultivated area is 30.076 million hectares and the yield is 212 million tons. Rice straw is an abundant agricultural byproduct resource, and most of it is currently burned or discarded, causing serious environmental pollution and more emissions of greenhouse gases. Rice straw has a high lignocellulosic content and poor palatability. It could not be directly fed as roughage like corn straw and would be difficult to digest for livestock. One of the effective ways to alleviate the problems is to feed rice straw by ensiling [1]. It can not only solve the above problems but also retain the nutrients of rice straw and ensure the supplement of roughage for livestock all year around. Moreover, making rice straw as silage contributes to the sustainable development of agricultural resources.

Ensiling is a traditional method for the conservation of fresh forages. It is based on lactic acid bacteria converting water-soluble carbohydrates into lactic acid in anaerobic conditions, resulting in a decrease in pH and inhibition of undesirable microbial activities so that nutrients of forage can be well preserved [2]. One of the important factors influencing

silage quality is the degree of compacting [3]. The importance of ensiling density is mainly reflected during the early stages of ensiling and aerobic exposure.

During the early stages of ensiling, there is still residual air in the silo, and this leads to plant respiration and aerobic microbial activities. According to Ruppel [4], the degree of compacting directly affects the amount of oxygen remaining in the silo, the respiration of plant tissues and the activities of aerobic bacteria, which would cause the loss of both nutrients and fermentation substrates. A higher degree of compacting allowed for more conservation of water-soluble carbohydrates and converted to lactic acid by lactic acid bacteria [5,6]. Therefore, the exclusion of air during the early stage of ensiling is an efficient strategy to improve fermentation quality.

During aerobic exposure, silage is unstable and rapidly heats and spoils due to the growth of aerobic bacteria, yeast, mold, etc. [2]. In general, lactate-assimilating yeasts initiate aerobic deterioration, and then, the aerobic bacteria and mold proliferate. They consume water-soluble carbohydrates, lactic acid and other substrates, increasing pH and ammonia nitrogen because of the proteolysis [7]. Aerobic deterioration increases the proliferation of pathogenic or undesirable microorganisms. Deteriorated silage causes a serious risk to the quality and safety of animal products and to animal health [8].

The low ensiling density often makes it easier for the air to infiltrate into silage mass, resulting in aerobic deterioration during aerobic exposure. Thus, it could improve silage aerobic stability during aerobic exposure by a high degree of compacting to prevent air infiltration [9]. In addition, the increasing degree of compacting has another benefit to reducing the cost of storage [6].

Rice straw has high crude fiber and low moisture content. It is difficult to be compactable while ensiling, which will easily lead to more residual air in the silo. In addition, the low water-soluble carbohydrate content in rice straw results in insufficient fermentation and slows down fermentation. Sarnklong [1] reported that using ligninolytic fungi, including their enzymes during ensiling, could improve the nutritive value of rice straw and is more environmentally friendly.

At present, the rice straw ensiling technology mainly piles the cut rice straw into the silo for compaction, and there is no accurate standard for ensiling density. The experiment aimed to evaluate the effects of different ensiling densities on fermentation quality and aerobic stability of rice straw and provide a reference for the ensiling density of rice straw silage.

2. Materials and Methods

2.1. Silage Preparation

The rice (Ningxiangjing No. 9 with low fiber content) was planted in the Baima experimental field of Nanjing Agricultural University (32.04° N, 118.88° E, 20 m asl, Nanjing, China), and the mature rice was harvested on 30 October 2021. Baima experimental field has a subtropical monsoon climate with an average temperature of 15.7 °C and mean annual precipitation of 1105 mm [10]. The rice straw was obtained after threshing and cut to 1~2 cm with the laboratory fodder chopper. Then, the chemical composition and microbial population were determined immediately (Table 1).

The chopped rice straw was uniformly mixed and filled into silos at different ensiling densities. The treatments were as follows: 700 g/L for high density (H-D), 600 g/L for medium density (M-D) and 500 g/L for low density (L-D), respectively. The material at 500 g/L represents the normal compaction state, 600 g/L indicates a relatively compacted material, while 700 g/L represents the maximum density the laboratory silo could bear. The chopped rice straw was filled into 10 L laboratory silos (polyethylene bottle with a diameter of 10 cm and height of 35 cm Lantian biological experimental instrument Co., Ltd., Jiangsu, Nantong, China) with 5 replicates for each density gradient and then was compacted and sealed. Anaerobic fermentation was conducted at ambient temperature (17–22 °C) for 3, 5, 7, 14, 30 and 60 days, respectively. A total of 90 silos (3 treatments × 6 ensiling days × 5 replicates per ensiling density) were sampled for fermentation quality analysis.

Table 1. Characteristics of rice straw.

Items	Rice Straw
Chemical composition	
DM (g/kg FW)	457 ± 0.13
TN (g/kg DM)	41.8 ± 0.07
WSC (g/kg DM)	28.5 ± 0.09
NDF (g/kg DM)	606 ± 0.23
ADF (g/kg DM)	375 ± 1.65
pH value	5.88 ± 2.34
BC (mEq/kg DM)	64.1 ± 3.47
Microbial population	
Lactic acid bacteria (log_{10} cfu/g FW)	8.78 ± 0.03
Yeasts (log_{10} cfu/g FW)	7.88 ± 0.08
Aerobic bacteria (log_{10} cfu/g FW)	10.6 ± 0.11

Data were means of five replicates; DM, dry matter; FW, fresh weight; TN, total nitrogen; WSC, water-soluble carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; BC, buffering capacity; mEq, milligram equivalent; cfu, colony forming units.

2.2. Chemical Component Analysis

The buffering capacity (BC) of rice straw was titrated by Playne's hydrochloric acid and sodium hydroxide method [11]. The silage samples (300 g) were oven-dried at 65 °C for more than 60 h to constant weight to determine dry matter (DM) content and then ground to pass a 1 mm screen with a laboratory knife mill (93ZT-300; Xingrong Co. Ltd., Guangzhou, China) and stored for subsequent analysis. Total nitrogen (TN) was determined by the Kjeldahl method [12]. The crude protein (CP) was calculated by multiplying TN by 6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the methods described by Van Soest et al. [13]. Water-soluble carbohydrates (WSC) were determined following the method of colorimetry after reaction with anthrone reagent [14].

The silage sample (30 g) was mixed with 90 mL of deionized water and extracted at 4 °C for 24 h. The extract samples were filtered through two layers of cheesecloth and a layer of filter paper (Xinhua Co., Ltd., Hangzhou, China), and the filtrate was stored in a −20 °C refrigerator (Haier Co., Ltd., Qingdao, China), and used for later analysis. The pH was determined with a glass electrode pH meter (HANNA HI 2221; Hanna Instruments Italia Srl, Villafranca Padovana, Italy). The ammonia nitrogen (NH_3–N) was determined by the method of phenol–hypochlorite reaction [15]. The organic acids (lactic acid, acetic acid, propionic acid and butyric acid) and ethanol were determined by an Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a refractive index detector (Carbomix® H-NP5 column, 2.5 mM H_2SO_4, 0.5 mL/min). Flieg's point (FP) (1) was calculated as mentioned by Abo-Donia [16]. An FP value >80 represents a very good quality of silage, 61–80 good quality, 41–60 moderate quality, 21–40 satisfying quality, and <20 worthless.

$$\text{Flieg's point} = 220 + (2 \times \%\text{DM-15}) - 40 \times \text{pH} \quad (1)$$

2.3. Microbial Population Analysis

The plating method was adopted for microbial counting. Briefly, 10 g of silage was placed in a 250 mL conical flask and immersed in 90 mL 0.85% sterilized saline solution. Then, the conical flask was sealed with plastic wrap and kept in the orbital shaker (Boxun Co., Ltd., Shanghai, China) at 120 rpm for 1 h. The counts of lactic acid bacteria (LAB), aerobic bacteria (AB), mold and yeast were determined in reference to the method of Wang [10]. All microbial data were transformed to log_{10} for presentation and statistical analysis.

2.4. Aerobic Stability

After 60 days of ensiling, a total of 45 silos (3 treatments × 3 aerobic exposure days × 5 replicates per ensiling density) were opened for the aerobic stability test. The opened laboratory

silos allowed air to enter, and the silos were covered with two layers of gauze. The temperature sensors of a data logger (MDL-1048A; Tianhe Automatic Instrument Co., Ltd., Shanghai, China) were placed in the geometric center of laboratory silos, and 6 probes were placed in the air to record the ambient temperature per 30 min. Aerobic stability is defined as the hours needed when the silage mass temperature is 2 °C above the ambient temperature [17]. The changes in chemical components and microbial counts were measured at 2, 4 and 6 days of aerobic exposure, and then the data were analyzed as indicators of aerobic deterioration.

2.5. Statistical Analysis

All analyses were conducted using the mixed linear model procedure of SPSS 26.0 (IBM Inc., Armonk, NY, USA) with the model:

$$Y_{ijk} = \mu + T_i + rep_{ji} + D_k + (T \times D)_{ik} + \varepsilon_{ijk}$$

where Y_{ijk} is the dependent variable; μ is the overall mean; T_i is fixed effect of ensiling density; rep_{ji} is the random error (a); D_k is fixed effect of ensiling days or aerobic exposure days; $(T \times D)_{ik}$ is the effect of interaction between ensiling density and ensiling days (aerobic exposure days); and the ε_{ijk} is the random error (b). Tukey's multiple comparisons were used to determine the statistical difference among means. Significant differences were declared at $p < 0.05$, and extremely significant differences were declared at $p < 0.001$.

3. Results

3.1. Fermentation Quality of Rice Straw

The dynamics of the pH, LA, AA and LA/AA contents during ensiling are shown in Table 2. The ensiling density, ensiling days and their interaction significantly affected the pH and LA content ($p < 0.05$). The pH decreased linearly ($p < 0.001$), while the LA content increased linearly ($p < 0.001$) during ensiling in all silage. However, the H-D and M-D had higher LA content and lower pH during ensiling compared to the L-D. On day 60 of ensiling, the LA contents of the H-D, M-D and L-D silage were 28.2 g/kg DM, 23.7 g/kg DM and 22.9 g/kg DM, and the pH decreased to 4.16, 4.32 and 4.58, respectively. The AA content of H-D and M-D was lower than that of L-D. The ratio of lactic acid to acetic acid tended to increase during ensiling, and the H-D had higher LA/AA compared to the M-D and L-D at the end of ensiling. There was not a large difference in the PA, BA and ethanol contents, and the contents of PA and BA were trace amounts among all silage (Table 3). The ethanol contents in all silage increased gradually on day 14 of ensiling. The ensiling density significantly ($p < 0.05$) affected Flieg's point. On day 60 of ensiling, Flieg's points of H-D and M-D were 125 and 119, respectively. They were higher than L-D (107).

Table 2. Effect of ensiling density on pH, LA, AA and LA/AA contents during ensiling.

Items	Ensiling Density	Ensiling Days						SEM	*p*-Value	Significance of Main Effects and Interactions		
		3	5	7	14	30	60			T	D	T × D
pH	L-D	5.16 ^{Aa}	5.14 ^{Aa}	4.98 ^{Aa}	4.78 ^{a}	4.69 ^{a}	4.58 ^{a}	0.220	0.050	<0.001	<0.001	0.015
	M-D	4.82 ^{Ba}	4.75 ^{ABa}	4.64 ^{Ba}	4.52 ^{a}	4.41 ^{a}	4.32 ^{b}	0.178	0.033			
	H-D	4.79 ^{Ba}	4.61 ^{Ba}	4.50 ^{Bab}	4.42 ^{b}	4.28 ^{b}	4.16 ^{b}	0.207	0.016			
	SEM	0.168	0.224	0.202	0.151	0.171	0.173					
	p-value	<0.001	<0.001	0.042	0.068	0.073	0.068					
LA (g/kg DM)	L-D	15.5 ^{Cd}	15.8 ^{Cd}	16.9 ^{Cc}	19.1 ^{b}	19.3 ^{Cb}	22.9 ^{Ca}	2.544	0.033	0.927	<0.001	0.661
	M-D	17.2 ^{Bb}	17.9 ^{Bb}	18.9 ^{Bab}	21.1 ^{ab}	22.7 ^{Ba}	23.7 ^{Ba}	2.425	<0.001			
	H-D	19.0 ^{Ad}	19.3 ^{Ad}	21.3 ^{Acd}	22.3 ^{c}	26.1 ^{Ab}	28.2 ^{Aa}	3.396	<0.001			
	SEM	1.429	1.438	1.799	1.319	2.776	2.333					
	p-value	0.003	0.038	0.045	0.053	0.040	0.044					
AA (g/kg DM)	L-D	5.05	4.26	5.71	8.78 ^{A}	6.54	4.45	1.538	0.131	0.194	0.067	0.686
	M-D	4.77	4.56	5.12 ^{B}	5.91 ^{B}	6.24	3.94	0.785	0.199			
	H-D	4.16	4.52	4.97	5.03 ^{B}	5.36	3.69	0.566	0.072			
	SEM	0.372	0.133	0.319	1.601	0.501	0.316					
	p-value	0.144	0.164	0.094	0.031	0.151	0.095					

Table 2. *Cont.*

Items	Ensiling Density	Ensiling Days						SEM	*p*-Value	Significance of Main Effects and Interactions		
		3	5	7	14	30	60			T	D	T × D
LA/AA	L-D	1.08 ^{Bc}	1.37 ^{Bbc}	1.20 ^{Cbc}	1.03 ^{Cc}	1.42 ^{Cb}	2.90 ^{Ca}	0.642	0.083	<0.001	<0.001	<0.001
	M-D	1.51 ^{Bc}	1.75 ^{Bc}	1.73 ^{Bc}	1.88 ^{Bc}	2.03 ^{Bb}	3.46 ^{Ba}	0.646	0.046			
	H-D	2.21 ^{Ac}	2.05 ^{Ac}	2.28 ^{Ac}	2.45 ^{Ac}	3.00 ^{Ab}	4.92 ^{Aa}	0.986	0.039			
	SEM	0.466	0.278	0.441	0.583	0.651	0.852					
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001					

Data were means of five replicates. Values with different lower-case letters show significant differences among ensiling days in the same density; values with different capital letters show significant differences among density in the same ensiling days ($p < 0.05$); L-D (low density) 500 g/L; M-D (medium density) 600 g/L; H-D (high density) 700 g/L; DM, dry matter; LA, Lactic acid; AA, acetic acid; SEM, standard error of means; T, treatments; D, ensiling days; T × D, interaction between treatments and ensiling days.

Table 3. Effect of ensiling density on PA, BA, ethanol contents and Flieg point during ensiling.

Items	Ensiling Density	Ensiling Days						SEM	*p*-Value	Significance of Main Effects and Interactions		
		3	5	7	14	30	60			T	D	T × D
PA (g/kg DM)	L-D	1.53	2.28	4.98	5.79	5.11	4.19	1.554	0.072	0.441	0.130	0.165
	M-D	3.74	3.33	3.67	5.02	4.89	3.42	0.682	0.215			
	H-D	2.71	3.15	3.39	5.03	3.85	2.98	0.763	0.131			
	SEM	0.903	0.458	0.693	0.361	0.549	0.500					
	p-value	0.444	0.643	0.572	0.951	0.295	0.245					
BA (g/kg DM)	L-D	0.81	0.85	0.80	0.97	0.81	0.45	0.159	0.566	0.497	0.602	0.484
	M-D	0.84	0.80	0.77	0.82	0.80	0.47	0.127	0.534			
	H-D	0.82	0.79	0.84	0.80	0.77	0.44	0.137	0.510			
	SEM	0.012	0.026	0.029	0.076	0.017	0.012					
	p-value	0.945	0.920	0.844	0.743	0.885	0.975					
Ethanol (g/kg DM)	L-D	7.85 ^{b}	8.19 ^{b}	10.1 ^{Aab}	12.1 ^{Aa}	11.5 ^{b}	10.9 ^{c}	1.597	0.046	0.777	0.139	0.861
	M-D	7.54 ^{ab}	6.64 ^{c}	7.99 ^{Bab}	8.56 ^{Ba}	9.29 ^{ab}	10.8 ^{c}	1.326	0.035			
	H-D	6.06 ^{bc}	7.72 ^{b}	7.45 ^{Bb}	9.43 ^{Ba}	9.70 ^{b}	10.8 ^{c}	1.595	0.023			
	SEM	0.781	0.649	1.143	1.506	0.959	0.047					
	p-value	0.544	0.128	0.045	0.032	0.310	0.951					
Flieg Point	L-D	85.7 ^{Bc}	85.9 ^{Cc}	95.5 ^{b}	98.8 ^{Bab}	105 ^{Ba}	107 ^{Ca}	8.343	<0.001	<0.001	0.423	0.251
	M-D	101 ^{Ad}	102 ^{Bcd}	106 ^{c}	113 ^{Ab}	117 ^{Aab}	119 ^{Ba}	7.063	<0.001			
	H-D	101 ^{Ac}	107 ^{Ac}	100 ^{bc}	113 ^{Aabc}	120 ^{Aab}	125 ^{BAa}	9.292	<0.001			
	SEM	7.212	9.003	4.301	6.694	6.481	7.483					
	p-value	<0.001	<0.001	0.010	<0.001	<0.001	<0.001					

Data were means of five replicates. Values with different lower-case letters show significant differences among ensiling days in the same density; values with different capital letters show significant differences among density in the same ensiling days ($p < 0.05$); L-D (low density) 500 g/L; M-D (medium density) 600 g/L; H-D (high density) 700 g/L; DM, dry matter; PA, Propionic acid; BA, Butyric acid; SEM, standard error of means; T, treatments; D, ensiling days; T × D, interaction between treatments and ensiling days.

As shown in Table 4, the ensiling density, ensiling days and their interaction significantly ($p < 0.05$) influenced the DM and NH_3-N. The ensiling days significantly ($p < 0.001$) affected the WSC. The DM decreased in all silage during ensiling, while the DM increased with ensiling density increasing ($p < 0.001$). The DM of H-D is higher ($p < 0.05$) in comparison to the L-D at the 60 days of ensiling. The WSC content continuously decreased during ensiling. The H-D, M-D and L-D silage reached the minimum value at the end of ensiling. The NH_3-N content continuously increased during ensiling, and the H-D, M-D and L-D silage reached the maximum value at the end of ensiling. The NH_3–N of H-D was significantly ($p < 0.05$) lower than that in the M-D and L-D.

Table 4. Effect of ensiling density on DM, WSC and NH_3-N during ensiling.

Items	Ensiling Density	Ensiling Day						SEM	*p*-Value	Significance of Main Effects and Interactions		
		3	5	7	14	30	60			T	D	T × D
DM (g/kg FW)	L-D	431 ^{B}	428 ^{C}	429 ^{B}	425 ^{B}	426 ^{C}	422 ^{C}	2.911	<0.001	<0.001	0.001	0.014
	M-D	439 ^{ABab}	436 ^{ABb}	432 ^{ABb}	431 ^{ABb}	431 ^{Ba}	429 ^{Bb}	3.416	<0.001			
	H-D	444 ^{A}	439 ^{A}	440 ^{A}	439 ^{A}	440 ^{A}	437 ^{A}	2.115	<0.001			
	SEM	5.354	4.643	4.643	5.735	5.793	6.128					
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001					
WSC (g/kg DM)	L-D	10.1 ^{a}	9.73 ^{ABa}	8.48 ^{ab}	7.42 ^{ABb}	5.11 ^{a}	4.43 ^{a}	2.153	<0.001	0.168	<0.001	0.002
	M-D	10.8 ^{ab}	9.64 ^{Aab}	8.65 ^{bc}	7.81 ^{Bc}	5.67 ^{a}	4.16 ^{ab}	2.269	<0.001			
	H-D	9.60 ^{bc}	8.35 ^{Bbc}	7.94 ^{bc}	6.16 ^{Ac}	5.32 ^{a}	4.06 ^{a}	1.897	<0.001			
	SEM	0.492	0.630	0.303	0.704	0.231	0.156					
	p-value	0.109	0.047	0.086	0.094	0.101	0.198					
NH_3-N (g/kg TN)	L-D	26.1 ^{c}	21.3 ^{Bc}	35.2 ^{bc}	66.2 ^{Aa}	65.1 ^{Aab}	72.8 ^{Aa}	20.796	<0.001	0.007	<0.001	0.106
	M-D	19.9 ^{c}	23.3 ^{ABbc}	31.9 ^{abc}	44.9 ^{Babc}	56.9 ^{ABab}	65.4 ^{Ba}	16.844	<0.001			
	H-D	19.3 ^{b}	32.1 ^{Aab}	30.4 ^{ab}	43.3 ^{Ba}	41.9 ^{Bab}	47.8 ^{Ca}	9.597	<0.001			
	SEM	3.074	4.691	2.005	10.438	9.606	10.486					
	p-value	0.051	<0.001	0.059	<0.001	<0.001	<0.001					

Data were means of five replicates. Values with different lower-case letters show significant differences among ensiling days in the same density; values with different capital letters show significant differences among density in the same ensiling days ($p < 0.05$); L-D (low density) 500 g/L; M-D (medium density) 600 g/L; H-D (high density) 700 g/L; DM, dry matter; FW, fresh weight; WSC, water-soluble carbohydrates; NH_3-N, ammonia nitrogen; TN, total nitrogen; SEM, standard error of means; T, treatments; D, ensiling days; T × D, the interaction between treatments and ensiling days.

3.2. Aerobic Stability

The aerobic stability of rice straw silage is shown in Figure 1. The aerobic stability of the H-D, M-D and L-D silage was 26, 24 and 13 h, respectively. The aerobic stability of the H-D and M-D was higher than that of the L-D.

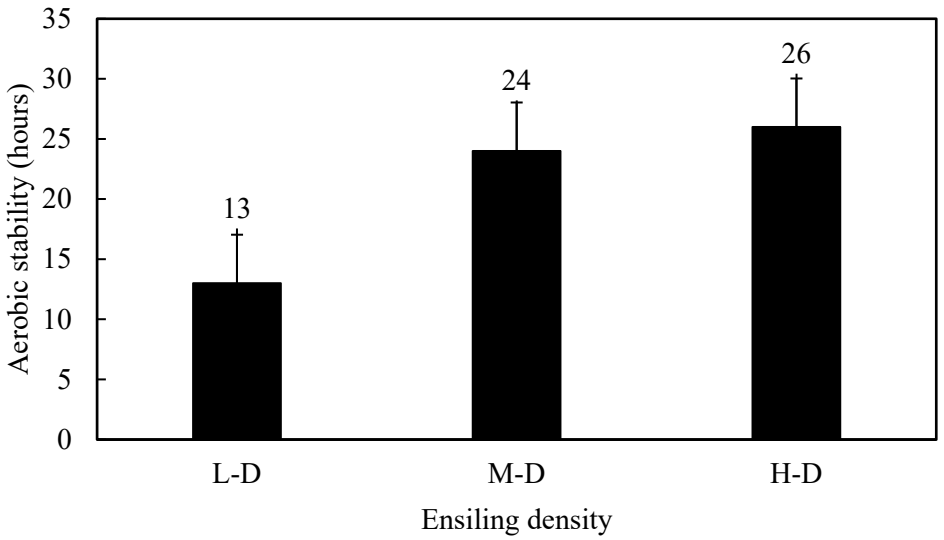

Figure 1. Effect of ensiling density on aerobic stability of rice straw silage. L-D, low density (500 g/L); M-D, medium density (600 g/L); H-D, high density (700 g/L). Columns with different superscripts are significantly different ($p < 0.05$).

Table 5 illustrates the dynamics of pH, NH_3-N and LA content of rice straw silage during aerobic exposure. Ensiling density, aerobic exposure days and their interaction significantly ($p < 0.05$) affected the pH and PA content. The aerobic exposure days significantly ($p < 0.05$) affected the pH and LA content. The LA content decreased, and the pH increased in all silage during aerobic exposure. The LA contents of the H-D, M-D and L-D silage decreased to 2.99 g/kg DM, 2.06 g/kg DM and 1.02 g/kg DM, the pH increased to 7.24,

7.27 and 7.57, respectively. Moreover, the LA content of the H-D and M-D is always higher, and the pH is lower than that of the L-D silage. The AA content remained at a stable value during aerobic exposure in all silage (Table 6). The low ethanol content and a trace amount of PA and BA were detected in all silage during aerobic exposure.

Table 5. Changes in pH, NH_3-N and LA contents of rice straw silage during air exposure.

Items	Ensiling Density	Aerobic Exposure Days				SEM	*p*-Value	Significance of Main Effects and Interactions		
		0	2	4	6			T	D	T × D
pH	L-D	4.58 ^{c}	7.24 ^{Ab}	7.39 ^{ab}	7.57 ^{a}	1.227	<0.001			
	M-D	4.32 ^{c}	6.68 ^{Bb}	7.40 ^{a}	7.27 ^{a}	1.241	<0.001	<0.001	<0.001	<0.001
	H-D	4.16 ^{c}	6.51 ^{Bb}	7.30 ^{a}	7.24 ^{a}	1.275	<0.001			
	SEM	0.173	0.312	0.045	0.149					
	p-value	0.354	<0.001	0.520	0.382					
NH_3-N (g/kg TN)	L-D	72.8 ^{Aa}	73.3 ^{Ab}	75.3 ^{Ab}	76.2 ^{Aa}	1.398	0.038			
	M-D	65.4 ^{Bb}	68.3 ^{Aa}	69.3 ^{Aa}	70.1 ^{Ba}	1.778	0.022	0.002	<0.001	0.596
	H-D	47.8 ^{Cc}	59.5 ^{Bab}	61.7 ^{Bab}	69.9 ^{Ba}	7.901	<0.001			
	SEM	10.486	5.705	5.565	2.924					
	p-value	<0.001	<0.001	<0.001	<0.001					
LA (g/kg DM)	L-D	22.9 ^{Ca}	6.45 ^{b}	3.11 ^{c}	1.02 ^{c}	8.610	<0.001			
	M-D	23.7 ^{Ba}	6.82 ^{b}	4.07 ^{c}	2.06 ^{c}	8.562	<0.001	0.733	<0.001	0.084
	H-D	28.2 ^{Aa}	7.27 ^{b}	4.97 ^{c}	2.99 ^{c}	10.127	<0.001			
	SEM	2.333	0.335	0.759	0.805					
	p-value	<0.001	0.059	0.139	0.158					

Data were means of five replicates. Values with different lower-case letters show significant differences among ensiling days in the same density; values with different capital letters show significant differences among density in the same ensiling days ($p < 0.05$); L-D (low density) 500 g/L; M-D (medium density) 600 g/L; H-D (high density) 700 g/L; DM, dry matter; NH3-N, ammonia nitrogen; TN, total nitrogen; LA, Lactic acid; SEM, standard error of means; T, treatments; D, ensiling days; T × D, interaction between treatments and ensiling days.

Table 6. Changes in AA, PA, BA and ethanol contents of rice straw silage during air exposure.

Items	Ensiling Density	Aerobic Exposure Days				SEM	*p*-Value	Significance of Main Effects and Interactions		
		0	2	4	6			T	D	T × D
AA (g/kg DM)	L-D	4.45 ^{A}	4.03	3.99	4.23	0.183	0.232			
	M-D	3.94 ^{Bb}	4.24 ^{a}	4.07 ^{b}	4.25 ^{a}	0.129	0.204	0.045	0.358	0.227
	H-D	3.69 ^{Bb}	4.15 ^{a}	4.06 ^{a}	4.16 ^{a}	0.192	0.046			
	SEM	0.316	0.086	0.036	0.039					
	p-value	0.032	0.261	0.378	0.259					
PA (g/kg DM)	L-D	4.19 ^{Aa}	3.21 ^{Ab}	1.76 ^{c}	1.59 ^{c}	1.072	<0.001			
	M-D	3.42 ^{Ba}	2.11 ^{Bb}	1.54 ^{b}	1.64 ^{b}	0.749	<0.001	<0.001	<0.001	0.016
	H-D	2.98 ^{Ba}	2.37 ^{Bb}	1.55 ^{c}	1.58 ^{c}	0.596	<0.001			
	SEM	0.500	0.469	0.101	0.026					
	p-value	0.034	0.025	0.536	0.299					
BA (g/kg DM)	L-D	0.45	0.84	0.82	0.85 ^{a}	0.168	0.184			
	M-D	0.47	0.81	0.83	0.83 ^{a}	0.153	0.171	0.686	<0.001	0.584
	H-D	0.44	0.78	0.81	0.87	0.168	0.169			
	SEM	0.012	0.024	0.008	0.016					
	p-value	0.712	0.207	0.821	0.776					
Ethanol (g/kg DM)	L-D	10.9	8.16	6.43	5.71	1.999	<0.001			
	M-D	10.8	8.78	7.60	6.91	1.475	<0.001	0.591	0.017	0.447
	H-D	10.8	8.59	7.52	6.97	1.466	<0.001			
	SEM	0.047	0.259	0.534	0.580					
	p-value	0.814	0.533	0.296	0.273					

Data were means of five replicates. Values with different lower-case letters show significant differences among ensiling days in the same density; values with different capital letters show significant differences among density in the same ensiling days ($p < 0.05$); L-D (low density) 500 g/L; M-D (medium density) 600 g/L; H-D (high density) 700 g/L; DM, dry matter; AA, Acetic acid; PA, Propionic acid; BA, Butyric acid; SEM, standard error of means; T, treatments; D, ensiling days; T × D, interaction between treatments and ensiling days.

Aerobic exposure days significantly ($p < 0.05$) affected the aerobic bacteria, yeasts and lactic acid bacteria counts (Table 7). The aerobic bacteria and yeasts count significantly ($p < 0.001$) increased in all silage during aerobic exposure. The aerobic bacteria and yeast count in the H-D and M-D were lower than in the L-D. The highest counts of aerobic bacteria and yeast were detected in the L-D silage. The lactic acid bacteria count significantly ($p < 0.05$) decreased during aerobic exposure, and the H-D silage had the highest lactic acid bacteria count (7.17 $\log_{10}$ cfu/g FW).

Table 7. Changes in microbial counts of rice straw silage during air exposure.

Items	Ensiling Density	Aerobic Exposure Days				SEM	*p*-Value	*p*-Value		
		0	2	4	6			T	D	T × D
Aerobic bacteria ($\log_{10}$ cfu/g FW)	L-D	8.56 ^{Ad}	9.75 ^{Ac}	11.6 ^{Ab}	14.5 ^{Aa}	2.241	<0.001	0.331	<0.001	0.742
	M-D	7.97 ^{Bd}	8.58 ^{Bc}	10.6 ^{Bb}	13.2 ^{Ba}	2.044	<0.001			
	H-D	7.58 ^{Bd}	8.32 ^{Bc}	10.2 ^{Bb}	13.1 ^{Ba}	2.131	<0.001			
	SEM	0.403	0.622	0.589	0.638					
	p-value	0.017	0.024	0.020	0.029					
Yeasts ($\log_{10}$ cfu/g FW)	L-D	6.19 ^{Ad}	9.38 ^{Ac}	10.5 ^{Ab}	12.5 ^{Aa}	2.285	<0.001	0.533	<0.001	0.001
	M-D	5.88 ^{Bc}	6.54 ^{Bb}	9.18 ^{Ba}	10.6 ^{Ba}	1.921	<0.001			
	H-D	5.54 ^{Bc}	7.29 ^{Bb}	8.58 ^{Ba}	10.3 ^{Ba}	1.744	<0.001			
	SEM	0.265	1.202	0.802	0.974					
	p-value	0.010	0.017	0.012	0.028					
Lactic acid bacteria ($\log_{10}$ cfu/g FW)	L-D	9.04 ^{Ba}	7.62 ^{Bb}	6.36 ^{Cc}	5.12 ^{Bd}	1.456	<0.001	0.077	0.007	0.275
	M-D	10.2 ^{Aa}	8.97 ^{Ab}	7.18 ^{Bc}	6.79 ^{Ad}	1.378	<0.001			
	H-D	10.8 ^{Aa}	9.02 ^{Ab}	8.07 ^{Ac}	7.17 ^{Ad}	1.345	<0.001			
	SEM	0.731	0.649	0.698	0.890					
	p-value	0.034	0.017	0.023	0.028					

Data were means of five replicates. Values with different lower-case letters show significant differences among ensiling days in the same density; values with different capital letters show significant differences among density in the same ensiling days ($p < 0.05$); L-D (low density) 500 g/L; M-D (medium density) 600 g/L; H-D (high density) 700 g/L; SEM, standard error of means; T, treatments; D, ensiling days; T × D, the interaction between treatments and ensiling days.

4. Discussion

It is well known that air residue in the silo enables plant respiration and aerobic microbial activity to take place during the early stage of ensiling, and causing a loss of both fermentable substrate and nutritive composition [2]. One of the important measures to quality silage making is to rapidly reduce the residual air in silo and prevent air infiltration during aerobic exposure. Increasing ensiling density can minimize WSC loss due to plant respiration and aerobic microbial activity during the early stages of ensiling. This is mainly manifested in the elimination of residual oxygen by high density, which creates an anaerobic environment for LA fermentation and promotes a rapid decline in pH [18]. In addition, high density could prevent air infiltration, thereby inhibiting the proliferation of aerobic bacteria during aerobic exposure [2].

4.1. The Effects of Ensiling Density on the Fermentation Quality of Rice Straw

Plant respiration and undesirable microbial activities are dependent on residual oxygen and result in a reduction in DM content [2]. Thus, the silage with a lower degree of compaction had higher DM loss.

The requirements for successful ensiling include adequate DM (300–400 g/kg FW) and WSC (>50 g/kg DM) content [19]. In this experiment, the WSC content of all silage was lower than 10 g/kg DM. The DM content was higher than 400 g/kg DM on the end of ensiling. This indicates that a proper amount of water and WSC should be added to promote fermentation during rice straw [2]. The LA content increased gradually, and the pH decreased slowly during the initial 5 days of ensiling. Then, the LA content increased

fast while the pH quickly decreased during the rest time of ensiling. These indicate that there was a time lag between the onset of the LA production and pH decline. The results agreed with Shao et al. [20]. Shao et al. [20] show that cell breakdown and the release of plant juices are prerequisites for the production of large amounts of LA, and the complete exclusion of air from the silage mass can promote cell breakdown within the initial stage of ensiling. While the production of lactic acid and other organic acids could inhibit harmful bacterial growth and improve the aerobic stability of silage [2]. Zhang and Yu [21] assessed the effects of the two ensiling densities (500 and 600 kg/m^3) on the fermentation quality of *Leymus chinensis* silage. They observed that high density (600 kg/m^3) had higher lactic acid content, and lower pH, butyric acid, ammoniacal nitrogen content.

The rice straw had high cellulose and hemicellulose contents and rigid physical properties that would make the cell breakdown and release of plant juices more difficult and slower. Therefore, fermentation was restricted in all silage during the initial 5 days of ensiling. The H-D and M-D silage showed larger LA production for the rest of the time of ensiling. This could be explained by the fact that the H-D and M-D silage provides a larger pressure to crush the plant tissue, promoting more juice release from grass mass, and thus, stimulating epiphytic lactic acid bacteria activity to increase the production of LA [22]. That is why the WSC content of H-D was lower than that in the M-D and L-D during ensiling. The AA content in the H-D and M-D silage is lower than that in the L-D silage. The ratio of LA/AA in the H-D is higher than the M-D and L-D during ensiling. This indicates that homofermentative lactic bacteria were the dominant bacteria in the H-D silage [10].

The NH_3-N content was low, and trace amounts of PA and BA were detected during ensiling. The Flieg's points were higher than 100 in all silage. This indicates that all silage had good conservation [16]. The high DM content and low WSC content not only inhibit the growth of clostridia and other undesirable microorganisms but also somewhat inhibit the growth of lactic acid bacteria [23]. The H-D and M-D silage generally showed higher contents of organic acids as compared with the L-D silage during ensiling, which indicates that the H-D and M-D silage had a faster and larger juice release. As a result, the H-D and M-D silage had more extensive fermentation than the L-D silage.

Plant proteases and microbial activity play crucial roles in proteolysis, and high levels of NH_3-N indicate a high occurrence of protein degradation during ensiling [24,25]. During 60 days of ensiling, the NH_3–N kept on increasing and the maximum NH_3–N among all silage was <80 g/kg DM [26]. This was due to high DM suppressing the activity of plant proteases and undesirable microorganisms to inhibit proteolysis during ensiling [27]. The H-D silage showed lower NH_3-N than the M-D and L-D silage at the end of ensiling. It indicates that the higher LA content and lower pH suppress undesirable microorganisms rather than the M-D and L-D silage. The results of this experiment are consistent with Sucu [6]. It shows that increasing the ensiling density could reduce the ammoniacal nitrogen content and fermentation loss.

4.2. The Effects of Ensiling Density on the Aerobic Stability of Rice Straw Silage

Aerobic stability is a very important factor in determining its subsequent nutritional quality and feed value [28]. The silage is inevitably exposed to air during the feedout phase, leading to the rapid proliferation of aerobic microbes, such as yeasts and molds, resulting in a rise in temperature and a deterioration of the silage [2]. Air penetrates silage mass by diffusion owing to differences in air pressure and density between the external air and internal pressure [17]. Permeability is affected by both ensiling density and moisture [17].

In the experiment, the H-D and M-D silage showed a longer period of aerobic stability compared with the L-D. This is mainly due to the H-D and M-D silage having lower porosity and permeability than the L-D. Low ensiling density as a result of inadequate compaction is reflected in higher porosity [29], which allows more rapid ingress of air into the silage mass during aerobic exposure. A low degree of compaction results in more extensive exposure of the silage microflora to oxygen during initial exposure to air than a high degree of compaction. As long as considerable air ingress occurs, higher

temperatures will be measured in a low degree of compaction as compared with a high degree of compaction [30]. A low degree of compaction provides a suitable environment for aerobic bacteria to proliferate.

Aerobic deterioration is initiated by acid-tolerant yeasts during aerobic exposure and then is accompanied by pH rises and proliferation of aerobic bacteria [31]. The aerobic bacteria and yeast count of the L-D silage are higher than the H-D and M-D silage at the beginning of aerobic exposure. The result was similar to the research of Kung Jr. [32], who stated that the lower compaction slowed down the fermentation rate and aided the counts of aerobic bacteria and yeasts produced in the silage during silo opening.

Aerobic deterioration of silage not only caused losses of nutrient compositions but also increased the risk of proliferation of undesirable microorganisms [33]. The pH, yeast and mold count increased, while the WSC, LA and AA contents decreased in all silage during aerobic exposure. These indicate that all silage underwent aerobic deterioration to different extents.

There are many limitations to this experiment. The rice straw had low WSC, the DM and fiber contents. These characteristics resulted in slow fermentation of ensiling. In subsequent experiments, we will add fermentation promoters, such as molasses, and adopt next-generation sequencing technology to study the effects of different ensiling densities on fermentation quality and microbial communities.

5. Conclusions

The high ensiling density could improve the fermentation quality and aerobic stability of rice straw silage, as indicated by the relatively high lactic acid content, Flieg's point and lactic acid bacteria count, low pH value and ammonia nitrogen content. During aerobic exposure, the aerobic stability of high ensiling density is higher than others. It was suggested that if the ensiling density is higher than 600 g/L, it could effectively improve the fermentation quality and aerobic stability of rice straw. In general, adequate ensiling density for rice straw silage could improve the sustainable utilization of rice straw.

Author Contributions: Methodology and software, X.L. and J.H.; validation, formal analysis, J.Z. and G.X.; investigation, resources and data curation, H.L. and D.D.; writing—review and editing, H.L.; supervision, Y.J. and T.S.; funding acquisition, Y.J. and T.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Major Science and Technology Special Project of Tibet-Technology Mode Innovation and Demonstration of Spatio-temporal Expansion of Tibet Prataculture (XZ202101ZD0003N) and Research on Key Technologies of Digestion of Anti-nutrient Factors and Palatability Improvement of Forage (2021YFD1300302).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article. The data that support the findings of this study are available from the corresponding author, TS, upon reasonable request.

Conflicts of Interest: The authors report no conflict of interest in this work.

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