agronomy

MDPI

Article

Effects of Ensiling Density on the Fermentation Profile and Aerobic Stability of Wilted Alfalfa Silage

Haopeng Liu [1], Xinbao Li [1], Feifei Yang [2], Junfeng Hu [3], Yushan Jia [4] and Tao Shao [1,*]

[1] Institute of Ensiling and Processing of Grass, College of Agro-Grassland Science, Nanjing Agricultural University, Nanjing 210095, China; m18061752580@163.com (H.L.); lxb2510@163.com (X.L.)
[2] Animal Production and Disease Control Specialty, Lianyungang Biological Engineering Specialized Secondary School, Lianyungang 222000, China; yangff2006@163.com
[3] Agricultural and Rural Office of Hemudu Town, Ningbo 315414, China; 18569414374@163.com
[4] Key Laboratory of Forage Cultivation, Processing and Highly Efficient Utilization, Ministry of Agriculture, Inner Mongolia Agricultural University, Hohhot 010018, China; jys_nm@sina.com
* Correspondence: taoshaolan@163.com

Abstract: Silage quality and aerobic stability are the key factors affecting the utilization efficiency of silage feed, and ensiling density stands as the fundamental principle of silage making. The experiment presented here evaluates the effects of ensiling density on the silage quality and aerobic stability of alfalfa silage. In this experiment, alfalfa was harvested, wilted, chopped, and subsequently packed into 10 L laboratory silos. The ensiling densities were set to 800 g/L, 700 g/L, and 600 g/L, respectively, with three replicates in each group. Sampling and analysis were carried out at 45 days of silage and 8 days of aerobic exposure. The results showed that ensiling density significantly ($p < 0.05$) affected the content of ammonia nitrogen, Flieg score, the counts of yeast, and mold. After 45 days of ensiling, 800 g/L silage had the highest contents of dry matter, water-soluble carbohydrates, crude protein, lactic acid, and total organic acids, and the lowest pH and ammonia nitrogen compared to the 700 g/L and 600 g/L silage ($p < 0.05$). The Flieg score of 800 g/L silage was higher than those of the 700 g/L and 600 g/L silage. The counts of yeast and mold of 800 g/L silage was significantly lower than those of the 700 g/L and 600 g/L silage. During aerobic exposure, a consistent decrease in lactic acid and an increase in pH were observed among all silages. The aerobic stability of 800 g/L silage (156 h) was significantly higher than that in the 700 g/L (136 h) and 600 g/L silage (111 h). It was suggested that the increasing ensiling density above 800 g/L was an effective method to improve both the silage quality and the aerobic stability of alfalfa silage.

Keywords: alfalfa; ensiling density; silage fermentation; aerobic stability

check for updates

Citation: Liu, H.; Li, X.; Yang, F.; Hu, J.; Jia, Y.; Shao, T. Effects of Ensiling Density on the Fermentation Profile and Aerobic Stability of Wilted Alfalfa Silage. *Agronomy* **2024**, *14*, 1143. https://doi.org/10.3390/agronomy14061143

Academic Editors: Martin Gierus and Fujiang Hou

Received: 2 April 2024
Revised: 11 May 2024
Accepted: 25 May 2024
Published: 27 May 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Ensiling is an effective method of preserving nutrients by maximizing lactic acid production under anaerobic conditions. During ensiling, the ensiled material undergoes four aerobic stages: the field stage, the initial aerobic stage during ensiling process, the air-infiltration stage, and the aerobic deterioration stage after opening [1]. In the early stages of ensiling, oxygen still exists within the silo, which stimulates the activity of aerobic microorganisms such as yeasts and molds, while also stimulating plant respiration (continuing metabolism of plant cells), causing a loss of both fermentation substrates and nutrients [2]. In addition, inadequate compaction increases the volume of air trapped in silos and promotes the growth of undesirable bacteria. These undesirable bacteria vie with lactic acid bacteria for water-soluble carbohydrates and degrading proteins, causing an unpleasant odor and reduced palatability [3]. Moreover, feeding spoiled silage to ruminants would cause reductions in intake and affect the health of livestock [4]. During ensiling and feed-out, air also permeates in the peripheral areas of a silo; this could promote the growth of aerobic bacteria results in decreased aerobic stability [5]. The high ensiling density

could prevent air infiltration to silage mass and improve aerobic stability during aerobic exposure [6]. Ruppel [7] found that, as packing density increased from 160 to 320 kg·m^3, the dry matter losses decreased from 20% to 10%. Therefore, we can infer that packing density is negatively correlated with storage loss.

Alfalfa (*Medicago sativa* L.) is a high-quality leguminous forage in China, with high crude protein, vitamins, and a variety of minerals. It also has good palatability and high yield, so it is widely cultivated in many countries, and is considered an important diet for ruminants [3]. Alfalfa is primarily used for producing silages to be fed to livestock [8]. However, achieving high-quality silage in practice poses challenges due to its low dry matter and water-soluble carbohydrate contents, as well as its high buffering capacity [9]. Therefore, proper wilt and adequate compaction are crucial measures for the production of high-quality alfalfa silage; these measures could reduce the moisture content of alfalfa and the amount of oxygen in the silos, thus inhibiting the growth of undesirable bacteria.

Ensiling density refers to the compactness or how tightly packed the forage is within a silo or bunker during ensiling [1]. It is a critical factor that significantly influences the quality and preservation of silage. Ensiling density is determined by several key factors, such as the moisture content and the physical structure of ensilage materials [10]. Diverse ensiling densities result in variations in the fermentation process and the quality of fermentation products [11]. Ensiling density is often overlooked in practice, and the operation is not standardized, leading to poor-quality silage. Therefore, achieving the right ensiling density is crucial for fermentation efficiency and optimal nutrient retention [12].

Based on previous research, the compaction density of alfalfa silage in mass production should be 200–250 kg DM/m^3, or there should be a clearance rate of less than 40%. This work proposes that higher ensiling densities lead to better quality and a longer aerobic stability time. Therefore, the purpose of this work to determine a suitable ensiling density for alfalfa silage, provide a reference for those engaged in production practices.

2. Materials and Methods

2.1. Preparation of Alfalfa Silage

The alfalfa was cultivated in Hemudu Town, Yuyao City (29.95° N, 121.35° E, 4 m asl, Zhejiang, China). The alfalfa was harvested (early bloom stage 5 November 2022) and wilted in the field (control the moisture content about 65%) and then carried to laboratory. The characteristics of wilted alfalfa are shown in Table 1. After chopping (2–3 cm) and mixing, the materials were loaded into 1 L and 10 L laboratory silos at different densities. The ensiling densities were set as 800 g/L, 700 g/L, and 600 g/L. Each density was replicated five times and stored at room temperature (16–21 °C). After 45 days of ensiling, the 1 L silos were opened to analyze the fermentation parameters and the aerobic stability of the 10 L silos was analyzed.

Table 1. Characteristics of wilted alfalfa, prior to ensiling.

Items	Value ± SD
pH value	6.42 ± 0.13
Dry matter (g/kg FW)	256 ± 1.16
Crude protein (g/kg DM)	255 ± 8.47
Neutral detergent fiber (g/kg DM)	404 ± 5.88
Acid detergent fiber (g/kg DM)	279 ± 6.92
Buffering capacity (mEq/kg DM)	271 ± 7.03
Water-soluble carbohydrates (g/kg DM)	79.0 ± 3.60
Lactic acid bacteria (log_{10} cfu/g FW)	6.73 ± 0.12
Yeast (log_{10} cfu/g FW)	6.24 ± 0.09
Aerobic bacteria (log_{10} cfu/g FW)	9.36 ± 0.07

DM, dry matter; FW, fresh weight; mEq, milligram equivalent; log_{10}: decimal logarithm; cfu, colony-forming units.

2.2. Chemical Component and Microbial Population Analysis

The silage sample was divided into two parts. Part one was placed in the oven at 65 °C for 48 h to dry to a constant weight; then, it was ground through a flour mill and passed through 40 mm and 100 mm screens. The dry matter (DM) was determined by Liu [6]. Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) contents were determined by an ANKOM (A2000i) fiber analyzer. Water-soluble carbohydrate (WSC) content was determined by Zhao [13].

A measure of 200 g of silage was mixed with 600 mL of distilled water and left to soak at 4 °C for 24 h. Afterward, the mixture was filtered through two layers of gauze and a layer of filter paper. The resulting extract was then utilized to measure pH using a pH meter. Determination of lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA), and ethanol contents was conducted by an HPLC system (Agilent 1260 Infinity, Agilent Technologies, Inc., Waldbronn, Germany). The content of ammonia nitrogen (NH_3-N) was determined by phenol-hypochlorite colorimetry [13].

Flieg's point was calculated by Abo-Donia [14]. The formula is as follows:

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

The lactic acid bacteria (LAB), aerobic bacteria (AB), mold, and yeast were counted using plate counting [15]. All microbial data were transformed to $\log_{10}$ for presentation and statistical analysis.

2.3. Aerobic Stability Test

After 45 days of ensiling, 45 silos were opened, and air was allowed to enter. Two layers of gauze were used to cover the tops of the silos, and the six temperature sensors of a data logger were placed in different geometric positions of the silos to record the temperature at 30 min intervals [16]. Six probes were placed in the environment as blanks. The aerobic stability of silage was assessed by comparing the time required for the silage to exceed room temperature by 2 °C, and the fermentation parameters were determined during aerobic exposure at 2, 4, and 6 days.

2.4. Statistical Analysis

Statistical analysis was conducted using SPSS (version 13.0 for Windows; SPSS Inc., Chicago, IL, USA). Fermentation characteristics and microbial counts were analyzed via one-way analysis of variance (ANOVA) using the following model:

$$Y_i = \mu + T_i + e_{ij} \quad (2)$$

In this model, Y_i represents the dependent variable, μ denotes the overall mean, T_i represents the effect of ensiling density, and e_{ij} signifies the residual error term.

A factorial treatment design model was employed to analyze aerobic stability parameters via two-way analysis of variance, outlined as follows:

$$Y_{ij} = \mu + T_i + D_j + (T \times D)_{ij} + e_{ij} \quad (3)$$

where Y_{ij} represents the dependent variable, μ denotes the overall mean, T_i signifies the impact of ensiling density, D_j indicates the effect of exposure days, $(T \times D)_{ij}$ represents the interaction effect between ensiling densities and exposure days, and e_{ij} is the residual error term.

3. Results

3.1. Fermentation Quality of Alfalfa

Table 2 displays the fermentation parameters, microbial composition, and chemical compositions of alfalfa silage following a 45-day ensiling period. Ensiling density significantly affected ($p < 0.05$) the NH_3-N content, Flieg score, and molds count. After 45 days

of ensiling, the WSC content of 800 g/L silage (23.4 g/kg DM) was higher than that of the 700 g/L silage (20.1 g/kg DM) and the 600 g/L (12.9 g/kg DM) silage; the NH_3-N contents of the 800 g/L and 700 g/L silages were significantly ($p < 0.05$) lower than that of the 600 g/L silage; the CP contents of the 800 g/L and 700 g/L silages were significantly ($p < 0.05$) higher than that of the 600 g/L silage. Ensiling density had little effect on the contents of DM, NDF, ADF, and PA. The 800 g/L silage had the lowest pH value (4.30), BA content (3.01 g/kg DM), and ethanol content (9.45 g/kg DM), and had the highest LA content (78.5 g/kg DM) and AA content (21.0 g/kg DM). The LA/AA values of the 800 g/L, 700 g/L, and 600 g/L silages were 3.63, 3.22, and 3.26, respectively.

Table 2. Fermentation profile and microbial and chemical compositions of alfalfa silage after 45 days of ensiling.

Items	Ensiling Density			SEM	*p*-Value
	600 g/L	700 g/L	800 g/L		
Fermentation profile (g/kg DM)					
Dry matter (g/kg FW)	207	212	221	2.772	0.193
Water-soluble carbohydrates	12.9 ^{b}	20.1 ^{ab}	23.4 ^{a}	2.009	0.071
NH_3-N (g/kg TN)	116 ^{a}	85.6 ^{b}	73.2 ^{b}	7.072	0.005
Crude protein	53.4 ^{b}	61.0 ^{ab}	85.3 ^{a}	6.333	0.076
Neutral detergent fiber	318	308	305	8.003	0.882
Acid detergent fiber	226	221	220	7.666	0.927
pH	4.89	4.52	4.30	0.051	0.136
Lactic acid	71.6	75.8	78.5	2.044	0.449
Acetic acid	26.6	23.8	21.0	1.882	0.547
Propionic acid	3.42	4.17	4.97	0.346	0.199
Butyric acid	4.46	3.75	3.01	0.584	0.802
Ethanol	14.8	12.7	9.45	1.357	0.310
Lactic acid/acetic acid	3.26	3.22	3.63	0.342	0.894
Flieg Score	50.8 ^{b}	66.6 ^{ab}	77.2 ^{a}	1.725	<0.001
Microbial numbers (log_{10} cfu/g FW)					
Lactic acid bacteria	11.6	13.8	16.4	1.148	0.249
Yeasts	3.84 ^{a}	2.49 ^{ab}	0.98 ^{b}	0.517	0.047
Molds	1.99 ^{a}	1.09 ^{b}	-	0.299	<0.001

Data were means of five replicates. ^{a,b} means in the same row followed by different letters are significantly different ($p < 0.05$). DM, dry matter; FW, fresh weight; TN, total nitrogen; log_{10}: decimal logarithm; cfu, colony-forming units. SEM, standard error of means.

The Flieg scores of the 800 g/L and 700 g/L silages were 77.2 and 66.6, which are higher than the Flieg score of the 600 g/L silage (50.8). The LAB count of the 800 g/L silage (16.4 g/kg FW) was higher than those of the 700 g/L (13.8 g/kg FW) and 600 g/L (11.6 g/kg FW) silages. The yeast count of the 800 g/L silage (0.98 g/kg FW) was significantly ($p < 0.05$) lower than those of the 700 g/L (2.49 g/kg FW) and 600 g/L (3.84 g/kg FW) silages. The mold count of the 800 g/L silage was below the detected level.

3.2. Aerobic Stability of Alfalfa Silage

The alterations in fermentation parameters during exposure to aerobic conditions are depicted in Table 3. After 4 days of aerobic exposure, there were no significant ($p > 0.05$) changes in the pH of 800 g/L and 700 g/L silages. However, the pH of the 600 g/L silage increased sharply ($p < 0.05$) from 4.49 to 6.82. In all silages, the content of LA and AA gradually decreased; the NH3-N increased during aerobic exposure. On day 6 of aerobic exposure, the following changes were found: the LA contents of the 800 g/L, 700 g/L, and 600 g/L silages decreased by 57.5, 51.6, and 39.2 g/kg DM; the AA contents decreased by 11.4, 16.1, and 20.7 g/kg DM; the NH3-N contents increased by 129, 135, and 153 g/kg TN. The content of PA and BA increased slowly in all silages during aerobic exposure.

Table 3. Changes in pH, NH3-N, organic acid, and ethanol of alfalfa silage during air exposure.

Items	Ensiling Density	Exposure Day				SEM	*p*-Value		
		0	2	4	6		T	D	T × D
pH	600 g/L	4.89 ^{c}	5.06 ^{bc}	5.23 ^{b}	6.82 ^{a}	0.882	<0.001	<0.001	<0.001
	700 g/L	4.52	4.85	5.04	5.65				
	800 g/L	4.30	4.52	4.84	5.14				
NH_3-N (g/kg TN)	600 g/L	116	114	129	153	5.821	0.006	<0.001	0.862
	700 g/L	85.6	104	107	135				
	800 g/L	73.2	108	106	129				
Lactic acid (g/kg DM)	600 g/L	71.6	62.1	54.8	39.2	1.570	<0.001	<0.001	0.022
	700 g/L	75.8	70.8	68.9	51.6				
	800 g/L	78.5	73.9	69.5	57.5				
Acetic acid (g/kg DM)	600 g/L	26.6	20.4	24.3	20.7	1.966	0.009	0.010	0.882
	700 g/L	23.8	15.3	15.9	16.1				
	800 g/L	21.0	15.2	12.4	11.4				
Propionic acid (g/kg DM)	600 g/L	3.42	4.47	5.48	7.00	0.201	0.001	<0.001	0.337
	700 g/L	4.17	5.77	5.80	6.59				
	800 g/L	4.97	6.52	6.75	6.18				
Butyric acid (g/kg DM)	600 g/L	4.46	3.76	3.87	4.27	0.184	0.001	0.001	0.083
	700 g/L	3.75	3.46	3.21	4.04				
	800 g/L	4.81	3.50	3.45	2.57				

Data were means of five replicates. $^{a–c}$ means in the same row followed by different letters are significantly different ($p < 0.05$). DM, dry matter; TN, total nitrogen; SEM, standard error of means; T, treatments; D, aerobic exposure days; T × D, interaction between treatments and aerobic exposure days.

The changes in microbic counts during aerobic exposure are shown in Table 4. The LAB count had a decreasing trend and the AB and yeast counts increased continually in all silages. The 800 g/L silage had the highest LAB count (10.7 $\log_{10}$ cfu/g FW) and the lowest AB and yeast counts (10.1 and 6.32 $\log_{10}$ cfu/g FW) during aerobic exposure.

Table 4. Changes in the microbial counts of alfalfa silage during air exposure.

Items	Ensiling Density	Exposure Day				SEM	*p*-Value		
		0	2	4	6		T	D	T × D
LAB ($\log_{10}$ cfu/g FW)	600 g/L	11.6	13.9	9.54	7.31	0.746	<0.001	<0.001	0.879
	700 g/L	13.8	15.2	10.5	9.64				
	800 g/L	16.4	18.1	12.1	10.7				
AB ($\log_{10}$ cfu/g FW)	600 g/L	7.93 ^{b}	9.17 ^{ab}	10.5 ^{a}	11.7 ^{a}	0.234	<0.001	<0.001	0.520
	700 g/L	7.27 ^{b}	8.62 ^{ab}	9.81 ^{ab}	10.6 ^{a}				
	800 g/L	7.24 ^{c}	8.48 ^{b}	9.58 ^{b}	10.1 ^{a}				
Yeast ($\log_{10}$ cfu/g FW)	600 g/L	5.58 ^{c}	6.07 ^{b}	6.87 ^{ab}	7.93 ^{a}	0.192	<0.001	<0.001	0.638
	700 g/L	5.42 ^{c}	5.25 ^{c}	5.84 ^{b}	6.82 ^{a}				
	800 g/L	4.64 ^{c}	5.31 ^{b}	5.59 ^{b}	6.32 ^{a}				

Data were means of five replicates. $^{a–c}$ means in the same row followed by different letters are significantly different ($p < 0.05$). FM, fresh matter; LAB, Lactic acid bacteria; AB, Acetic acid bacteria. SEM, standard error of means; T, treatments; D, aerobic exposure days; T × D, interaction between treatments and aerobic exposure days.

Figure 1 illustrates the aerobic stability of alfalfa silages. The silage temperatures of the 800 g/L, 700 g/L, and 600 g/L silages were different by more than 2 °C from the ambient temperature after 156, 146, and 111 h during aerobic exposure, respectively.

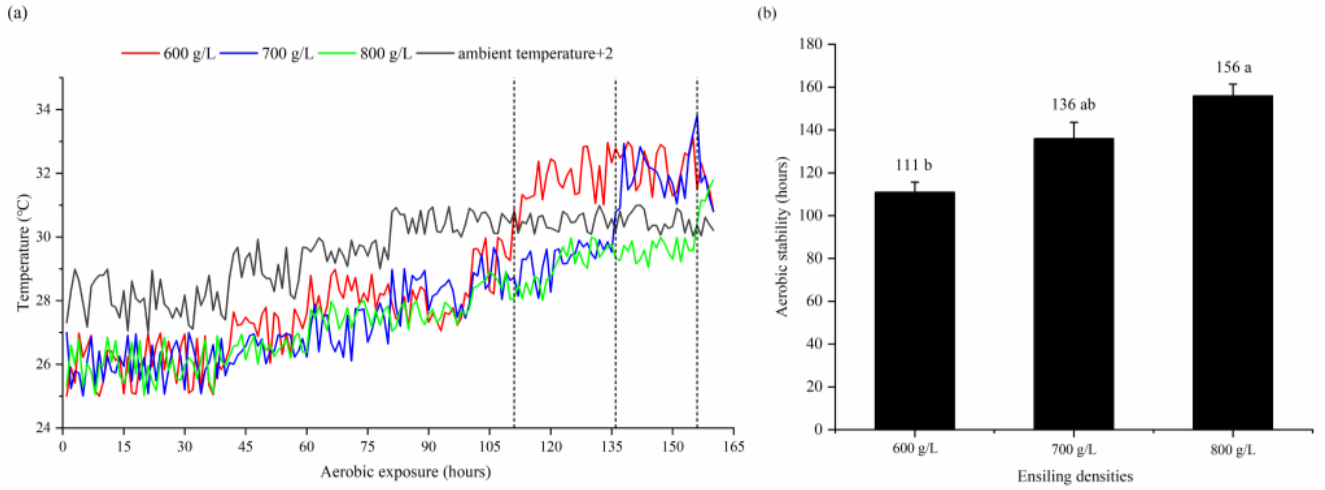


Figure 1. Temperature changes (**a**) and aerobic stability (**b**) of alfalfa silages after exposure to air. ab means different letters are significantly different ($p < 0.05$).

3.3. Dimension Reduction Analyses

Principal component analysis (PCA) was employed to enhance the visualization of the correlation among chemical compositions, microbial counts, and aerobic stability (Figure 2). Principal component 1 (PC1) and principal component 2 (PC2) accounted for 42.6% and 23.4% (totaling 66%) of the total variance, demonstrating that these two principal components effectively captured the sample information. The high density had a higher contribution to the water-soluble carbohydrates content, LAB count, and aerobic stability.

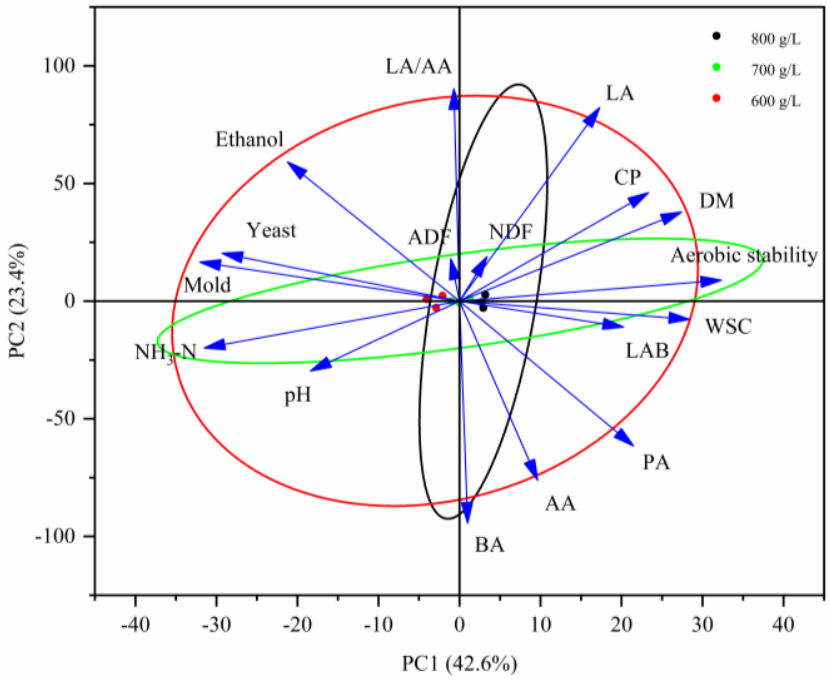


Figure 2. Principal coordinate analysis (PCA) plots for chemical compositions, microbial counts, and aerobic stability of alfalfa silage after 45 days. PC1 explains 42.6% of the variance; PC2 explains 23.4% of the variance. Black triangles are high-density silage; red triangles are low-density silage; green triangles are medium-density silage.

4. Discussion

During the early stage of ensiling, the DM and WSC contents are influenced by the microbial activities and respiration of plant cells [1]. Thus, the higher-density silage had a lower DM loss [7]. The 800 g/L silage had the highest WSC and DM contents; this is due to the fact that high density could reduce the volume of air in the silo, which reduced the consumption of WSC and other nutrients by plant cell respiration and aerobic bacteria [17]. NH_3-N production is indicative of the degree of protein degradation within silage; this reflects the extent of proteolysis and the fact that the NH_3-N content of well-preserved silages should be lower than 80 g/kg TN [1]. The results show that the 800 g/L silage had good conservation, and the high-density silage had the highest CP and the lowest NH_3-N contents. This indicated that the high density inhibited the growth of undesirable bacteria and thus reduced the degradation of proteins [18]. Compared with raw materials, there were differences in CP, NDF, and ADF contents among all silages at 45 days of ensiling; this was probably because the different ensiling conditions stimulated the release of plant enzymes and promoted the degradation of cellulose and hemicellulose [19]. In addition, the ensiling process usually produces a certain temperature, which is conductive to the growth and activity of microorganisms; higher temperatures may promote protein breakdown and volatilization, thus reducing the crude protein content in plant materials [1].

The epiphytic LAB values present on fresh alfalfa exceed the minimum threshold required for fermentation (10^5 cfu/g FW) [20]. The high compaction could promote plant cell rupture and juice exudation and might promote LAB fermentation, quickly producing more LA and leading to a decreased pH [21]. It was found that the 800 g/L silage had a higher LA content and a lower pH than the 700 g/L and 600 g/L silages [22]. The LA/AA values of all silages were found to be above 3; this indicates that the fermentation process was predominantly homolactic [1], resulting in reduced fermentation losses, particularly in silages with high ensiling densities [23]. The research showed the ensiling density could not change the type of fermentation of the silage; silages with different ensiling densities were found to have similar bacterial communities [19]. However, the 800 g/L silage had the highest count of LAB and the lowest count of yeast and mold. This finding indicated that high ensiling density rapidly created an anaerobic environment for the growth of LAB. Meanwhile, the highly acidified environment inhibited the growth of aerobic bacteria and yeast [24]. Kung [23] showed that a lower packing density slowed down the rate of fermentation and acidification; the abundance of yeast was found to increase during ensiling. The Flieg score, derived from the dry matter content and the pH observed in the silages, serves as a method for evaluating the fermentation quality of silage. These factors do not take into account organic acids, the extent of protein degradation, or the aerobic stability of the silage [25]. In the work, the Flieg scores of all silages were higher than 61, indicating that all silages had been well preserved [14].

During the process of opening and feeding silage, air exposure is unavoidable. Aerobic microorganisms such as yeast and mold can proliferate rapidly. These aerobic microorganisms metabolize carbohydrates and organic acids to produce carbon dioxide and water, generating heat and causing a rapid increase in pH [16]. Therefore, changes in pH and temperature are used as crucial indicators of aerobic deterioration. In the experiment, the pH, the contents of NH_3-N and PA, and the counts of AB and yeast increased; meanwhile, the contents of LA and AA and the LAB count decreased in all silages during air exposure. These results showed that all silages experienced varying degrees of aerobic deterioration [26]. The 600 g/L silage exhibited elevated levels of LAB, AA, and yeast compared to the other silages. Among these microorganisms, AA and yeast are primarily responsible for initiating aerobic deterioration in silage, leading to nutrient degradation and the release of noxious odors and harmful substances. Additionally, during aerobic spoilage, mold-produced mycotoxins pose risks to livestock health, potentially impairing liver function and weakening the immune system. Furthermore, through the food chain, these mycotoxins can accumulate and threaten human food safety.

The 800 g/L, 700 g/L, and 600 g/L silages surpassed the ambient temperature by 2 °C after 156, 136, and 111 h, respectively; these results indicated that tightly packed silage was more stable than loosely packed silage [27]. Exposure of silages to air triggers heightened aerobic microbial activity, with lactic-acid-assimilating yeasts initiating the process of aerobic deterioration. This phenomenon culminates in the generation of excessive heat [28]. This corresponds to the results which showed that lactic acid content decreased, and the temperature of the silage mass increased during aerobic exposure. The 800 g/L silage exhibited significantly greater aerobic stability compared to the 700 g/L and 600 g/L silages. This illustrates that high ensiling density correlates with a longer duration of aerobic stability compared with the low ensiling density. This is primarily due to the lower porosity and permeability of high ensiling density, which makes it difficult for oxygen to penetrate in the interior of the silage [29].

In this work, 800 g/L is the maximum capacity of the laboratory silo; the silo is quickly filled, densely packed, and sealed immediately during ensiling to minimize the exposure of forage and silage to air. However, it is difficult to completely exclude air from the silage mass during silo filling and prevent its infiltration during storage in farm-scale silos. Therefore, producers have to know the capacity of their silos to determine the maximum amount of forage that the silos should be filled with. In addition, producers need to pay attention to the degree of crushing for forage; pre-processing treatments such as shredding appear advantageous for the production of high-quality silages [30]. Generally, producers follow the standard operation method to ensure the production of high-quality silage.

5. Conclusions

A high ensiling density effectively enhances the fermentation quality and aerobic stability of silages; this was evidenced by higher dry matter and lactic acid content, along with lower pH and NH_3-N levels. The Flieg score of all alfalfa silages was greater than 60 points, indicating that all silages underwent satisfactory fermentation. In conclusion, the experiment confirms the previous hypothesis, and it was suggested that increasing the ensiling density above 800 g/L was an effective approach for enhancing both the fermentation quality and the aerobic stability of alfalfa silage.

Author Contributions: Conceptualization, H.L.; methodology, F.Y.; software, J.H.; formal analysis, X.L.; investigation, X.L. and F.Y.; resources, J.H.; data curation, J.H.; writing—original draft preparation, H.L.; writing—review and editing, H.L.; visualization, H.L.; supervision, Y.J. and T.S.; project administration, Y.J. and T.S.; funding acquisition, T.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Demonstration of Ecological Grass Husbandry Technology in Tibet High-cold Region (XZ202301YD0012C).

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

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. McDonald, P.; Henderson, A.R.; Heron, S.J.E. *The Biochemistry of Silage*; Chalcomble Publications: Marlow, UK, 1991.
2. Woolford, M.K. The detrimental effects of air on silage. *J. Appl. Bacteriol.* **1990**, *68*, 101–116. [CrossRef] [PubMed]
3. Su, R.N.; Ke, W.C.; Usman, S.; Bai, J.; Kharazian, Z.A.; Guo, X.S. Dry matter content and inoculant alter the metabolome and bacterial community of alfalfa ensiled at high temperature. *Appl. Microbiol. Biotechnol.* **2023**, *107*, 3443–3457. [CrossRef] [PubMed]
4. Gerlach, K.; Ross, F.; Weiss, K.; Büscher, W.; Südekum, K.H. Changes in maize silage fermentation products during aerobic deterioration and effects on dry matter intake by goats. *Agric. Food Sci.* **2013**, *22*, 168–181. [CrossRef]
5. Borreani, G.; Tabacco, E. Quantifying the extent of aerobic deterioration in corn bunker and pile silages at a farm level. In Proceedings of the 15th International Silage Conference, Madison, WI, USA, 27–29 July 2009; pp. 321–322.
6. 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. [CrossRef]

7. Ruppel, K.A.; Pitt, R.E.; Chase, L.E.; Galton, D.M. Bunker Silo Management and Its Relationship to Forage Preservation on Dairy Farms. *J. Dairy Sci.* **1995**, *78*, 141–153. [CrossRef]
8. Yang, L.L.; Yuan, X.J.; Li, J.F.; Dong, Z.H.; Shao, T. Dynamics of microbial community and fermentation quality during ensiling of sterile and nonsterile alfalfa with or without Lactobacillus plantarum inoculant. *Bioresour. Technol.* **2019**, *275*, 280–287. [CrossRef] [PubMed]
9. Nkosi, B.D.; Meeske, R.; Langa, T.; Motiang, M.D.; Modiba, S.; Mkhize, N.R.; Groenewald, I.B. Effects of ensiling forage soybean (*Glycine max* (L.) Merr.) with or without bacterial inoculants on the fermentation characteristics, aerobic stability and nutrient digestion of the silage by Damara rams. *Small Rumin. Res.* **2016**, *134*, 90–96. [CrossRef]
10. Sucu, E.; Kalkan, H.; Canbolat, O.; Filya, I. Effects of ensiling density on nutritive value of maize and sorghum silages. *Rev. Bras. Zootec.-Braz. J. Anim. Sci.* **2016**, *45*, 596–603. [CrossRef]
11. Catchpoole, V.R. Silage and silage-making from tropical herbage species. *Herb. Abstr.* **1971**, *41*, 213–219.
12. Kung, L.; Savage, R.M.; da Silva, E.B.; Polukis, S.A.; Smith, M.L.; Johnson, A.C.B.; Miller, M.A. The effects of air stress during storage and low packing density on the fermentation and aerobic stability of corn silage inoculated with Lactobacillus buchneri 40788. *J. Dairy Sci.* **2021**, *104*, 4206–4222. [CrossRef]
13. Zhao, J.; Dong, Z.H.; Li, J.F.; Chen, L.; Bai, Y.F.; Jia, Y.S.; Shao, T. Ensiling as pretreatment of rice straw: The effect of hemicellulase and *Lactobacillus plantarum* on hemicellulose degradation and cellulose conversion. *Bioresour. Technol.* **2018**, *266*, 158–165. [CrossRef] [PubMed]
14. Abo-Donia, F.M.; Ahmed El-Shora, M.; Abd-Elaziz Riad, W.; Basuony Elgamal, N.; Abdel-Menaem El-Hamady, W. Improve the nutritional value and utilization of rice straw via an ensiling process with different sources of energy and nitrogen enrichment. *J. Appl. Anim. Res.* **2022**, *50*, 333–341. [CrossRef]
15. Zhao, J.; Yin, X.J.; Wang, S.R.; Li, J.F.; Dong, Z.H.; Shao, T. Changes in the fermentation products, taxonomic and functional profiles of microbiota during high-moisture sweet sorghum silage fermentation. *Front. Microbiol* **2022**, *13*, 967624. [CrossRef] [PubMed]
16. Wilkinson, J.M.; Davies, D.R. The aerobic stability of silage: Key findings and recent developments. *Grass Forage Sci.* **2013**, *68*, 1–19. [CrossRef]
17. Sun, L.; Na, N.; Li, X.M.; Li, Z.Q.; Wang, C.; Wu, X.G.; Xiao, Y.Z.; Yin, G.M.; Liu, S.B.; Liu, Z.P.; et al. Impact of Packing Density on the Bacterial Community, Fermentation, and In Vitro Digestibility of Whole-Crop Barley Silage. *Agriculture* **2021**, *11*, 672. [CrossRef]
18. Tian, J.; Xu, N.; Liu, B.; Huan, H.; Gu, H.; Dong, C.; Ding, C. Interaction effect of silo density and additives on the fermentation quality, microbial counts, chemical composition and in vitro degradability of rice straw silage. *Bioresour. Technol.* **2020**, *297*, 122412. [CrossRef] [PubMed]
19. Xu, H.W.; Wu, N.; Na, N.; Sun, L.; Zhao, Y.; Ding, H.J.; Fang, Y.Y.; Wang, T.W.; Xue, Y.L.; Zhong, J. Fermentation weight loss, fermentation quality, and bacterial community of ensiling of sweet sorghum with lactic acid bacteria at different silo densities. *Front. Microbiol.* **2022**, *13*, 1013913. [CrossRef]
20. Ni, K.K.; Wang, F.F.; Zhu, B.G.; Yang, J.X.; Zhou, G.A.; Pan, Y.; Tao, Y.; Zhong, J. Effects of lactic acid bacteria and molasses additives on the microbial community and fermentation quality of soybean silage. *Bioresour. Technol.* **2017**, *238*, 706–715. [CrossRef] [PubMed]
21. Shao, T.; Oba, N.; Shimojo, M.; Masuda, Y. Changes in Mono-and Disaccharides Compositions of Guineagrass (Panicum maximum Jacq.) Silage During Early Stages of Ensiling. *J. Fac. Agric. Kyushu Univ.* **2003**, *47*, 333–339. [CrossRef]
22. Krüger, A.M.; Lima, P.d.M.T.; Abdalla Filho, A.L.; Moro, J.D.G.; De Carvalho, I.Q.; Abdalla, A.L.; Jobim, C.C. Dry matter concentration and corn silage density: Effects on forage quality. *Trop. Grassl.-Forrajes Trop.* **2020**, *8*, 20–27. [CrossRef]
23. Kung, L., Jr. Aerobic stability of silage. In Proceedings of the 2010 California Alfalfa and Forage Symposium and Corn/Cereal Silage Conference, Visalia, CA, USA, 1–2 December 2010.
24. Xie, Y.X.; Wang, L.; Li, W.Q.; Xu, S.Y.; Bao, J.Z.; Deng, J.J.; Wu, Z.; Yu, Z. Fermentation Quality, In Vitro Digestibility, and Aerobic Stability of Total Mixed Ration Silage in Response to Varying Proportion Alfalfa Silage. *Animals* **2022**, *12*, 1039. [CrossRef] [PubMed]
25. Moselhy, M.A.; Borba, J.P.; Borba, A.E.S. Improving the nutritive value, in vitro digestibility and aerobic stability of *Hedychium gardnerianum* silage through application of additives at ensiling time. *Anim. Feed Sci. Technol.* **2015**, *206*, 8–18. [CrossRef]
26. Bayat, J. Effects of microbial inoculant on composition, aerobic stability, in situ ruminal degradability and in vitro gas production of corn silage. *Int. J. Agrisci.* **2012**, *2*, 774–786.
27. Tabacco, E.; Piano, S.; Cavallarin, L.; Bernardes, T.F.; Borreani, G. Clostridia spore formation during aerobic deterioration of maize and sorghum silages as influenced by *Lactobacillus buchneri* and *Lactobacillus plantarum* inoculants. *J. Appl. Microbiol.* **2009**, *107*, 1632–1641. [CrossRef] [PubMed]
28. Kleinschmit, D.H.; Schmidt, R.J.; Kung, L. The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. *J. Dairy Sci.* **2005**, *88*, 2130–2139. [CrossRef]

29. Holmes, B.J.; Muck, R.E. Packing Bunkers and Piles to Maximize Forage Preservation. In Proceedings of the American Society of Agricultural and Biological Engineers Sixth International Dairy Housing Conference Proceeding, Minneapolis, MN, USA, 16–18 June 2007.
30. Samarasinghe, M.B.; Larsen, M.; Johansen, M.; Waldemar, P.; Weisbjerg, M.R. Effects of shredding on silage density and fermentation quality. *Grass Forage Sci.* **2019**, *74*, 244–253. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.