

Effects of Chopping Length and Additive on the Fermentation Quality and Aerobic Stability in Silage of *Leymus chinensis*

Authors:

Zhiqiang Sun, Tingting Jia, Run Gao, Shengyang Xu, Zhe Wu, Bing Wang, Zhu Yu

Date Submitted: 2021-04-29

Keywords: *Lactobacillus plantarum*, aerobic stability, *Leymus chinensis*, fermentation quality, silage processing

Abstract:

The objective of this experiment was to evaluate the effects of the chopping length and additive on the fermentation characteristics and aerobic stability in silage of *Leymus chinensis*. *L. chinensis* was chopped to 1?2 cm and 4?5 cm, and immediately ensiled with the three treatments, i.e., 2% sucrose (fresh weight basis; SU), 1×10^5 cfu/g *Lactobacillus plantarum* (LP) or 1×10^5 cfu/g LP plus 2% sucrose (SU+LP). Silage treated with distilled water served as the control. After silage processing for 30 and 90 d, the fermentation quality of *L. chinensis* silage was evaluated. The composition of the fermentation products and the pH value in the silage were determined at 1, 3, 5 and 7 d after opening the silo. The results showed that in *L. chinensis* silage there was a lower pH value, higher lactic acid content and better aerobic stability at the 1?2 cm length than those at the 4?5 cm ($p < 0.001$). When the chopping length was 4?5 cm, the addition of either LP or SU+LP increased the content of lactic acid and acetic acid, and decreased the pH value and butyric acid content, compared to those of the control and SU treatment ($p < 0.001$). Furthermore, combination treatment of SU+LP performed better than LP alone, and the aerobic stability time of *L. chinensis* silage at 4?5 cm without any additives was the worst. In conclusion, enhanced fermentation quality and aerobic stability can be obtained by processing *L. chinensis* silage with the shorter length. When the *L. chinensis* is cut longer, e.g., 4?5 cm in this study, LP or SU+LP could be used as an effective method to improve the fermentation quality and aerobic stability of *L. chinensis* silage.

Record Type: Published Article

Submitted To: LAPSE (Living Archive for Process Systems Engineering)

Citation (overall record, always the latest version):

LAPSE:2021.0276

Citation (this specific file, latest version):

LAPSE:2021.0276-1

Citation (this specific file, this version):




LAPSE:2021.0276-1v1

DOI of Published Version: <https://doi.org/10.3390/pr8101283>

License: Creative Commons Attribution 4.0 International (CC BY 4.0)

Article

Effects of Chopping Length and Additive on the Fermentation Quality and Aerobic Stability in Silage of *Leymus chinensis*

Zhiqiang Sun ¹, Tingting Jia ¹, Run Gao ¹, Shengyang Xu ¹, Zhe Wu ¹, Bing Wang ² and Zhu Yu ^{1,*}

¹ College of Grassland Science and Technology, China Agricultural University, Beijing 100193, China; BS20193040405@cau.edu.cn (Z.S.); B20163040303@cau.edu.cn (T.J.); gaorun@cau.edu.cn (R.G.); sy20193040640@cau.edu.cn (S.X.); wuzhe@cau.edu.cn (Z.W.)

² State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China; wangb@cau.edu.cn

* Correspondence: 02059@cau.edu.cn; Tel.: +86-010-6273-3414

Received: 12 September 2020; Accepted: 12 October 2020; Published: 14 October 2020



Abstract: The objective of this experiment was to evaluate the effects of the chopping length and additive on the fermentation characteristics and aerobic stability in silage of *Leymus chinensis*. *L. chinensis* was chopped to 1–2 cm and 4–5 cm, and immediately ensiled with the three treatments, i.e., 2% sucrose (fresh weight basis; SU), 1×10^5 cfu/g *Lactobacillus plantarum* (LP) or 1×10^5 cfu/g LP plus 2% sucrose (SU+LP). Silage treated with distilled water served as the control. After silage processing for 30 and 90 d, the fermentation quality of *L. chinensis* silage was evaluated. The composition of the fermentation products and the pH value in the silage were determined at 1, 3, 5 and 7 d after opening the silo. The results showed that in *L. chinensis* silage there was a lower pH value, higher lactic acid content and better aerobic stability at the 1–2 cm length than those at the 4–5 cm ($p < 0.001$). When the chopping length was 4–5 cm, the addition of either LP or SU+LP increased the content of lactic acid and acetic acid, and decreased the pH value and butyric acid content, compared to those of the control and SU treatment ($p < 0.001$). Furthermore, combination treatment of SU+LP performed better than LP alone, and the aerobic stability time of *L. chinensis* silage at 4–5 cm without any additives was the worst. In conclusion, enhanced fermentation quality and aerobic stability can be obtained by processing *L. chinensis* silage with the shorter length. When the *L. chinensis* is cut longer, e.g., 4–5 cm in this study, LP or SU+LP could be used as an effective method to improve the fermentation quality and aerobic stability of *L. chinensis* silage.

Keywords: silage processing; fermentation quality; *Leymus chinensis*; aerobic stability; *Lactobacillus plantarum*

1. Introduction

Leymus chinensis (Trin.) Tzvel., which is commonly known as Chinese wildrye, is a native cool-season perennial grass in the Gramineae tribe and a good forage source for ruminants [1]. Nowadays, it is widely distributed on the Eurasian Steppe, including the eastern Inner Mongolian Plateau and the Songnen Plain in China [2,3]. Fresh tender stems and leaves have good palatability, and higher levels of digestible fiber in *L. chinensis* [4]. Generally, *L. chinensis* is usually processed for hay and silage [5]. The hay preparation includes processing and storage, and during these processes the loss of nutrients can reach 28%–32% [6]. Moreover, hay preparation is easily affected by external factors such as the mowing period, weather and others, and especially rain causes mildew growth and deterioration in forage grass [7,8]. Therefore, processing *L. chinensis* for silage is an effective way

to utilize natural grassland. The processing of silage under sealed anaerobic conditions can reduce the loss of nutrients, which in turn provides high-quality fermented green forage for livestock in winter [9]. However, *L. chinensis* silage is not easily processed for ensiling because of its relatively low concentration of lactic acid bacteria (LAB) and water-soluble carbohydrate (WSC) content during ensiling [1,10]. Moreover, the hollow and hard stems impede the creation of anaerobic conditions for successful ensiling [11]. In addition, the raw material characteristics of *L. chinensis* and mechanical limitations prevent the cutting of *L. chinensis* into short segments, which increases the quantity of oxygen entrapped in the silage and may result in poor silage preservation [12]. *L. chinensis* silage also deteriorates easily when exposed to air [1]. Thus, more efforts are needed to improve the fermentation and aerobic stability of *L. chinensis* silage.

In our previous studies, organic acids, chemical additives and LAB strains were used to improve the fermentation quality and aerobic stability of forage silage [1,10]. It is well documented that homofermentative bacterial inoculants ferment WSC into organic acids, particularly lactic acid (LA), which improve the fermentation of silage [13]. Sucrose is usually used as an additive to improve the fermentation quality of grass silage or to reduce the aerobic deterioration of silage, especially for plants with lower WSC contents [14,15]. Applying a combination of these two types of additives may be more effective than using one type of additive alone [16]. In addition, the cutting length also influences the density of compaction and the followed fermentation characteristics [12,17]. However, to our knowledge, only a few studies have investigated the effects of proper lengths and additives on improving the fermentation quality and suppressing aerobic deterioration of *L. chinensis* silage.

The aim of our study was to detect the effects of chopping length and additives on the fermentation quality of *L. chinensis* silage. Furthermore, the levels of in acidic substances associated with the aerobic stability during the aerobic exposure were also measured.

2. Materials and Methods

2.1. Forage Harvest and Silage Preparation

The heading stage of *L. chinensis* was harvested in July 2018 at Guyuan, Hebei Province, China (41°42′–41°57′ N, 115°32′–115°59′ E, altitude 1430 m). The materials of *L. chinensis* were collected and chopped to 1–2 cm or 4–5 cm, and immediately ensiled with the three treatments, i.e., sucrose at 2% of the silage fresh weight (SU), 1×10^5 cfu/g *Lactobacillus plantarum* KR107060 (LP), which was isolated from *L. chinensis* by Zhang et al. [10] or 1×10^5 cfu/g LP plus sucrose at 2% of the silage fresh weight (SU+LP). At the same time, silages treated with distilled water served as the control. The *L. chinensis* from each pile was packed in 1 L bucket silos and sealed with plastic lids. The targeted packing density was 600 kg/m³. Laboratory silos were stored at room temperature for ensiling 30 d and 90 d. The three replicates were included in each treatment, with 48 silos in all.

2.2. Fermentation Quality and Microbial Counts

Upon silo opening, 20 g of the silage from each bucket was diluted with 180 mL of sterilized distilled water, homogenized for 1 min via 30 pulses of 2 s using a plant tissue crusher (WaringTM-8010S; Waring Laboratory Science, Kendall, TX, USA) and then filtered through four layers of cheesecloth and a qualitative filter paper. The pH of the filtrate was measured with a glass-electrode pH meter (PHS-3C; Shanghai Precision & Scientific Instrument Co. Ltd., Shanghai, China). The filtrate was further processed with a dialyzer of 0.22 µm and then kept at –20 °C for organic acid analysis. The LA, acetic acid (AA), propionic acid (PA) and butyric acid (BA) contents were determined by high-performance liquid chromatography (HPLC; Shimadzu, Tokyo, Japan) [18]. The HPLC conditions were as follows: column, Shodex RSpak KC-811S-DVB gel C (8.0 mm × 30 cm; Shimadzu, Tokyo, Japan); oven temperature, 50 °C; mobile phase, 3 mmol/L HClO₄; flowrate, 1.0 mL/min; injection volume, 5 µL; and detector, SPD-M10AVP. For the microbial counts, 10 g of the sample was ten-fold serially diluted with sterilized saline solution (0.85% NaCl). The numbers of mold and yeast were

counted according to Zhang et al. [10,19]. And the microbial counts were transformed to \log_{10} and presented on a fresh matter (FM) basis.

2.3. Chemical Composition Analyzes

The pre-ensiled materials of *L. chinensis* were collected for chemical analysis. The dry matter (DM) content was determined after oven-drying the samples for 48 h at 65 °C. The dried samples were ground to 1 mm screen using a microplant grinding machine. Using an automatic fiber analyzer (Ankom 2000i full; Ankom Tech Co., Macedon, NY, USA), the neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the methods by Van Soest [20]. The crude protein (CP) content was analyzed by using method of the Association of Official Analytical Chemists [21]. The content of WSC were determined by the anthrone-sulfuric acid method [22].

2.4. Aerobic Stability

After ensiling for 90 d, the plastic buckets were opened, and 300 g of *L. chinensis* silages were placed loosely back into 1-L plastic buckets. The aerobic stability was defined as the number of hours before the silage temperatures increased to 2 °C above the ambient temperature [23]. The ambient temperature and silage temperature were measured at 5-min intervals using a temperature recorder (SMOWO MDL-1048A, Shanghai Tianhe Automation Instrument Co., Ltd., Shanghai, China). Three buckets were used for temperature recording over 7 d of exposure to air. Other silage samples were collected from another three buckets, and the pH value and composition of the fermentation products of the *L. chinensis* silages were determined at 1, 3, 5 and 7 d after silo opening.

2.5. Statistical Analysis

The data were collated in Excel 2010. Duncan's multiple comparison method was used to analyze the two factors of different additives and lengths. Significance was declared at $p < 0.05$. All of the above statistical analyzes were performed with the GLM procedure in SPSS 19.0. The data are presented as the means and standard error of the mean (SEM).

3. Results

3.1. Chemical Composition of Fresh Forage

As shown in Table 1, the DM content of *L. chinensis* before ensiling was 450.06 g/kg, the WSC content was 62.86 g/kg DM, and the CP content was 67.08 g/kg DM. The NDF and ADF contents were 619.75 g/kg DM and 329.62 g/kg DM, respectively.

Table 1. Chemical composition of *L. chinensis* material before ensiling (\pm SD).

Item	<i>L. chinensis</i>
Dry matter (g/kg)	450.06 \pm 0.77
Crude protein (g/kg DM)	67.08 \pm 0.34
Water soluble carbohydrates (g/kg DM)	62.86 \pm 1.59
Neutral detergent fiber (g/kg DM)	619.75 \pm 12.01
Acid detergent fiber (g/kg DM)	329.62 \pm 5.81

SD, standard deviation; DM, dry matter.

3.2. Fermentation Traits of *L. chinensis* Silage for Ensiling 30 d

The fermentation quality of *L. chinensis* silage with different chopping lengths and additives ensiled for 30 d is shown in Table 2. The chopping length and additives both affected the pH value of the silage, and shorter silage lengths resulting in lower pH values ($p < 0.001$). At a length of 1–2 cm, the pH value of *L. chinensis* silage treated with all additives was lower than that of the control ($p < 0.001$). At a length of 5–6 cm, pH value did not differ between the control and SU but decreased under the LP and SU+LP

treatment ($p < 0.001$). The pH values in the SU+LP silages were the lowest at all lengths ($p < 0.001$). The silage of shorter-length *L. chinensis* had a higher LA content compared to the longer-length silage for the SU, LP and control treatments ($p < 0.001$). The LA content of the LP treatment was higher than that of the control for all lengths tested ($p < 0.001$). In contrast, the shorter-length *L. chinensis* silage had a lower AA content compared to the longer-length silage ($p < 0.001$). At the 1–2 cm length, SU+LP increased the AA content of *L. chinensis* silage ($p < 0.001$). At both 1–2 cm and 5–6 cm lengths, there was no significant difference ($p > 0.05$) in the PA and BA contents in SU and SU+LP, compared to the control. At the 1–2 cm length, LP significantly decreased the PA content ($p < 0.05$). At the 5–6 cm length, significant differences were not observed in the PA content ($p > 0.05$). BA was detected in all treatments, but the contents were close to zero.

Table 2. The pH value and fermentation products of *L. chinensis* silage with different chopping lengths and additives after ensiling for 30 d (Dry matter basis).

Item	Length	Additive				SEM	p-Value		
		Control	SU	LP	SU+LP		Length	Additive	Interaction
pH	1–2 cm	4.20 ^{aB}	4.00 ^{bB}	4.06 ^{bB}	3.88 ^{cB}	0.014	<0.001	<0.001	<0.001
	4–5 cm	4.64 ^{aA}	4.58 ^{aA}	4.14 ^{bA}	3.99 ^{bA}				
Lactic acid (g/kg)	1–2 cm	32.53 ^{bA}	40.20 ^{bA}	51.77 ^{aA}	38.50 ^b	0.801	<0.001	<0.001	<0.001
	4–5 cm	4.60 ^{bB}	3.77 ^{bB}	34.36 ^{aB}	37.03 ^a				
Acetic acid (g/kg)	1–2 cm	6.37 ^{bB}	3.53 ^{bB}	5.97 ^{bB}	13.50 ^a	0.433	<0.001	0.007	0.002
	4–5 cm	18.70 ^A	19.83 ^A	21.90 ^A	19.33				
Propionic acid (g/kg)	1–2 cm	10.47 ^a	8.87 ^b	7.90 ^{bB}	9.33 ^{ab}	0.186	0.017	0.589	0.008
	4–5 cm	9.57	9.70	11.27 ^A	9.97				
Butyric acid (g/kg)	1–2 cm	1.23	1.53	1.76	1.56	0.212	0.672	0.235	0.678
	4–5 cm	0.70 ^b	1.00 ^{ab}	2.63 ^a	1.03 ^{ab}				

Means with in the same row (^{a–c}) or with in the same column (^{A,B}) with different superscripts differ significantly ($p < 0.05$); SU, sucrose; LP, *Lactobacillus plantarum*; SEM, standard error of the mean.

3.3. Fermentation Traits of *L. chinensis* Silage for Ensiling 90 d

The fermentation quality of *L. chinensis* silage ensiled for 90 d is shown in Table 3. The addition of LP or SU+LP significantly decreased the pH value, and the pH values of the SU+LP silages were the lowest for all lengths tested ($p < 0.001$). Furthermore, similar to the case for silage ensiled for 30 d, the silage pH value of the 1–2 cm-length was significantly lower than that of the 4–5 cm-length silage for all the additives ($p < 0.001$). When the chopping length was 1–2 cm, there was no significant effect on the LA content in all treatments ($p > 0.05$). However, at the 4–5 cm length, with the addition of LP and SU+LP, the LA content increased from 12.30 g/kg DM to 30.27 g/kg DM and 26.25 g/kg DM, respectively. Moreover, the silage of both LP and SU+LP had a higher level of AA for all chopping lengths. Treatment with SU+LP resulted in lower PA contents for all lengths ($p < 0.001$). With no additives, differences were not observed in the PA contents for all chopping lengths tested ($p > 0.05$). Compared with silage ensiled for 30 d, higher BA was detected with the addition of SU at all chopping lengths and with no additives at the 4–5 cm length. The yeast counts in *L. chinensis* silage treated with LP were $1 \times 10^{2.71}$ and $1 \times 10^{2.56}$ cfu/g FM at the 1–2 cm length and the 4–5 cm length, respectively. At the 1–2 cm length, lower yeast and mold counts were observed of *L. chinensis* silage compared to the 4–5 cm length. At the 4–5 cm length, SU+LP decreased the yeast and mold counts of *L. chinensis* silage compared to the control.

Table 3. The pH value and fermentation products of *L. chinensis* silage with different chopping lengths and additives after ensiling for 90 d (Dry matter basis).

Item	Length	Additive				SEM	p-Value		
		Control	SU	LP	SU+LP		Length	Additive	Interaction
pH	1–2 cm	4.11 ^{aB}	4.07 ^{aB}	4.00 ^b	3.85 ^c	0.028	<0.001	<0.001	<0.001
	4–5 cm	4.94 ^{aA}	4.61 ^{aA}	4.01 ^b	3.91 ^b				
Lactic acid (g/kg)	1–2 cm	34.72 ^A	27.42 ^A	38.69 ^A	35.44	1.046	<0.001	<0.001	0.054
	4–5 cm	12.30 ^{bB}	7.32 ^{bB}	30.27 ^{aB}	26.25 ^a				
Acetic acid (g/kg)	1–2 cm	8.03 ^b	16.79 ^{aA}	21.44 ^a	8.09 ^{bB}	0.557	0.899	<0.001	<0.001
	4–5 cm	9.67 ^c	4.01 ^{dB}	22.65 ^a	18.62 ^{bA}				
Propionic acid (g/kg)	1–2 cm	10.67 ^a	8.19 ^b	11.10 ^{aA}	6.22 ^c	0.194	0.262	<0.001	0.156
	4–5 cm	9.70 ^a	8.22 ^{ab}	9.45 ^{aB}	7.00 ^b				
Butyric acid (g/kg)	1–2 cm	ND	16.87 ^{aB}	0.38 ^b	ND	3.450	0.072	0.106	0.299
	4–5 cm	33.26	34.69 ^A	ND	ND				
Yeast (Log ₁₀ cfu/g FM)	1–2 cm	<2	<2	2.71	<2				
	4–5 cm	3.63	<2	2.56	<2				
Mold (Log ₁₀ cfu/g FM)	1–2 cm	<2	<2	<2	<2				
	4–5 cm	2.45	<2	2.03	<2				

Means with in the same row ^(a-c) or with in the same column ^(A,B) with different superscripts differ significantly from each other ($p < 0.05$); SU, sucrose; LP, *Lactobacillus plantarum*; ND, no detected; SEM, standard error of means; cfu, colony-forming units; FM, fresh matter.

3.4. Aerobic Stability of *L. chinensis* Silage

The temperature changes of *L. chinensis* silage were examined with the time of aerobic exposure at lengths of 1–2 cm (Figure 1) and 4–5 cm (Figure 2). We found that the temperature of *L. chinensis* silage increased firstly and then decreased with the increased of aerobic exposure time. At the 1–2 cm length, after 120 h of aerobic exposure, the temperature of silage treated with sucrose was 2 °C higher than the ambient temperature. Silage treated with SU+LP and the control almost exceeded the ambient temperature by 2 °C at the same time. Under this test condition, the temperature of *L. chinensis* silage treated with LP was always lower than the ambient temperature by 2 °C after aerobic exposure. At the 4–5 cm length, after 50 h of aerobic exposure, the temperature of *L. chinensis* silage with no additive exceeded the ambient temperature by 2 °C, and after 70 h of aerobic exposure, the highest temperature was observed. The aerobic stability of *L. chinensis* silage can be improved with the addition of LP, SU or SU+LP. When exposed to air, the temperature of *L. chinensis* treated with SU and LP exceeded the ambient temperature by 2 °C after 120 h and 160 h, respectively. A factorial analysis revealed that the length, additive and their interactions had significant effects ($p < 0.001$) on the aerobic stability and the time required to reach the maximum temperature of *L. chinensis* silage (Table 4).

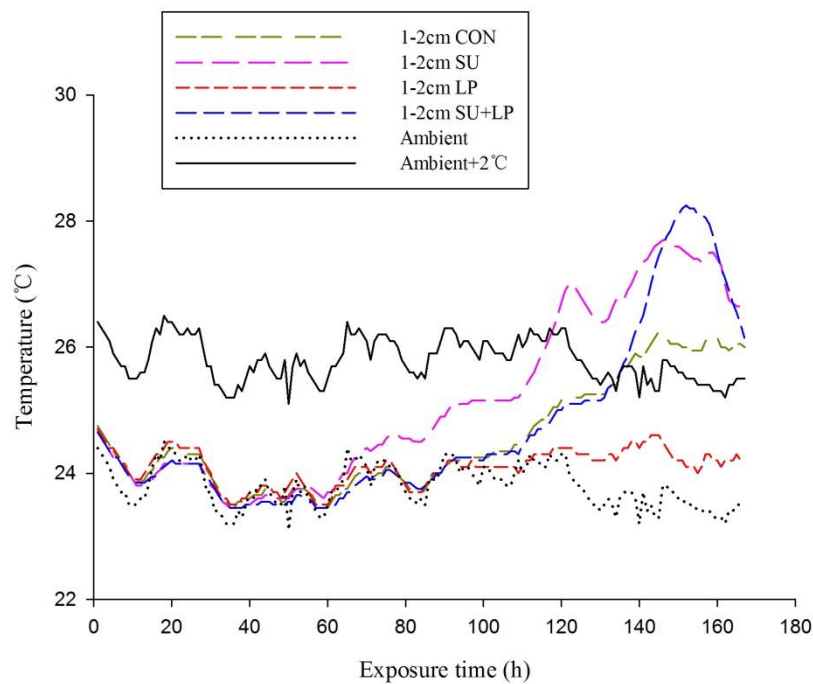


Figure 1. The temperature changes of *L. chinensis* silage at a length of 1–2 cm with the time of aerobic exposure. CON, control; SU, sucrose; LP, *Lactobacillus plantarum*.

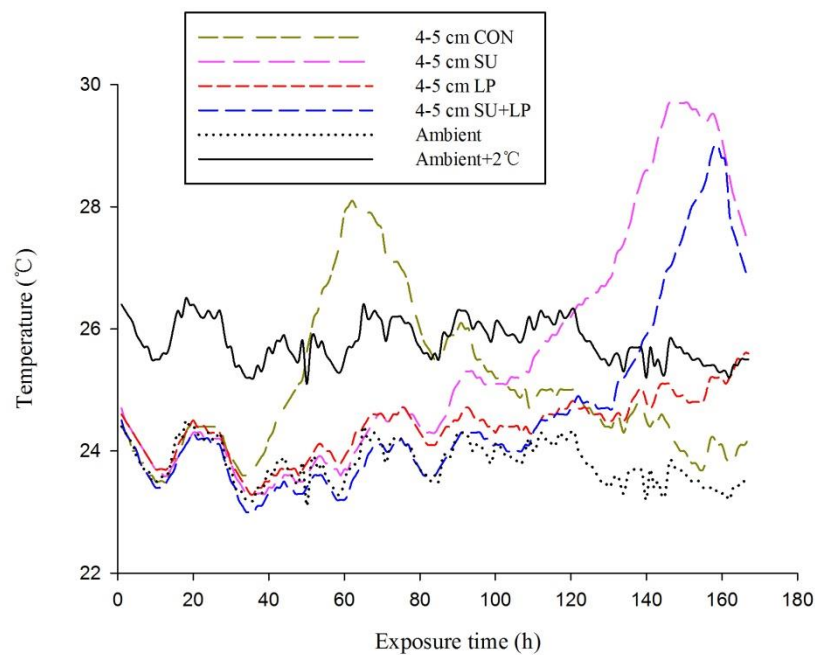


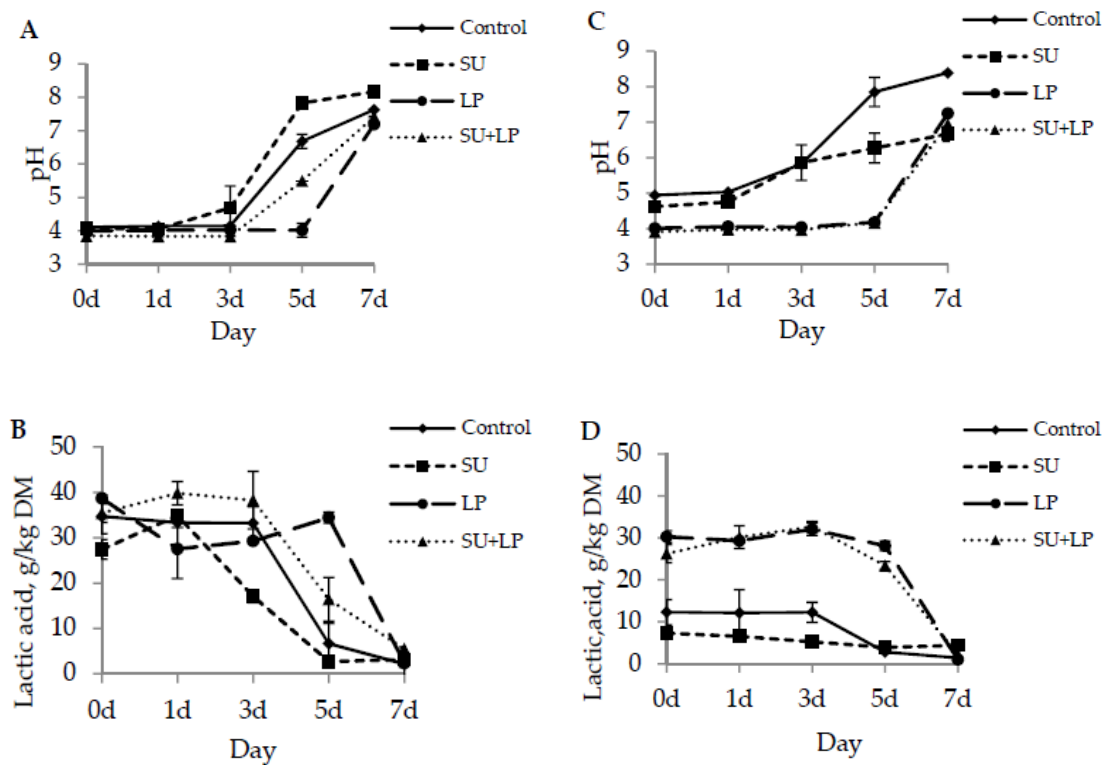
Figure 2. The temperature changes of *L. chinensis* silage at a length of 4–5 cm with the time of aerobic exposure. CON, control; SU, sucrose; LP, *Lactobacillus plantarum*.

Table 4. Significant analyzes of length, additive, and their interactions on aerobic stability, maximum temperature and the time to maximum temperature of *L. chinensis* silage.

	Treatments	Aerobic Stability (h)	Maximum Temperature (°C)	Time to Maximum Temperature (h)
	Length	<0.001	0.088	<0.001
<i>p</i> -Value	Additive	<0.001	0.010	<0.001
	Interaction	<0.001	0.825	<0.001

3.5. Changes of pH Value and Acidic Substances of *L. chinensis* Silage

The dynamic changes in the pH value and LA content during the process of aerobic exposure of *L. chinensis* silage with different chopping lengths and different additives are given in Figure 3. At 1–2 cm, with no additives, the pH value remained stable after the silo had been opened for 3 d, and it increased 5 d after exposure to air. With the addition of LP, the pH value did not change after the silo had been opened for 5 d, but it increased 7 d after exposure to air (Figure 3A); after exposure to air for 3 d, the LA content of the control silage with no additives and the LP- and SU+LP-treated silage remained stable. With the addition of SU, the LA content decreased from 27.42 g/kg DM to 17.12 g/kg DM. Additionally, the LA content was close to 0 during the 7-d spoilage test for all treatments (Figure 3B). At 4–5 cm, with the addition of LP or SU+LP, the pH value was lower compared to the control and remained stable after the silo had been opened for 5 d and increased 7 d after exposure to air. With the addition of SU or with no additives, the pH value tended to increase after 3 d of exposure to air (Figure 3C); the LA content of *L. chinensis* silage treated with LP or SU+LP was higher than that of the control or silage treated with SU on 0 to 5 d of aerobic exposure. After aerobic exposure for 7 d, the LA content was close to 0 for all treatments (Figure 3D).

**Figure 3.** Dynamic changes in the pH value during aerobic exposure of *L. chinensis* silage treated with different additives at 1–2 cm length (A) and 4–5 cm length (C). Dynamic changes in the lactic acid content during aerobic exposure of *L. chinensis* silage with different additives at 1–2 cm length (B) and 4–5 cm length (D). SU, sucrose; LP, *Lactobacillus plantarum*; DM, dry matter.

The dynamic changes in the acetic acid, propionic acid and butyric acid contents during aerobic exposure of *L. chinensis* silage with different chopping lengths and different additives are given in Figure 4. At 1–2 cm, the AA content decreased after silo opening during the 7-d spoilage test. After 7 d of aerobic exposure, the AA content of *L. chinensis* silage treated with LP was 11.72 g/kg DM, which in the other treatments was close to 0 (Figure 4A); the PA content fluctuated from 5 g/kg DM to 15 g/kg DM and decreased after 7 d of aerobic exposure (Figure 4B); after exposure to air for 3 d, BA was detected on all the days with the addition of SU. In contrast, BA was not detected at any number of days of aerobic exposure with the addition of LP (Figure 4C). At 4–5 cm, the AA content of *L. chinensis* silage treated with LP and SU+LP was higher than that of control and SU at 0 to 7 d after aerobic exposure. After aerobic exposure for 7 d, the AA content was close to 0 for all treatments (Figure 4D); no regular changes of the PA content were detected after the silo had been opened for 7 d (Figure 4E); BA was detected at 7 d of aerobic exposure in the SU and control, but was not detected in LP and SU+LP treatments at 0 to 5 d of aerobic exposure (Figure 4F).

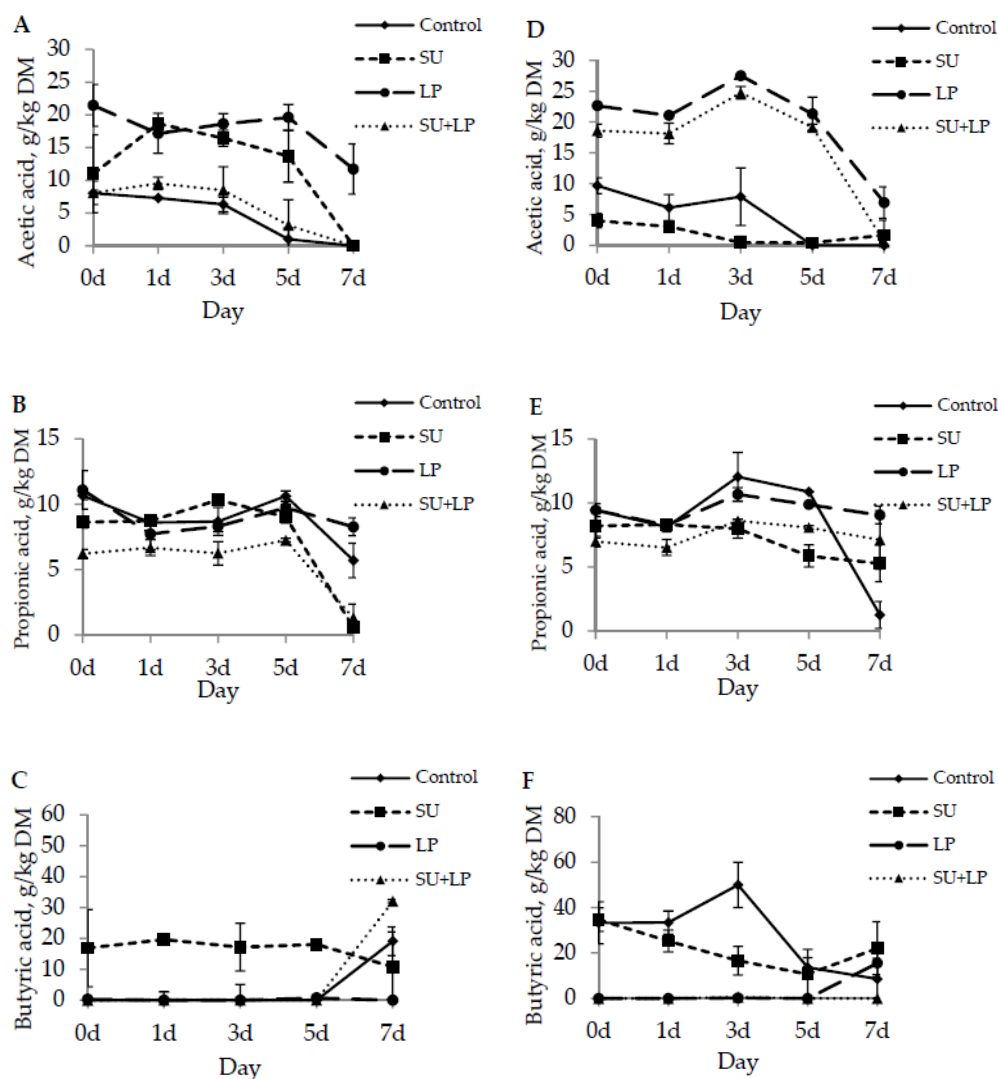


Figure 4. Dynamic changes in the acetic acid content during aerobic exposure of *L. chinensis* silage with different additives at 1–2 cm length (A) and 4–5 cm length (D). Dynamic changes in the propionic acid content during aerobic exposure of *L. chinensis* silage with different additives at 1–2 cm length (B) and 4–5 cm length (E). The dynamic changes in the butyric acid content during aerobic exposure of *L. chinensis* silage with different additives at 1–2 cm length (C) and 4–5 cm length (F). SU, sucrose; LP, *Lactobacillus plantarum*; DM, dry matter.

4. Discussion

The contents of WSC and LAB are critical for successful ensilage. Previous studies showed that with a low WSC content, inoculants will not produce enough LA due to the limitations of the substrates, and the presence of sufficient LAB can convert WSCs into LA and lower the pH value in an anaerobic environment [24,25]. However, previous research also showed that relatively low contents of WSC and LAB were observed in *L. chinensis* [10,26]. Therefore, in order to promote fermentation, it is necessary to supplement WSC or LAB inoculants. In addition, the *L. chinensis* stalk is hollow and hard; thus, removing all the air when making silage is difficult [10]. Compacting and chopping were used to exhaust the air from *L. chinensis* stems to make high-quality *L. chinensis* silage. In our experiments, SU, LP and SU+LP were added to *L. chinensis* silage, which was cut into different lengths. The pH value is an important factor that reflects the silage quality, and a pH value below 4.20 is considered an important key indicator for inhibiting the growth of contaminating microorganisms [27]. For processing silage, the key factors that determine the pH value are the LA and AA contents. Lower pH value and higher LA content were obtained in *L. chinensis* silage with the shorter chopping length, which was consistent with the results of Savoie et al. [12]. Additionally, lower pH value and higher LA content were detected with the SU+LP treatment at all lengths. Moreover, the results of ensiling for 30 d and 90 d were similar. Usually, both biological (mainly consisting of homofermentative LAB) and chemical additives are used to improve silage quality [28,29]. The addition of homofermentative LAB to forage at ensiling can enhance the fermentation process by producing high concentrations of LA and rapidly reducing the pH of the silage [17]. Similar results were found in our experiments. In addition, we found that when the cut length was 4–5 cm, all additives showed positive effects on *L. chinensis* preservation, and LP or SU+LP treatments resulted in lower pH values and higher LA contents. *Lactobacillus buchneri* (LB) were reported to increase the AA content, and *L. plantarum* (i.e., LP in this study) was shown to increase the LA content [23,30]. In our study, inoculation of the *L. chinensis* silage with LP increased the AA content and thereby affected the lactate:acetate ratio, which might be related to the lack of effect of inoculation on certain fermentation end products. However, the increased BA content upon the addition of SU with ensiling for 90 d was difficult to explain, and it may have arisen from *Clostridia* reproduction in the silage [31]. The yeast count of the 4–5 cm length with no additives was $1 \times 10^{3.63}$ cfu/g FM, which was higher than the count in the other treatments. The results showed that the yeast number was inhibited by the length of chopping and additives.

The aerobic deterioration of silage is a significant problem for farm profitability and feed quality throughout the world [32,33]. After the silo was opened, the anaerobic environment became aerobic immediately, and aerobic microorganisms began to reproduce. Generally, the aerobic deterioration of silage is mainly caused by the activities of aerobic microorganisms such as yeast and mold, which use the LA and residual carbohydrates, amino acids and proteins produced by silage fermentation to release heat from the silage and cause the deterioration of aerobic metamorphism [34]. In this study, when the cutting length was 4–5 cm, the yeast and mold levels of *L. chinensis* silage were higher than those in all the other treatments, and the aerobic stability of *L. chinensis* silage was poorest with no additives. Molasses and LAB have been reported to promote resistance to aerobic deterioration [35,36], and similar results were found in this study. When the chopping length was longer, the aerobic stability increased with the addition of SU, LP and SU+LP. In addition, we found that *L. chinensis* silage is resistant to aerobic deterioration for 5–7 d in all treatments at the 1–2 cm chopping length. Zhang et al. [10] investigated the aerobic stability of *L. chinensis* silage at 54.8 h and found that the stability was lower than that observed in our study. This finding may be due to the different moisture contents of the materials. In our experiment, the DM of the *L. chinensis* material was 450.06 g/kg, which was higher than 327.3 g/kg that observed in previous study [10].

In our study, we found that when the chopping length was shorter, the pH value of *L. chinensis* silage in all treatments remained stable after exposure to air for 3 d. The addition of LP increased the AA content only after silo opening. Combined with the influence of the aerobic temperature, the effect of additives on the aerobic stability of *L. chinensis* silage with a shorter length was not apparent.

Many microorganisms are able to aerobically metabolize sugars and LA. The content of LA has been reported to decrease with exposure time [10]. Similar results were observed in our study, and the LA content was close to 0 on the 7th day of aerobic exposure for all treatments. In addition, we found that BA could be detected with the addition of SU. This finding may be related to the use of sugar by *Clostridium* undergoing nutritional reproduction [35]. Considering the high BA content in silage treated with SU alone, SU+LP could be used to inhibit the aerobic deterioration of *L. chinensis* silage. When the chopping length was 4–5 cm, the addition of LP or SU+LP greatly increased the LA content of *L. chinensis* silage during anaerobic fermentation and enhanced AA production. Previous studies found that an increase in the AA content could improve aerobic stability [30]. In our study, when the chopping length was 4–5 cm, the addition of LP or SU+LP led to stable pH values after the silo was opened for 5 d, and the aerobic stabilities were 157 h and 143 h, respectively. Therefore, the enhanced aerobic stability observed in the silage treated with LP or SU+LP can be partly attributed to the increased AA content after the silos were opened [5]. In addition, at the 4–5 cm length, the content of BA was not detected in LP and SU+LP treatments at 0 to 5 d of aerobic exposure, which exhibited low pH values. This might be explained by the growth of microorganisms, such as yeast and coliform bacteria, which were inhibited at low pH values [14,37]. However, the microbial community was not measured in our study, which represents a limitation of the presented work. In the future, the microbial community of *L. chinensis* silage could be explored to determine the changes of acidic substances during ensiling and aerobic exposure.

5. Conclusions

The chopping length affected the fermentation quality on processing of *L. chinensis* silage, and the longer length resulted in negative effects. The lower pH value, higher LA content and better aerobic stability were obtained at 1–2 cm length. The additives of LP or SU+LP could be used to improve the fermentation quality and aerobic stability during processing of *L. chinensis* silage.

In summary, to ensure the high quality of *L. chinensis* silage, the recommended length of *L. chinensis* is 1–2 cm; otherwise, appropriate additives should be provided to improve the fermentation quality and aerobic stability.

Author Contributions: Z.Y. and T.J. designed the study. Z.S. and R.G. analyzed the data. Z.S. and T.J. wrote the manuscript. S.X., Z.W., and B.W. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The work was financially supported by the China Forage and Grass Research System (CARS-34), the Key Technologies Research and Demonstration for Efficient Utilization of Modern Artificial Grassland (2017BY082), and the Demonstration Project of Exploitation and Utilization of High Quality Green and Rough Feed Resources (16190051).

Acknowledgments: We thank Shangang Jia in College of Grassland Science and Technology in China Agricultural University, for his help in the revision of manuscript.

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

References

1. Zhang, Q.; Yu, Z.; Na, R.S. Effects of different additives on fermentation quality and aerobic stability of *Leymus chinensis* silage. *Grass Forage Sci.* **2018**, *73*, 413–419. [[CrossRef](#)]
2. Peng, X.; Zhang, L.; Zhang, L.; Liu, Z.; Cheng, L.; Yang, Y.; Shen, S.; Chen, S.; Liu, G. The transcriptional factor LcDREB2 cooperates with LcSAMDC2 to contribute to salt tolerance in *Leymus chinensis*. *Plant Cell Tiss. Org.* **2013**, *113*, 245–256. [[CrossRef](#)]
3. Chen, S.; Huang, X.; Yan, X.; Liang, Y.; Wang, Y.; Li, X.; Peng, X.; Ma, X.; Zhang, L.; Cai, Y. Transcriptome analysis in sheepgrass (*Leymus chinensis*): A dominant perennial grass of the Eurasian steppe. *PLoS ONE* **2013**, *8*, e67974. [[CrossRef](#)] [[PubMed](#)]

4. Niu, W.; He, Y.; Wang, H.; Xia, C.; Shi, H.; Cao, B.; Su, H. Effects of *Leymus chinensis* replacement with whole-crop wheat hay on blood parameters, fatty acid composition, and microbiomes of Holstein bulls. *J. Dairy Sci.* **2018**, *101*, 246–256. [[CrossRef](#)]
5. Zhang, Q.; Li, X.J.; Zhao, M.M.; Yu, Z. Isolating and evaluating LAB strains for effectiveness of *Leymus chinensis* silage fermentation. *Lett. Appl. Microbiol.* **2014**, *59*, 391–397. [[CrossRef](#)]
6. Bondarev, V.A.; Pobednov, Y.A. Improving technologies for fodder production and storage. *Kormoproizvodstvo* **2001**, *3*, 27–32.
7. McDonald, P.; Henderson, A.R.; Heron, S.J.E. *The Biochemistry of Silage*; Chalcombe Publications: Marlow, UK, 1991; 340p.
8. Coblenz, W.K.; Akins, M.S. Silage review: Recent advances and future technologies for baled silages. *J. Dairy Sci.* **2018**, *101*, 4075–4092. [[CrossRef](#)]
9. Tian, J.; Yu, Y.; Zhu, Y.; Tao, S.; Zhao, M. Effects of LAB inoculants and cellulase on fermentation quality and in vitro digestibility of *Leymus chinensis* silage. *Grassl. Sci.* **2014**, *60*, 199–205. [[CrossRef](#)]
10. Zhang, Q.; Li, X.; Zhao, M.; Yu, Z. Lactic acid bacteria strains for enhancing the fermentation quality and aerobic stability of *Leymus chinensis* silage. *Grass Forage Sci.* **2016**, *71*, 472–481. [[CrossRef](#)]
11. Zhang, Q.; Yu, Z. Characterization, identification and application of LAB isolated from *Leymus chinensis* silage. *Grassl. Sci.* **2017**, *63*, 111–117. [[CrossRef](#)]
12. Savoie, P.; Tremblay, D.; Tremblay, G.F.; Wauthy, J.M.; Flipot, P.M.; Theriault, R. Effect of length of cut on quality of stack silage and milk-production. *Can. J. Anim. Sci.* **1992**, *72*, 253–263. [[CrossRef](#)]
13. Huisden, C.M.; Adesogan, A.T.; Kim, S.C.; Ososanya, T. Effect of applying molasses or inoculants containing homofermentative or heterofermentative bacteria at two rates on the fermentation and aerobic stability of corn silage. *J. Dairy Sci.* **2009**, *92*, 690–697. [[CrossRef](#)] [[PubMed](#)]
14. Heinritz, S.N.; Martens, S.D.; Avila, P.; Hoedtke, S. The effect of inoculant and sucrose addition on the silage quality of tropical forage legumes with varying ensilability. *Anim. Feed Sci. Technol.* **2012**, *174*, 201–210. [[CrossRef](#)]
15. Wu, B.; Nishino, N. Identification and isolation of *Lactobacillus fructivorans* from wilted alfalfa silage with and without molasses. *J. Appl. Microbiol.* **2016**, *120*, 543–551. [[CrossRef](#)] [[PubMed](#)]
16. McEniry, J.; King, C.; O’Kiely, P. Silage fermentation characteristics of three common grassland species in response to advancing stage of maturity and additive application. *Grass Forage Sci.* **2014**, *69*, 393–404. [[CrossRef](#)]
17. Thomson, A.L.; Humphries, D.J.; Kliem, K.E.; Dittmann, M.T.; Reynolds, C.K. Effects of replacing maize silage with lucerne silage and lucerne silage chop length on rumen function and milk fatty acid composition. *J. Dairy Sci.* **2017**, *100*, 7127–7138. [[CrossRef](#)] [[PubMed](#)]
18. Xu, Q.F.; Yu, Z.; Han, J.G. HPLC method for organic acid of alfalfa silages. *Grassl. Turf.* **2007**, *2*, 63–67. [[CrossRef](#)]
19. Zhang, Q.; Yu, Z.; Yang, H.; Na, R.S. The effects of stage of growth and additives with or without cellulase on fermentation and invitro degradation characteristics of *Leymus chinensis* silage. *Grass Forage Sci.* **2016**, *71*, 595–606. [[CrossRef](#)]
20. Vansoest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [[CrossRef](#)]
21. AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed.; The Association: Arlington, VA, USA, 1990.
22. McDonald, P.; Henderson, A.R. Determination of water-soluble carbohydrates in grass. *J. Sci. Food Agric.* **1964**, *15*, 395–398. [[CrossRef](#)]
23. Ranjit, N.K.; Kung, L. The effect of *Lactobacillus buchneri*, *Lactobacillus plantarum*, or a chemical preservative on the fermentation and aerobic stability of corn silage. *J. Dairy Sci.* **2000**, *83*, 526–535. [[CrossRef](#)]
24. Seale, D.R.; Henderson, A.R.; Pettersson, K.O.; Lowe, J.F. The effect of addition of sugar and inoculation with 2 commercial inoculants on the fermentation of lucerne silage in laboratory silos. *Grass Forage Sci.* **1986**, *41*, 61–70. [[CrossRef](#)]
25. Nadeau, E.M.G.; Buxton, D.R.; Russell, J.R.; Allison, M.J.; Young, J.W. Enzyme, bacterial inoculant, and formic acid effects on silage composition of orchardgrass and alfalfa. *J. Dairy Sci.* **2000**, *83*, 1487–1502. [[CrossRef](#)]

26. Xue, Y.; Bai, C.; Sun, J.; Sun, L.; Chang, S.; Sun, Q.; Yu, Z.; Yin, G.; Zhao, H.; Ding, H. Effects of locations and growth stages on nutritive value and silage fermentation quality of *Leymus chinensis* in Eurasian steppe of northern China. *Grassl. Sci.* **2018**, *64*, 40–50. [[CrossRef](#)]
27. Randby, A.T.; Gismervik, K.; Andersen, A.; Skaar, I. Effect of invasive slug populations (*Anon vulgaris*) on grass silage I. Fermentation quality, in-silo losses and aerobic stability. *Anim. Feed Sci. Technol.* **2015**, *199*, 10–19. [[CrossRef](#)]
28. Muck, R.E.; Nadeau, E.M.G.; McAllister, T.A.; Contreras-Govea, F.E.; Santos, M.C.; Kung, L., Jr. Silage review: Recent advances and future uses of silage additives. *J. Dairy Sci.* **2018**, *101*, 3980–4000. [[CrossRef](#)]
29. Queiroz, O.C.M.; Arriola, K.G.; Daniel, J.L.P.; Adesogan, A.T. Effects of 8 chemical and bacterial additives on the quality of corn silage. *J. Dairy Sci.* **2013**, *96*, 5836–5843. [[CrossRef](#)]
30. Danner, H.; Holzer, M.; Mayrhuber, E.; Braun, R. Acetic acid increases stability of silage under aerobic conditions. *Appl. Environ. Microbiol.* **2003**, *69*, 562–567. [[CrossRef](#)]
31. Spoelstra, S.F. Comparison of the content of *Clostridial* spores in wilted grass-silage ensiled in either laboratory, pilot-scale or farm silos. *Neth. J. Agr. Sci.* **1990**, *38*, 423–434. [[CrossRef](#)]
32. Tabacco, E.; Righi, F.; Quarantelli, A.; Borreani, G. Dry matter and nutritional losses during aerobic deterioration of corn and sorghum silages as influenced by different LAB inocula. *J. Dairy Sci.* **2011**, *94*, 1409–1419. [[CrossRef](#)]
33. Wilkinson, J.M.; Davies, D.R. The aerobic stability of silage: Key findings and recent developments. *Grass Forage Sci.* **2013**, *68*, 1–19. [[CrossRef](#)]
34. Hu, W.; Schmidt, R.J.; McDonnell, E.E.; Klingerman, C.M.; Kung, L., Jr. The effect of *Lactobacillus buchneri* 40788 or *Lactobacillus plantarum* MTD-1 on the fermentation and aerobic stability of corn silages ensiled at two dry matter contents. *J. Dairy Sci.* **2009**, *92*, 3907–3914. [[CrossRef](#)] [[PubMed](#)]
35. Jonsson, A. Growth of *clostridium-tyrobutyricum* during fermentation and aerobic deterioration of grass-silage. *J. Sci. Food Agric.* **1991**, *54*, 557–568. [[CrossRef](#)]
36. Ferrero, F.; Piano, S.; Tabacco, E.; Borreani, G. Effects of conservation period and *Lactobacillus hilgardii* inoculum on the fermentation profile and aerobic stability of whole corn and sorghum silages. *J. Sci. Food Agric.* **2019**, *99*, 2530–2540. [[CrossRef](#)]
37. Arriola, K.G.; Kim, S.C.; Adesogan, A.T. Effect of applying inoculants with heterolactic or homolactic and heterolactic bacteria on the fermentation and quality of corn silage. *J. Dairy Sci.* **2011**, *94*, 1511–1516. [[CrossRef](#)]

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 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 (<http://creativecommons.org/licenses/by/4.0/>).