fermentation

MDPI

Article

Effects of *Neolamarckia cadamba* Leaf Extract on Dynamic Fermentation Characteristics and Bacterial Community of *Stylosanthes guianensis* Silage

Peishan Huang [1], Mengmeng Chen [2], Dekui Chen [3], Meiqi Zang [1], Weiling Zhang [1], Xiyue Lin [1], Hongyan Han [4] and Qing Zhang [1,*]

[1] College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou 510642, China; huangpeishan@stu.scau.edu.cn (P.H.); 3063632263@stu.scau.edu.cn (M.Z.); zwl2003@stu.scau.edu.cn (W.Z.); 2242697476@stu.scau.edu.cn (X.L.)
[2] Business School, Yancheng Polytechnic College, Yancheng 224005, China; chenmmeng@stu.scau.edu.cn
[3] College of Animal Science, South China Agricultural University, Guangzhou 510642, China; chendekui1996@163.com
[4] State Key Laboratory of Reproductinve Regulation & Breeding of Grassland Livestock, Inner Mongolia University, Hohhot 010020, China; hyhan1212@imu.edu.cn
* Correspondence: zqing@scau.edu.cn

Abstract: This study focused on exploring the effects of *Neolamarckia cadamba* leaf extract (NE) on the fermentation process and bacterial community of stylo (*Stylosanthes guianensis*) silage. Fresh raw materials of stylo were ensiled with 0%, 1%, and 2% NE, and various fermentation parameters, nitrogen components, and microbial compositions were analyzed at different time points (days 3, 7, 14, and 30) during the ensiling process. The experiment showed that, in comparison to the control group, incorporating NE into the ensiling process resulted in improved fermentation parameters, including increased lactic acid and acetic acid levels, as well as decreased pH, coliform population, and ammonia nitrogen concentration. Moreover, the relative abundances of *Lactobacillus* and *Pediococcus* were augmented, while the growth of Enterobacter was inhibited by the NE addition. These results suggest that NE has potential as a novel additive for silage, promoting a reduction in harmful bacteria and enhancements in the nutritional quality and fermentation characteristics of stylo silage.

Keywords: *Neolamarckia cadamba*; plant extracts; silage; fermentation characteristics; bacterial community

Citation: Huang, P.; Chen, M.; Chen, D.; Zang, M.; Zhang, W.; Lin, X.; Han, H.; Zhang, Q. Effects of *Neolamarckia cadamba* Leaf Extract on Dynamic Fermentation Characteristics and Bacterial Community of *Stylosanthes guianensis* Silage. *Fermentation* **2024**, *10*, 347. https://doi.org/10.3390/fermentation10070347

Academic Editor: Tao Shao and Fuyu Yang

Received: 10 April 2024
Revised: 31 May 2024
Accepted: 11 June 2024
Published: 2 July 2024

1. Introduction

Stylo (*Stylosanthes guianensis*), also called "Tropical alfalfa", is a very popular legume forage native to Central and South America, and it is widely used in the tropics and subtropics for its high yield and protein content [1,2]. A common way to preserve stylo in humid tropical and subtropical areas is through silage [3]. Stylo silage quality is poor in the absence of additives, as reported by Liu et al. [4]. High buffering capacity, low water-soluble carbohydrates (WSCs), and fewer lactic acid bacteria limit the fermentation of legume feed such as stylo [5]. Lactic acid bacteria, molasses, cellulase, organic acids, and some new additives are used in silage to improve silage preservation [6–8].

Plant extracts are natural active substances derived from plants, with antioxidant, anti-inflammatory, and flora-regulating functions, and various applications in medicine, food, and other industries [9–11]. Many studies have shown that plant extracts have good performance in improving the meat quality of animal products, enhancing the immune function of animals, and improving animal growth and development. They also have an excellent effect in reducing greenhouse gas emissions from animals, making them important feed additives [12–14]. In addition, research has found that some plant extracts improve silage fermentation and bacterial communities. For example, ellagic acid enhances

fermentation quality and protein retention, while ethanol extracts of oregano and thyme reduce mycotoxin levels in maize silage [15,16].

Typically found in tropical and subtropical regions, *Neolamarckia cadamba* is fast-growing [17]. *Neolamarckia cadamba* leaves have been studied as a woody forage in recent years due to their rapid growth, high protein content, and high biomass [18]. Moreover, the leaves are rich in alkaloids, flavonoids, tannins, and phenolic acids, which have antioxidant, antibacterial, anticancer, and therapeutic effects on various diseases [19–21]. Some phytochemicals, such as chlorogenic acid, dihydrocadambine, and β-sitosterol, have broad-spectrum antibacterial activity [22]. These extracts have been reported to have significant antibacterial activity against *Acinetobacter*, *Escherichia*, *Klebsiella*, and *Pseudomonas*, which are also abundant in silage [23–25]. As a result, the extraction of natural compounds from *Neolamarckia cadamba* leaves may be beneficial for improving silage quality. There is currently little research on the role of *Neolamarckia cadamba* leaf extract in silage, which requires further investigation.

In this study, it was hypothesized that the addition of *Neolamarckia cadamba* leaf extract (NE) would prevent undesirable fermentation during the ensiling of forage. NE contains rich active ingredients and biological activity, which can help improve silage quality. In order to experiment with this hypothesis, stylo underwent ensiling with either 0%, 1%, or 2% NE added. Assessments were conducted at various intervals (days 3, 7, 14, and 30) to assess the ensiling characteristics, nitrogen fractions, and bacterial community of the ensiling fermentation to evaluate the potential for improving stylo silage quality through NE addition.

2. Materials and Methods

2.1. Preparation of N. cadamba Leaf Powder

The leaves of *N. cadamba* were harvested from an experimental plot located at the South China Agricultural University (113°35′ E, 23°16′ N, Guangzhou, China), and all leaves were air-dried at 30 °C in fume hoods until they reached a constant weight. The dry leaves were ground using a Taisite Instrument Co. grinder (FW100, Tianjin, China) and sifted through a 1 mm screen. The powder was sealed in an enclosed bag and stored in a shaded place until the NE extraction.

2.2. Extraction of NE

The dry leaf powder was thoroughly mixed with 70% methanol solution (solid-to-liquid ratio: 1:30) and extracted in an ultrasonic cleaner tank (JM-15D-40, Shenzhen, China). Afterward, the solvent was filtered using filter paper and the evaporation was carried out at 50 °C and −90 kPa. The concentrated extract was freeze-dried using a Biocool Experimental Instrument Co. lyophilizer (PILot5-8S, Beijing, China) to obtain the final NE extract. The NE extract was sealed in an enclosed bag and stored in a −20 °C refrigerator until silage experiments were conducted.

2.3. Stylo Silage Preparation

The fresh stylo (CIAT 184) was harvested from the above-mentioned experimental plot. The forage materials were chopped into pieces of around 2 cm using a forage chopper. The chopped stylo was mixed split into 3 treatments: no additives (CK), 1% NE (NE1), and 2% NE (NE2). NE was added to stylo based on fresh matter (FM) level. All additives were mixed homogenously with stylo, and then approximately 200 g of chopped stylo was put into an vacuum-sealed plastic bag (20 cm × 30 cm), as referenced in Zi et al. [26]. In total, 36 bags (3 treatments × 4 ensiling periods × 3 replicates) were prepared and stored at room temperature (28 to 30 °C). For each treatment, three bags were randomly selected for the analysis of fermentation performance at room temperature after 3, 7, 14, and 30 days.

2.4. Microbial Counts and Chemical Composition Analysis

Referring to the methodology of Wang et al. [2], 20 g of each sample was randomly taken and combined with 180 mL of sterile saline solution and diluted stepwise to 10^{-6} times to obtain 6 gradients of sample extract. The different bacteria and fungi in the samples were counted on specific agar media: Man–Rogosa–Sharpe agar for LAB, Violet Red Bile agar for coliform bacteria, and Rose Bengal agar for yeasts and molds. All the media used in the experiment were purchased from Guangdong Huankai Bio-tech. For every sample, 20 g was added to 80 mL of distilled water. After an overnight refrigeration at 4 °C, filter paper was used to filter the mixture. The pH, organic acid concentrations, and ammonia nitrogen (AN) were measured using the filtrate. The pH of the filtrate was determined using a pH meter. Levels of lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA) were assessed using high-performance liquid chromatography to determine the concentration of organic acids. The determination of ammoniacal nitrogen was carried out following the procedure outlined by Broderick et al. [27].

For the purpose of determining dry matter (DM), each silage sample weighing about 100 g was dried in an oven at 65 °C for 48 h. For subsequent chemical analyses, dried silage samples were ground into powder to pass through a 1 mm screen. The determination of the DM loss was based on the method described by Köhler and colleagues [28]. The anthrone method was used to quantify the content of water-soluble carbohydrates (WSCs) [29]. Crude protein (CP) and true protein (TP) were analyzed using a Kjeldahl nitrogen analyzer(Kjeltec 2300 Auto-Analyser, FOSS Analytical AB, Hoganas, Sweden) and their difference was used to calculate non-protein nitrogen (NPN) content [30]. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed according to the procedures by Van Soest et al. [31].

2.5. Bacterial Community Analyses

Following Yu et al. [32], bacterial DNA was extracted using a soil DNA kit (Magen, Guangzhou, China). The 16S rDNA V3-V4 region was amplified by PCR. According to the manufacturer's instructions, a DNA gel extraction kit (Axygen Biosciences, Union City, CA, USA) was used to extract the amplicons, and these were quantified in an ABI StepOnePlus Real-Time PCR System (Life Technologies, Foster City, CA, USA). The PCR products were purified and sequenced using the Illumina platform. Liu et al.'s [33] method was used to assemble the raw reads.

2.6. Statistical Analysis

Two-way analysis of variance was performed using SPSS v 22.0 software (SPSS Inc., Chicago, IL, USA) to assess the impact of treatments, storage days, and their interaction on stylo silage. Multiple comparisons were performed using Duncan's test, with $p < 0.05$ designating a significant difference. A free web tool (http://www.omicshare.com/tools) was used to analyze the sequencing data and perform correlation analyses, accessed on 10 December 2023. The figures were made using GraphPad Prism 9.3 and OriginPro 9.1 software and further enhanced using Adobe Illustrator 2022 v26.0.1.

3. Results

3.1. Characteristics of Raw Stylo Materials Prior to Ensiling

In Table 1, the characteristics of raw stylo materials are shown. The DM content of stylo at harvest was around 32% FM, and the CP and WSC contents were approximately 10% and 1% DM, respectively. The quantity of lactic acid bacteria (LAB) was similar to that of coliform bacteria, with both exceeding 4 log10 colony-forming units (cfu) g^{-1} FM.

Table 1. The characteristics of silage materials (n = 3, ±SD).

Item	Stylo
DM (% FM)	32.23 ± 0.30
CP (% DM)	10.74 ± 0.45
NDF (% DM)	62.38 ± 0.17
ADF (% DM)	47.01 ± 1.12
WSC (% DM)	1.79 ± 0.06
Lactic acid bacteria (l g cfu·g^{-1} FM)	4.52 ± 0.26
Coliform (lg cfu·g^{-1} FM)	4.80 ± 0.10
Yeasts (lg cfu·g^{-1} FM)	3.32 ± 0.11
Molds (lg cfu·g^{-1} FM)	2.98 ± 0.05

Note: cfu: colony-forming units; SD: standard deviation; DM: dry matter; FM: fresh matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; WSC: water-soluble carbohydrates; the same as below.

3.2. Fermentation Quality and Microbial Population

Table 2 shows that NE and storage days significantly affected DM, DM loss, pH, and counts of LAB and coliform ($p < 0.05$). NE caused a significant decrease ($p < 0.05$) in pH levels and coliform counts on each day of storage. Storage days resulted in significantly increased DM loss and decreased coliform counts in silage at each treatment ($p < 0.05$). There were no molds or yeasts found in the silage during the ensiling process. The LAB count was lower in the treatment group after 30 days of ensiling compared to that after 3 days of ensiling. NE had no significant influence ($p > 0.05$) on DM content after 30 days of silage.

Table 2. The effect of NE on the dynamic fermentation characteristics and microbial population of stylo silage.

Items	Treatments	Storage Days				SEM	*p*-Value		
		3	7	14	30		T	D	T × D
DM (% FM)	CK	32.01 ^{b}	31.98 ^{c}	31.69 ^{b}	32.32	0.86	<0.01	<0.01	0.03
	NE1	33.59 ^{aA}	33.23 ^{bA}	32.01 ^{bB}	32.77 ^{AB}				
	NE2	33.67 ^{a}	34.23 ^{a}	33.25 ^{a}	33.10				
DM loss (%)	CK	0.31 ^{aD}	0.61 ^{aC}	0.96 ^{aB}	1.97 ^{aA}	0.59	<0.01	<0.01	<0.01
	NE1	0.27 ^{abD}	0.49 ^{bC}	0.87 ^{abB}	1.73 ^{bA}				
	NE2	0.22 ^{bD}	0.41 ^{cC}	0.73 ^{bB}	1.65 ^{cA}				
pH	CK	5.42 ^{aA}	5.25 ^{aB}	5.25 ^{aB}	5.09 ^{aC}	0.28	<0.01	<0.01	<0.01
	NE1	5.00 ^{bA}	4.83 ^{bB}	4.75 ^{bBC}	4.71 ^{bC}				
	NE2	5.06 ^{bA}	4.69 ^{cB}	4.56 ^{cC}	4.58 ^{cC}				
Lactic acid bacteria (lg cfu·g^{-1} FM)	CK	8.88 ^{A}	8.40 ^{cAB}	8.81 ^{A}	8.25 ^{bB}	0.35	<0.01	<0.01	<0.01
	NE1	8.89 ^{B}	9.41 ^{aA}	8.81 ^{BC}	8.57 ^{aC}				
	NE2	8.86 ^{B}	9.29 ^{bA}	8.88 ^{B}	8.59 ^{aC}				
Coliform (lg cfu·g^{-1} FM)	CK	7.83 ^{aA}	7.15 ^{aB}	5.45 ^{aC}	2.95 ^{aD}	2.01	<0.01	<0.01	<0.01
	NE1	7.26 ^{bA}	5.76 ^{bB}	3.71 ^{bC}	2.36 ^{bD}				
	NE2	6.73 ^{cA}	5.45 ^{bB}	2.83 ^{cC}	2.20 ^{bD}				
Molds (lg cfu·g^{-1} FM)	CK	<2.00	<2.00	<2.00	<2.00	-	-	-	-
	NE1	<2.00	<2.00	<2.00	<2.00				
	NE2	<2.00	<2.00	<2.00	<2.00				
Yeasts (lg cfu·g^{-1} FM)	CK	<2.00	<2.00	<2.00	<2.00	-	-	-	-
	NE1	<2.00	<2.00	<2.00	<2.00				
	NE2	<2.00	<2.00	<2.00	<2.00				

Note: Significant differences in the same column followed by different lowercase letters ($p < 0.05$); significant differences in the same row followed by different capital letters ($p < 0.05$); T: treatments; D: storage days; SEM: standard error of the mean; the same as below.

3.3. Organic Acids and Nutritional Quality

The effects of NE on organic acids and nutritional quality are shown in Table 3. In addition to crude protein and true protein content, significant effects of NE and storage days on the content of LA, AA, AN, NPN, NDF, and ADF were observed in stylo silage. NE significantly boosted ($p < 0.05$) LA and AA contents and dramatically reduced ($p < 0.05$) the AN content of the silage with each storage day. Storage days dramatically boosted ($p < 0.05$) the AN content of the silage with each treatment. After 30 days of silage, the addition of 2% NE tended to increase the TP content and decrease the NDF and ADF contents compared to the control. In addition, no PA or BA content was detected.

Table 3. The effect of NE on organic acids and nutritional quality of stylo silage.

Items	Treatments	Storage Days				SEM	*p*-Value		
		3	7	14	30		T	D	T × D
LA (% DM)	CK	1.19 ^{b}	1.09 ^{c}	1.13 ^{b}	1.21 ^{b}	0.44	<0.01	<0.01	0.01
	NE1	1.47 ^{aB}	1.49 ^{bB}	2.06 ^{aA}	2.20 ^{aA}				
	NE2	1.63 ^{aB}	1.77 ^{aB}	2.06 ^{aA}	2.23 ^{aA}				
AA (% DM)	CK	0.12 ^{cC}	0.35 ^{cA}	0.19 ^{cB}	0.17 ^{cB}	0.13	<0.01	<0.01	<0.01
	NE1	0.20 ^{bC}	0.45 ^{bA}	0.43 ^{aA}	0.30 ^{bB}				
	NE2	0.39 ^{aB}	0.54 ^{aA}	0.29 ^{bC}	0.41 ^{aB}				
CP (% DM)	CK	10.51 ^{A}	10.36 ^{A}	10.66 ^{A}	9.92 ^{B}	0.43	0.46	<0.01	0.97
	NE1	10.34	10.15	10.55	9.92				
	NE2	10.28 ^{AB}	10.03 ^{AB}	10.70 ^{A}	9.70 ^{B}				
TP (% DM)	CK	7.73 ^{A}	7.24 ^{B}	6.98 ^{B}	6.46 ^{bC}	0.41	0.12	<0.01	0.04
	NE1	7.62 ^{A}	7.14 ^{AB}	7.41 ^{AB}	6.80 ^{abB}				
	NE2	7.43	7.24	7.39	7.29 ^{a}				
NPN (% DM)	CK	2.78 ^{C}	3.12 ^{BC}	3.68 ^{aA}	3.47 ^{AB}	0.45	0.02	0.01	0.14
	NE1	2.72	3.01	3.13 ^{b}	3.13				
	NE2	2.85 ^{AB}	2.79 ^{AB}	3.32 ^{abA}	2.41 ^{B}				
AN (% TN)	CK	2.67 ^{aD}	5.03 ^{aC}	6.95 ^{aB}	8.27 ^{aA}	2.13	<0.01	<0.01	<0.01
	NE1	1.37 ^{bC}	3.04 ^{bB}	3.94 ^{bAB}	4.52 ^{bA}				
	NE2	0.99 ^{bD}	2.17 ^{cC}	2.81 ^{cB}	4.12 ^{bA}				
NDF (% DM)	CK	60.55 ^{aB}	61.72 ^{AB}	60.49 ^{B}	63.64 ^{aA}	1.94	<0.01	<0.01	0.14
	NE1	60.27 ^{aAB}	61.30 ^{AB}	58.05 ^{B}	62.00 ^{abA}				
	NE2	57.45 ^{bB}	59.79 ^{A}	59.32 ^{AB}	59.98 ^{bA}				
ADF (% DM)	CK	48.86 ^{aB}	50.32 ^{AB}	50.90 ^{aA}	49.05 ^{aAB}	2.61	<0.01	<0.01	<0.01
	NE1	46.62 ^{abB}	50.23 ^{A}	44.40 ^{bB}	47.22 ^{abB}				
	NE2	44.97 ^{bBC}	49.05 ^{A}	43.52 ^{bC}	46.51 ^{bAB}				

Note: LA: lactic acid; AA: acetic acid; TP: true protein; NPN: non-protein nitrogen; AN: ammonia nitrogen; TN: total nitrogen; the same as below.

3.4. Microbial Community of Stylo Silage during Ensiling

Table 4 displays the bacterial diversity found in stylo silage. All the treatments had good coverage values exceeding 0.995. In comparison to the CK group, the NE treatment group exhibited higher Chao1 and Ace indices at various storage days, while the Shannon and Simpson indices were comparatively lower. Figure 1 illustrates the differences in the bacterial populations under the various treatments. With longer ensiling days, the variances in the bacterial community between the three treatments became apparent. The NE-treated samples were clearly separated from the CK samples. Principle components 1 (PC1) and 2 (PC2) were partially responsible for 66.42% and 17.94% of the variation.

Table 4. Alpha diversity of bacterial community of ensiled stylo.

Items		Sobs	Shannon	Simpson	Chao1	Ace	Good's Coverage
Storage Days	Treatments						
3	CK	703	4.40	0.90	823	829	0.997
	NE1	812	4.36	0.88	903	918	0.998
	NE2	770	4.03	0.85	914	921	0.998
7	CK	604	4.21	0.89	717	711	0.998
	NE1	723	4.18	0.89	849	857	0.998
	NE2	866	4.48	0.90	986	985	0.998
14	CK	696	4.69	0.91	774	788	0.999
	NE1	699	4.38	0.90	800	804	0.998
	NE2	825	4.42	0.89	901	896	0.999
30	CK	549	4.52	0.91	617	617	0.999
	NE1	676	4.24	0.88	759	748	0.999
	NE2	618	4.24	0.87	669	666	0.999

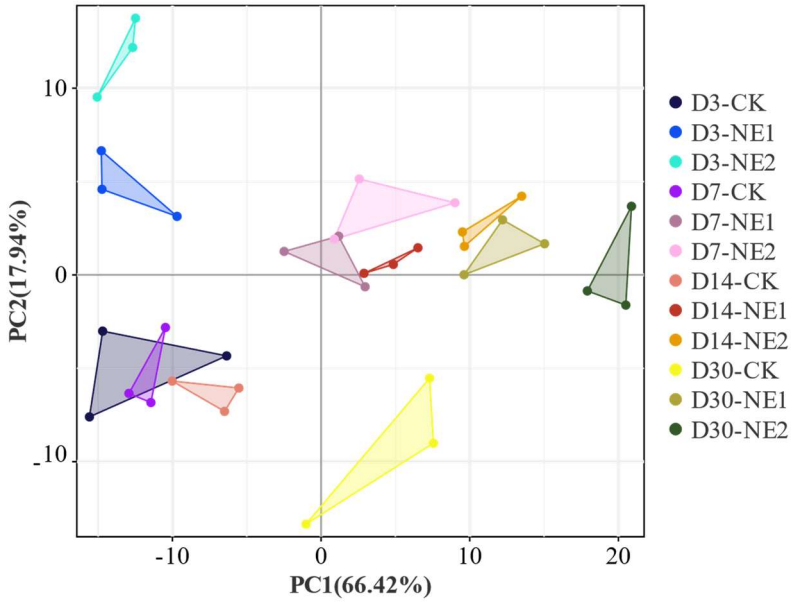

Figure 1. Principal component analysis of bacterial communities for stylo silage (CK: the control; NE1: 1% *Neolamarckia cadamba* leaf extracts; NE2: 2% *Neolamarckia cadamba* leaf extracts; D3, D7, D14, and D30 refer to being stored for 3, 7, 14, and 30 days, respectively).

Figure 2 illustrates the distribution of bacterial communities in the stylo silage, both at the phylum and genus levels. The bacterial communities had similar phylum-level components, including mainly *Firmicutes*, *Proteobacteria*, *Cyanobacteria*, and *Bacteroidetes*. Among all groups, *Firmicutes* were the most abundant bacteria (47.46–61.28%), and their population increased when treated with NE. *Proteobacteria* were the second most abundant (24.77–38.46%), but decreased when treated with NE. As the ensiling days were progressed, there was a decrease in the overall level of *Cyanobacteria*. *Lactobacillus* (4.51–36.73%), *Weissella* (6.68–27.05%), *Enterobacter* (7.59–18.52%), *Pediococcus* (8.05–22.77%), and *Lactococcus* (3.13–13.04%) were the five most dominant bacteria in this study. *Enterobacter* and *Weissella* were the two predominant bacterial genera in the CK group at D3, D7, and D14 of silage. After 30 days of silage, *Enterobacter* (18.52%) and *Lactobacillus* (19.49%) were the two predominant bacterial genera in the CK group. One of the most dominant bacterial genera in the NE group throughout the first 7 days of ensiling was *Weissella*. Beginning on the 14th day of silage, *Pediococcus* and *Lactobacillus* became the dominant bacterial genera in the NE group. Figure 3 clearly demonstrates a decrease in the proportion of *Weissella*

and *Lactococcus*, and an increase in *Lactobacillus*, *Enterobacter*, and *Pediococcus* at D30 compared to D3. Throughout the fermentation process, *Pediococcus* and *Lactobacillus* were more abundant in the NE group compared to the CK group, while *Enterobacter* and *Lactococcus* were less abundant in the NE group than in the CK group. Meanwhile, starting from day 7, the presence of NE resulted in a decline in *Weissella* abundance. As the number of days increased, *Lactobacillus* showed an increasing trend, while *Lactococcus* and *Weissella* showed a decreasing trend. Throughout the fermentation process, the NE treatment had a lower abundance of *Enterobacter* and a higher abundance of *Pediococcus* than the CK treatment.

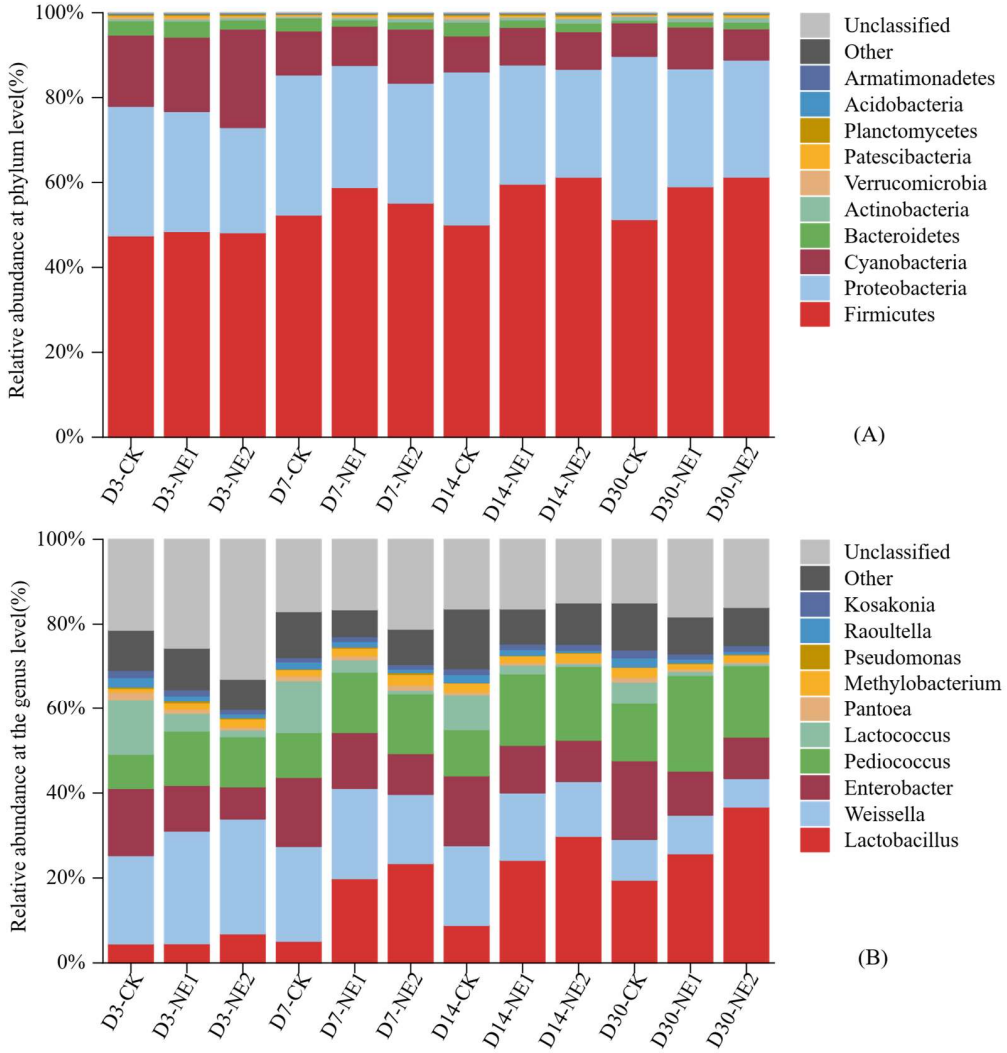

Figure 2. The distribution of bacterial communities based on their phylum (**A**) and genus (**B**) level. (CK: the control; NE1: 1% *Neolamarckia cadamba* leaf extracts; NE2: 2% *Neolamarckia cadamba* leaf extracts; D3, D7, D14, and D30 refer to being stored for 3, 7, 14, and 30 days, respectively).

A correlation heatmap displaying the relationship between the main bacterial genera and silage characteristics is presented in Figure 4. *Enterobacter*, *Weissella*, *Pantoea*, *Raoultella*, and *Lactococcus* all showed a substantial negative connection ($p < 0.001$) with pH, but *Pediococcus* and *Lactobacillus* showed a positive association ($p < 0.001$) with pH. *Enterobacter* and AN had a considerably positive correlation ($p < 0.001$), while *Weissella* and *Pseudomonas* showed a different significantly negative correlation with AN, respectively. LA had a negative connection ($p < 0.001$) with *Enterobacter*, *Weissella*, *Pantoea*, *Raoultella*, and *Lactococcus*, but a substantial positive correlation ($p < 0.001$) with *Pediococcus* and *Lactobacillus*. AA had a different significantly negative correlation with *Lactococcus*, *Raoultella*, and *Enterobacter*, but a positive correlation ($p < 0.05$) with *Lactobacillus*.

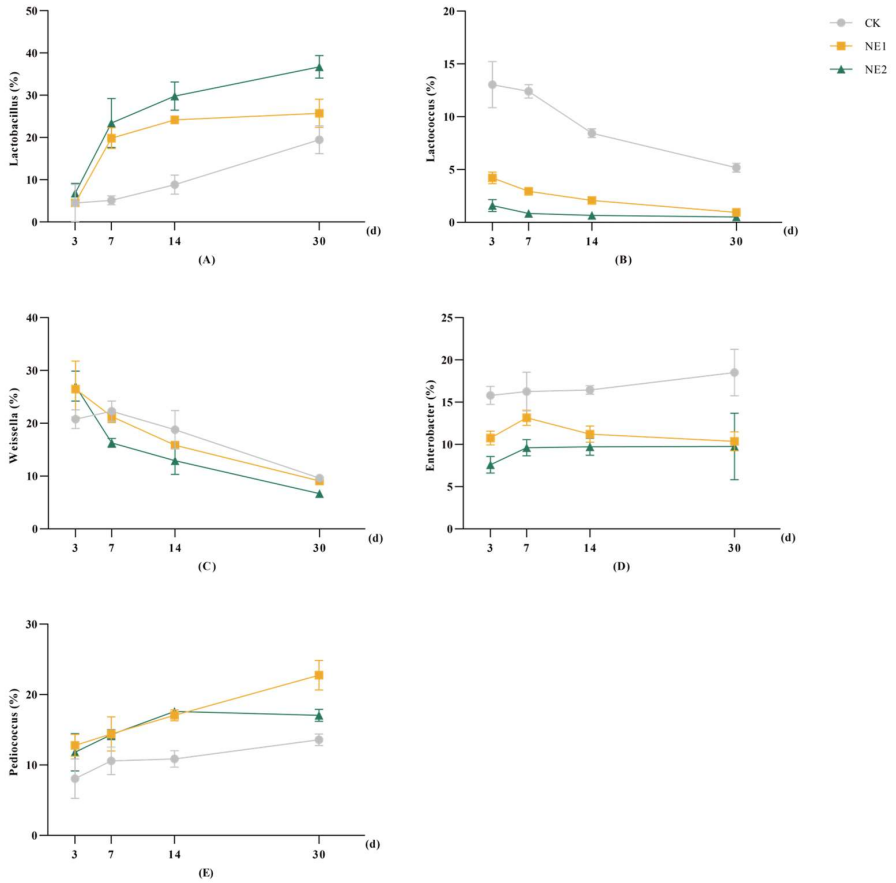

Figure 3. Changes in the relative abundance of five dominant bacterial genera. (**A**–**E**) represent changes in relative abundance of *Lactobacillus*, *Lactococcus*, *Weissella*, *Enterobacter* and *Pediococcus*, respectively. (d) represents storage days.

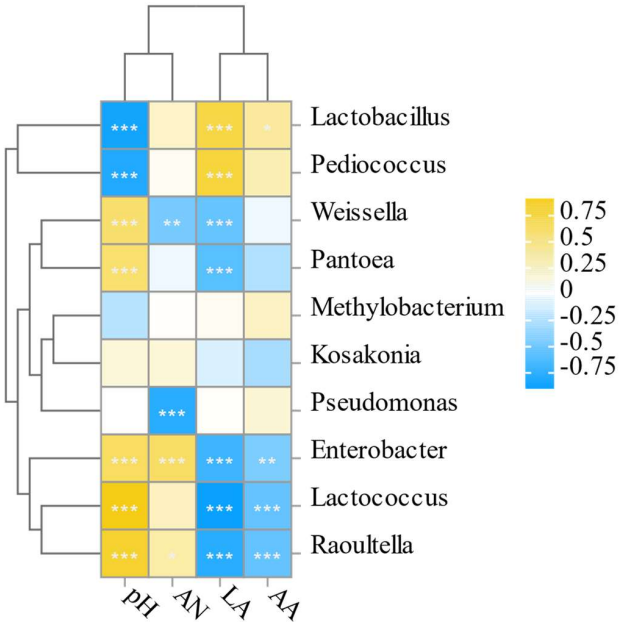

Figure 4. The correlation between microorganisms and fermentation parameters at the genus level. Corresponding value of middle heat map is Spearman correlation coefficient r, which ranges between 1 and −1; r < 0 indicates a negative correlation (blue) and r > 0 indicates a positive correlation (yellow). * significance at $p < 0.05$; ** significance at $p < 0.01$; *** significance at $p < 0.001$.

4. Discussion

4.1. Characteristics of Raw Stylo Materials

The CP content of stylo in the current study was similar to the findings published by Liu and colleagues [34], but less than those of Da et al. [35]. The contents of NDF and ADF were higher than the results reported by Bureenok et al. [36]. This variation could be due to planting conditions, location, and climate. The DM content of stylo was 32.23%, which is in line with the dry matter content requirement for good silage [37]. The ideal DM content promotes lactic acid bacteria growth and inhibits harmful microorganisms, such as *Clostridium*, from fermenting [38]. In addition, the main parameters affecting the quality of silage feed fermentation are WSC content and the quantity of lactic acid bacteria. In general, well-preserved silage feed requires lactic acid bacteria ≥ 5 lg cfu·g^{-1} FM and a WSC content over 5% DM in raw materials [39,40]. The DM content of the raw materials for stylo was relatively optimal for the ensiling process. However, the lactic acid bacteria count was below the standard for quality silage, with an abundance of harmful microorganisms like *Escherichia coli*, and low WSC content. These factors might lead to an inadequate fermentation of stylo silage, necessitating the use of silage additives.

4.2. Microbial Population and Quality of Fermentation in Stylo Silage

Dry matter losses occurred during each of the stages of the ensiling process [41]. This primarily occurred due to plant respiration during the initial stages of silage and microbial metabolism during the middle and later stages [42]. Consequently, it was evident that as the duration of silage increased, the rate of dry matter loss also increased in all treated groups. After 30 days of ensiling, adding NE resulted in a reduction in DM loss and was beneficial for feed preservation in comparison with the CK group. Throughout the ensiling time, groups treated with NE had lower pH values than the CK group, with NE2 causing the greatest pH reduction of all the treatments. According to the findings by Ni et al. [43], pH decline mostly happened within the first 7 to 14 days of ensiling, and no additional substantial pH decrease was seen with extended ensiling time. A pH of less than 4.2 is frequently used to determine if silage is well fermented. In this experiment, the pH of the direct silage of stylo was above 5.0, while the pH of the NE treatment groups was below 5.0 but above 4.2. This could be because lowering the pH value is challenging due to the large buffering capacity [5]. However, the pH of the NE group was within the acceptable pH range (4.3–5.0) for producing leguminous forage silage [44]. The number of lactic acid bacteria almost reached a maximum on the 7th day of silage. The low pH environment may prevent some acid-tolerant lactic acid bacteria from growing, while the absence of fermentable substrate may prevent lactic acid bacteria from being active [45,46]. Following 30 days of ensiling, the inclusion of NE notably enhanced the quantity of lactic acid microbes and facilitated the buildup of lactic acid and acetic acid, which also corresponded to a relatively low pH. Coliform bacteria declined as the duration of ensiling progressed, which was in line with Zou et al.'s [15] findings. In addition, there was a reduction in the number of coliforms with the addition of NE. This might be due to the bacteriostatic effect of NE on coliform [24]. In this experiment, the counts of both molds and yeasts were lower than 2 lg cfu·g^{-1} FM. This may be due to the vacuum-sealed plastic bag creating a good vacuum environment, making the anaerobic conditions in the fermentation environment more adequate, and thus inhibiting the growth of molds and yeasts. Alternatively, it could be because they could not compete with other bacteria in the fermentation environment.

4.3. Organic Acids and Nitrogen Fractions of Stylo Silage

Organic acids in silage are produced mainly by the metabolic activity of microorganisms. Lactic acid bacteria use WSCs to manufacture lactic acid, but some heterofermentative lactic acid bacteria use them to produce acetic acid [47]. In the present study, LA accumulated with increasing days, indicating that lactic acid bacteria dominated the fermentation. Following 30 days of silage, compared to the CK group, LA and AA contents were high in

the NE-treated group. This suggests that the incorporation of NE had a positive impact on the metabolic activities of lactic acid bacteria. Notably, no PA or BA levels were identified. This result was also found in the study by Zou et al. [15]. It is possible that the activities of Clostridium and Propionibacterium were suppressed in the silage environment of this experiment, and therefore PA and BA were not detected. Clostridium metabolism produces butyric acid, which causes DM loss and reduced protein value, while propionic acid production by propionic acid bacteria increases aerobic stability [48]. Therefore, it is beneficial for silage samples to be without butyric acid. Although the absence of propionic acid may result in reduced aerobic stability, the presence of acetic acid may compensate for this [44].

It is well known that plant protein hydrolysis in silage is unavoidable. Proteases and microbes hydrolyze proteins to produce free amino acids, peptides, amides, amines, and ammonia [49]. The CP and TP contents of the treatments tended to decrease with increasing fermenting days. However, the NE treatment group effectively increased the TP content at D30 of the silage. There was a relatively low efficiency of non-protein nitrogen utilization by ruminants compared to the true protein utilization efficiency [50]. The addition of NE tended to reduce the NPN content, but not significantly. Limiting the hydrolysis of proteins could improve the fermentation quality to a certain extent. The ratio of ammonium nitrogen to total nitrogen reflected the degree of protein hydrolysis in the silage [51]. The ratio of ammonia-N to total nitrogen increased with prolonged ensilage time, but it was consistently lower in the NE group than in the CK group. Following 30 days of fermentation, the CK group had an ammonium nitrogen content of 8.27% TN, which exceeded the criterion for quality silage material with an ammoniacal nitrogen content below 8% TN [52]. With the addition of NE, the ammonia-N content was lower than 5% TN in all cases. Furthermore, the NE2 group's true protein content showed a significant increase. This suggests that adding NE helped preserve stylo silage proteins to some extent. This might be attributed to the high concentration of polyphenols, such as tannins, in NE [53]. Tannins affect the deamidation of free amino acids, reduce the conversion of free amino acids, and inhibit the production of ammonia-N, which together restrict proteolysis in ensiled forage [54]. It is worth noting that the addition of NE resulted in a reduction in NDF and ADF content, suggesting that NE might help improve silage digestibility [55].

4.4. Bacterial Diversity of Stylo Silage

The composition and structure of the silage bacterial community have a major impact on the quality of silage fermentation. Understanding the silage fermentation process and the alterations in the bacterial community can be achieved through the analysis of the bacterial community. Nowadays, 16S rDNA sequencing technology is widely used for investigating bacterial communities, including community diversity and microbiological identification. Good's coverage values in this study reached over 99%, suggesting that the majority of microorganisms were sufficiently caught through sequencing. The result was a good representation of the bacterial community. In stylo silage, NE application resulted in an increase in Chao1 and Ace, but a decrease in Shannon and Simpson. This suggests a decline in diversity but an increase in the richness of the bacterial community. Ogunade et al. [56] discovered that microbial communities are less diverse when dominant bacteria are more abundant. It could be inferred that NE increases the richness of dominant bacterial genera and hinders the growth of harmful bacteria. The alterations in bacterial communities were additionally shown by the β-diversity analysis. In stylo silage, samples from the CK group appeared to be isolated from those from the NE-treated groups, indicating that NE had a notable impact on the bacterial community. The addition of NE increased the samples' discreteness, and there was some sample overlap between the two groups that received NE treatment. Similar outcomes were likewise obtained with the addition of GA in the study conducted by He et al. [50]. On the other hand, the samples from the CK group at various times were tightly grouped within a small range. It is proposed that the addition of NE during ensiling is thought to influence the successional process of the bacterial community in stylo silage.

4.5. Bacterial Abundance in Stylo Silage

Proteobacteria, *Firmicutes*, and *Cyanobacteria* were the three phyla that predominated in all of the stylo samples in this investigation, which is in line with other research on stylo silage [15]. During the fermentation of silage, *Firmicutes* and *Proteobacteria* were the most prevalent phyla [57]. After ensiling for 30 days, the relative abundance of *Firmicutes* rose. According to the report by Keshri et al. [58], in settings of anaerobic and acidic fermentation, *Firmicutes* might grow and reproduce more readily. Adding NE reduced the abundance of *Proteobacteria*, which can utilize lactic acid to cause nutrition loss [59]. The use of NE may therefore be beneficial in maintaining silage nutrition. Similar findings were made in the current investigation as well as by He et al. [55], who discovered that extended fermentation decreased the relative abundance of *Cyanobacteria*.

There was found to be a distinction in the bacterial communities during ensiling between the control and NE-treated groups, according to the further analysis of the bacterial population at the genus level. In the early stages of fermentation under anaerobic conditions, lactic acid bacteria, such as *Lactobacillus*, *Pediococcus*, *Weissella*, and *Lactococcus* in the silage, converted WSCs to lactic acid and inhibited enterobacteria, clostridia, and other microorganisms by lowering the pH. This reduced proteolysis and fermentation DM losses [47]. *Lactococcus* and *Weissella* were common lactic acid bacteria abundant during the early stages of fermentation. However, when the pH of the silage dropped, the acid-tolerant *Lactobacillus* progressively took over as the dominant genus [39]. In this investigation, the abundance of *Lactococcus* and *Weissella* reduced with fermentation, whereas *Lactobacillus* eventually became the dominant genus. This demonstrated that extremely acid-tolerant, homofermentative lactic acid bacteria promote the later phases of fermentation. *Lactobacillus* abundance increased significantly with the addition of NE, probably because NE is rich in polyphenols such as phenolic acids and flavonoids, which promote lactic acid bacterial growth and lactic acid concentration [60,61]. It is worth mentioning that *Pediococcus* was more prevalent after NE was added. This may be an indication that the pH may continue to decrease. *Enterobacter* are undesirable microbes in silage because they compete with lactic acid bacteria for fermentation substrate to produce ammonia-N, causing spoilage and lowering nutritional quality [62]. The *Enterobacter* abundance in the CK group increased, while that in the NE group was much lower than that in the CK group. This suggests that NE could successfully stop *Enterobacter* from growing and proliferating. Additionally, the abundance of Enterobacter decreased as the amount of NE increased. The NE group's decreased ammonia-N content was partially explained by the decline in *Enterobacter* abundance. *Pantoea* is also undesirable in silage, and is commonly found in stylo silage [3]. *Pantoea*, however, has reportedly been shown to lower the pH and ammonia-N concentration of silage [56]. More research is needed to determine *Pantoea's* role in stylo silage, despite the fact that its relative abundance in the experiment was quite low. In theory, *Methylobacterium* would be inhibited in prolonged silage due to anaerobic and low pH environments [63]. *Methylobacterium* was found in every sample, which could be a result of the stylo silage's comparatively high pH (>4.5). Wang et al. [2] also reported finding *Kosakonia* in stylo silage, which was classified from the genus *Enterobacter*. Furthermore, this was found to have a positive impact on the distribution of nitrogen by converting molecular nitrogen to ammonia-N, and mostly making protein after that [64]. Due to the potential for biogenic amine production, *Pseudomonas* may be undesirable in silage [65]. The good news is that *Pseudomonas* is not abundant in stylo silage.

4.6. Analysis of the Relationship between the Microbial Community Structure and the Quality of Fermentation in Stylo Silage

The fermentation of silage is a complex biological process heavily influenced by the activity of lactic acid bacteria, which are essential in promoting the production of lactic acid and helping to lower the pH levels of silage [66]. Just like the study mentioned earlier, in this research, we observed an inverse relationship between pH levels and the population of lactic acid bacteria, particularly *Lactobacillus* and *Pediococcus*. These two organisms are

homologous fermentation lactic acid bacteria that excel in fermenting and producing acids, as well as resisting acidic environments [67,68]. A strong positive correlation was observed between pH levels and the presence of *Weissella* and *Lactococcus* in this study. *Weissella* is a weak acid-producing heterologous fermenter, while *Lactococcus* is less acid-tolerant than *Lactobacillus*. Both undergo growth inhibition late in fermentation [58,69]. *Enterobacter* was positively correlated with AN content. This is due to the fact that, in silage, *Enterobacter* is in competition with lactic acid bacteria for the production of ammoniacal nitrogen [62]. Of particular interest was the inverse correlation between the quantities of *Weissella* and *Pseudomonas* and AN content. *Pseudomonas* is one of the undesirable genera in silage, and this study showed the ability to reduce AN content. Consequently, further exploration into the function of *Pseudomonas* in the fermentation process of silage is necessary.

5. Conclusions

This experiment was designed with three treatments, four silage time periods, and three replications to investigate the effects of NE on the dynamics of fermentation quality in stylo. The study demonstrated a decrease in pH value, ammonia-N content, and coliform bacteria count in stylo silage when NE was added. Conversely, there was an increase in lactic acid and acetic acid levels. Additionally, the abundance of *Enterobacter* decreased while lactate-producing bacteria increased. These findings indicate that NE may serve as an innovative supplement for enhancing the fermentation quality of stylo silage. Specifically, the addition of 2% NE showed superior efficacy in enhancing the silage quality of stylo.

Author Contributions: Conceptualization, Q.Z.; investigation, P.H. and M.C.; software, D.C.; validation, M.Z., W.Z. and X.L.; data curation, P.H. and D.C.; writing—original draft preparation, P.H.; writing—review and editing, P.H., M.C. and M.Z.; visualization, W.Z. and X.L.; supervision, H.H.; project administration, Q.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Chimelong Group Research Projects (F110020230509), National Natural Science Foundation of China (32371771) and the Science and Technology Major Project of Inner Mongolia Autonomous Region of China to the State Key Laboratory of Reproductive Regulation and Breeding of Grassland Livestock (2021ZD0048).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

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

References

1. Dong, C.; Liu, P.; Wang, X.; Zhang, W.; He, L. Effects of phenyllactic acid on fermentation parameters, nitrogen fractions and bacterial community of high-moisture stylo silage. *Fermentation* **2023**, *9*, 572. [CrossRef]
2. Wang, C.; Pian, R.; Chen, X.; Lv, H.; Zhou, W.; Zhang, Q. Beneficial effects of tannic acid on the quality of bacterial communities present in high-moisture mulberry leaf and stylo silage. *Front. Microbiol.* **2020**, *11*, 586412. [CrossRef] [PubMed]
3. Tian, J.; Huang, L.; Tian, R.; Wu, J.; Tang, R.; Zhang, J. Fermentation quality and bacterial community of delayed filling stylo silage in response to inoculating lactic acid bacteria strains and inoculating time. *Chemical and Biological Technologies in Agriculture* **2023**, *10*, 44. [CrossRef]
4. Liu, Q.; Chen, M.; Zhang, J.; Shi, S.; Cai, Y. Characteristics of isolated lactic acid bacteria and their effectiveness to improve stylo (*Stylosanthes guianensis* Sw.) silage quality at various temperatures. *Anim. Sci. J.* **2012**, *83*, 128–135. [CrossRef] [PubMed]
5. Denek, N.; Can, A.; Avci, M.; Aksu, T.; Durmaz, H. The effect of molasses-based pre-fermented juice on the fermentation quality of first-cut lucerne silage. *Grass Forage Sci.* **2011**, *66*, 243–250. [CrossRef]
6. Bao, J.; Wang, L.; Yu, Z. Effects of different moisture levels and additives on the ensiling characteristics and in vitro digestibility of *Stylosanthes* silage. *Animals* **2022**, *12*, 1555. [CrossRef] [PubMed]
7. Huang, Y.; Qiu, C.; Wang, Y.; Zhang, W.; He, L. Effect of tea polyphenols on the fermentation quality, protein preservation, antioxidant capacity and bacterial community of stylo silage. *Front. Microbiol.* **2022**, *13*, 993750. [CrossRef] [PubMed]

8. Li, M.; Zhou, H.; Zi, X.; Cai, Y. Silage fermentation and ruminal degradation of stylo prepared with lactic acid bacteria and cellulase. *Anim. Sci. J.* **2017**, *88*, 1531–1537. [CrossRef] [PubMed]
9. Ordonez, Y.F.; Miranda, E.; Lopez, M.F.; Ordonez, P.E. Antibacterial activity of plant extracts against Streptococcus equi subsp. zooepidemicus isolates from guinea pigs with lymphadenitis in Ecuador. *Heliyon* **2024**, *10*, e25226. [CrossRef] [PubMed]
10. Zhou, Y.; Wang, Q.; Wang, S. Effects of rosemary extract, grape seed extract and green tea polyphenol on the formation of N-nitrosamines and quality of western-style smoked sausage. *J. Food Process Pres.* **2020**, *44*, e14459. [CrossRef]
11. Haris, Z.; Ahmad, I. Evaluation of antioxidant-rich bioactive plant extracts with special reference to Moringa oleifera Lam. for their interference to bacterial quorum sensing and biofilm. *Phytomed. Plus* **2024**, *4*, 100511. [CrossRef]
12. Li, S.; Sui, M.; Wu, F.; Chen, X.; Chen, B.; Yao, L. Effects of Chinese herbal plant extracts on diarrhea rate, intestinal morphology, nutrient digestibility, and immunity of weaned piglets. *Can. J. Anim. Sci.* **2023**, *103*, 388–393. [CrossRef]
13. Zhang, Q.; Wu, S.; Zou, X.; Ruan, S.; Kholif, A.E.; Hu, L.; Chen, X.; Zhou, W. Effects of *Neolamarckia cadamba* leaves extract on methanogenesis, microbial community in the rumen and digestibility of stylo silage. *J. Clean. Prod.* **2022**, *369*, 133338. [CrossRef]
14. Adli, D.N.; Sugiharto, S.; Irawan, A.; Tribudi, Y.A.; Wibowo, S.; Azmi, A.; Sjofjan, O.; Jayanegara, A.; Tistiana, H.; Wahyono, T.; et al. The effects of herbal plant extract on the growth performance, blood parameters, nutrient digestibility and carcase quality of rabbits: A meta-analysis. *Heliyon* **2024**, *10*, e25724. [CrossRef] [PubMed]
15. Zou, X.; Chen, D.; Lv, H.; Zhang, Q.; Zheng, P. Effect of ellagic acid on fermentation quality and bacterial community of stylo silage. *Fermentation* **2021**, *7*, 256. [CrossRef]
16. Vaiciuliene, G.; Bakutis, B.; Jovaisiene, J.; Falkauskas, R.; Gerulis, G.; Bartkiene, E.; Klupsaite, D.; Klementaviciute, J.; Baliukoniene, V. Effects of Ethanol Extracts of Origanum vulgare and Thymus vulgaris on the Mycotoxin Concentrations and the Hygienic Quality of Maize (Zea mays L.) Silage. *Toxins* **2022**, *14*, 298. [CrossRef] [PubMed]
17. Lv, Y.; He, Z.; Xiao, Y.; Ouyang, K.; Wang, X.; Hu, X. Population structure and genetic diversity in the natural distribution of *Neolamarckia cadamba* in China. *Genes* **2023**, *14*, 855. [CrossRef] [PubMed]
18. He, L.; Zhou, W.; Wang, Y.; Wang, C.; Chen, X.; Zhang, Q. Effect of applying lactic acid bacteria and cellulase on the fermentation quality, nutritive value, tannins profile and in vitro digestibility of *Neolamarckia cadamba* leaves silage. *J. Anim. Physiol. Anim. Nutr.* **2018**, *102*, 1429–1436. [CrossRef] [PubMed]
19. Ahmed, F.; Rahman, S.; Ahmed, N.; Hossain, M.; Biswas, A.; Sarkar, S.; Banna, H.; Khatun, A.; Chowdhury, M.H.; Rahmatullah, M. Evaluation of *Neolamarckia Cadamba* (Roxb.) bosser leaf extract on glucose tolerance In glucose-induced hyperglycemic mice. *Afr. J. Tradit. Complement. Altern. Med.* **2011**, *8*, 79–81. [CrossRef] [PubMed]
20. Dwevedi, A.; Sharma, K.; Sharma, Y.K. Cadamba: A miraculous tree having enormous pharmacological implications. *Pharmacogn. Rev.* **2015**, *9*, 107–113. [CrossRef]
21. Mishra, A.; Maurya, S.K.; Singh, A.; Siddique, H.; Samanta, S.K.; Mishra, N. *Neolamarckia cadamba* (Roxb.) Bosser (Rubiaceae) extracts: Promising prospects for anticancer and antibacterial potential through in vitro and in silico studies. *Med. Oncol.* **2023**, *40*, 99. [CrossRef] [PubMed]
22. Chandel, M.; Sharma, U.; Kumar, N.; Singh, B.; Kaur, S. Antioxidant activity and identification of bioactive compounds from leaves of *Anthocephalus cadamba* by ultra-performance liquid chromatography/electrospray ionization quadrupole time of flight mass spectrometry. *Asian Pac. J. Trop. Med.* **2012**, *5*, 977–985. [CrossRef] [PubMed]
23. Dubey, D.; Sahu, M.C.; Rath, S.; Paty, B.P.; Debata, N.K.; Padhy, R.N. Antimicrobial activity of medicinal plants used by aborigines of Kalahandi, Orissa, India against multidrug resistant bacteria. *Asian Pac. J. Trop. Biomed.* **2012**, *2*, S846–S854. [CrossRef]
24. Khandelwal, V.; Bhatia, A.K.; Goel, A. Antimicrobial and antioxidant efficacy of aqueous extract of *Anthocephalus cadamba* leaves. *J. Pure Appl. Microbiol.* **2016**, *10*, 209.
25. Rajesh, T.; Roy, A.K.; Erumalla, V.R.; Goli, D.; Basha, S.J. Development and evaluation of antimicrobial ointment formulation containing extracts of *Ocimum sanctum*, *Anthocephalus cadamba*, *Allium sativum* and *Origanum vulgare*. *World J. Pharm. Res.* **2014**, *3*, 398–422.
26. Zi, X.; Liu, Y.; Chen, T.; Li, M.; Zhou, H.; Tang, J. Effects of Sucrose, Glucose and Molasses on Fermentation Quality and Bacterial Community of Stylo Silage. *Fermentation* **2022**, *8*, 191. [CrossRef]
27. Broderick, G.A.; Kang, J.H. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy. Sci.* **1980**, *63*, 64–75. [CrossRef]
28. Köhler, B.; Taube, F.; Ostertag, J.; Thurner, S.; Kluß, C.; Spiekers, H. Dry-matter losses and changes in nutrient concentrations in grass and maize silages stored in bunker silos. *Grass Forage Sci.* **2019**, *74*, 274–283. [CrossRef]
29. McDonald, P.; Henderson, A.R. Determination of water-soluble carbohydrates in grass. *J. Sci. Food Agric.* **1964**, *15*, 395–398. [CrossRef]
30. Licitra, G.; Hernandez, T.M.; Van Soest, P.J. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed. Sci. Tech.* **1996**, *57*, 347–358. [CrossRef]
31. Van Soest, 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] [PubMed]
32. Yu, Q.; Li, M.; Zhang, Y.; Xu, J.; Li, P.; Sun, H.; Xie, Y.; Dong, R.; Zheng, Y.; Chen, C. Effects of Different Cutting Stages and Additives on the Fermentation Quality and Microbial Community of Sudangrass (*Sorghum sudanense* Stapf.) Silages. *Fermentation* **2023**, *9*, 777. [CrossRef]

33. Liu, B.; Huan, H.; Gu, H.; Xu, N.; Shen, Q.; Ding, C. Dynamics of a microbial community during ensiling and upon aerobic exposure in lactic acid bacteria inoculation-treated and untreated barley silages. *Bioresour. Technol.* **2019**, *273*, 212–219. [CrossRef] [PubMed]
34. Liu, Q.; Zhang, J.; Shi, S.; Sun, Q. The effects of wilting and storage temperatures on the fermentation quality and aerobic stability of stylo silage. *Anim. Sci. J.* **2011**, *82*, 549–553. [CrossRef] [PubMed]
35. Da, S.J.; Ribeiro, K.G.; Pereira, O.G.; Mantovani, H.C.; Cecon, P.R.; Pereira, R.C.; Silva, J.L. Nutritive value and fermentation quality of palisadegrass and stylo mixed silages. *Anim. Sci. J.* **2018**, *89*, 72–78. [CrossRef]
36. Bureenok, S.; Sisaath, K.; Yuangklang, C.; Vasupen, K.; Schonewille, J.T. Ensiling characteristics of silages of Stylo legume (*Stylosanthes guianensis*), Guinea grass (*Panicum maximum*) and their mixture, treated with fermented juice of lactic bacteria, and feed intake and digestibility in goats of rations based on these silages. *Small Ruminant Res.* **2016**, *134*, 84–89. [CrossRef]
37. Guyader, J.; Baron, V.; Beauchemin, K. Corn Forage Yield and Quality for Silage in Short Growing Season Areas of the Canadian Prairies. *Agronomy* **2018**, *8*, 164. [CrossRef]
38. Fang, D.; Dong, Z.; Wang, D.; Li, B.; Shi, P.; Yan, J.; Zhuang, D.; Shao, T.; Wang, W.; Gu, M. Evaluating the fermentation quality and bacterial community of high-moisture whole-plant quinoa silage ensiled with different additives. *J. Appl. Microbiol.* **2022**, *132*, 3578–3589. [CrossRef] [PubMed]
39. Cai, Y.; Benno, Y.; Ogawa, M.; Ohmomo, S.; Kumai, S.; Nakase, T. Influence of *lactobacillus* spp. from an inoculant and of *weissella* and *leuconostoc* spp. from forage crops on silage fermentation. *Appl. Environ. Microb.* **1998**, *64*, 2982–2987. [CrossRef] [PubMed]
40. Kung, L.; Stough, E.C.; McDonell, E.E.; Schmidt, R.J.; Hofherr, M.W.; Reich, L.J.; Klingerman, C.M. The effect of wide swathing on wilting times and nutritive value of alfalfa haylage. *J. Dairy. Sci.* **2010**, *93*, 1770–1773. [CrossRef] [PubMed]
41. Borreani, G.; Tabacco, E.; Schmidt, R.J.; Holmes, B.J.; Muck, R.E. Silage review: Factors affecting dry matter and quality losses in silages. *J. Dairy. Sci.* **2018**, *101*, 3952–3979. [CrossRef] [PubMed]
42. Luo, C.; Li, Y.; Chen, Y.; Fu, C.; Long, W.; Xiao, X.; Liao, H.; Yang, Y. Bamboo lignocellulose degradation by gut symbiotic microbiota of the bamboo snout beetle *Cyrtotrachelus buqueti*. *Biotechnol. Biofuels* **2019**, *12*, 70. [CrossRef] [PubMed]
43. Ni, K.; Wang, F.; Zhu, B.; Yang, J.; Zhou, G.; Pan, Y.; Tao, Y.; Zhong, J. Effects of lactic acid bacteria and molasses additives on the microbial community and fermentation quality of soybean silage. *Bioresource Technol.* **2017**, *238*, 706–715. [CrossRef] [PubMed]
44. Kung, L.; Shaver, R.D.; Grant, R.J.; Schmidt, R.J. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *J. Dairy. Sci.* **2018**, *101*, 4020–4033. [CrossRef] [PubMed]
45. Cai, Y.; Kumai, S.; Zhang, J.; Benno, Y. Comparative studies of lactobacilli and enterococci associated with forage crops as silage inoculants. *Anim. Sci. J.* **1999**, *70*, 188–194. [CrossRef]
46. Ren, F.; He, R.; Zhou, X.; Gu, Q.; Xia, Z.; Liang, M.; Zhou, J.; Lin, B.; Zou, C. Dynamic changes in fermentation profiles and bacterial community composition during sugarcane top silage fermentation: A preliminary study. *Bioresource Technol.* **2019**, *285*, 121315. [CrossRef] [PubMed]
47. Muck, R.E.; Nadeau, E.M.G.; McAllister, T.A.; Contreras-Govea, F.E.; Santos, M.C.; Kung, L. Silage review: Recent advances and future uses of silage additives. *J. Dairy. Sci.* **2018**, *101*, 3980–4000. [CrossRef] [PubMed]
48. Bandla, N.; Südekum, K.; Gerlach, K. Review: Role of silage volatile organic compounds in influencing forage choice behavior and intake in ruminants. *Anim. Feed. Sci. Tech.* **2024**, *307*, 115853. [CrossRef]
49. Ohshima, M.; McDonald, P. A review of the changes in nitrogenous compounds of herbage during ensilage. *J. Sci. Food Agric.* **1978**, *29*, 497–505. [CrossRef]
50. He, L.; Chen, N.; Lv, H.; Wang, C.; Zhou, W.; Chen, X.; Zhang, Q. Gallic acid influencing fermentation quality, nitrogen distribution and bacterial community of high-moisture mulberry leaves and stylo silage. *Bioresource Technol.* **2020**, *295*, 122255. [CrossRef] [PubMed]
51. Zhang, H.; Cheng, X.; Elsabagh, M.; Lin, B.; Wang, H. Effects of formic acid and corn flour supplementation of banana pseudostem silages on nutritional quality of silage, growth, digestion, rumen fermentation and cellulolytic bacterial community of Nubian black goats. *J. Integr. Agric.* **2021**, *20*, 2214–2226. [CrossRef]
52. Henderson, N. Silage additives. *Anim. Feed. Sci. Tech.* **1993**, *45*, 35–56. [CrossRef]
53. Qureshi, A.K.; Liew, S.Y.; Othman, N.A.; Awang, K. Phytochemical constituents from *Neolamarckia cadamba* (Roxb.) Bosser. *Biochem. Syst. Ecol.* **2021**, *96*, 104257. [CrossRef]
54. He, L.; Lv, H.; Xing, Y.; Chen, X.; Zhang, Q. Intrinsic tannins affect ensiling characteristics and proteolysis of Neolamarckia cadamba leaf silage by largely altering bacterial community. *Bioresource Technol.* **2020**, *311*, 123496. [CrossRef]
55. Miller, M.D.; Kokko, C.; Ballard, C.S.; Dann, H.M.; Fustini, M.; Palmonari, A.; Formigoni, A.; Cotanch, K.W.; Grant, R.J. Influence of fiber degradability of corn silage in diets with lower and higher fiber content on lactational performance, nutrient digestibility, and ruminal characteristics in lactating Holstein cows. *J. Dairy. Sci.* **2021**, *104*, 1728–1743. [CrossRef] [PubMed]
56. Ogunade, I.M.; Jiang, Y.; Pech Cervantes, A.A.; Kim, D.H.; Oliveira, A.S.; Vyas, D.; Weinberg, Z.G.; Jeong, K.C.; Adesogan, A.T. Bacterial diversity and composition of alfalfa silage as analyzed by Illumina MiSeq sequencing: Effects of *Escherichia coli* O157:H7 and silage additives. *J. Dairy. Sci.* **2018**, *101*, 2048–2059. [CrossRef]
57. Li, S.; Bao, Y.; Lv, M.; Zhang, L.; Liu, L.; Liu, Y.; Lu, Q. Comparative Na+ and K+ profiling reveals microbial community assembly of alfalfa silage in different saline-alkali soils. *Fermentation* **2023**, *9*, 877. [CrossRef]
58. Keshri, J.; Chen, Y.; Pinto, R.; Kroupitski, Y.; Weinberg, Z.G.; Sela Saldinger, S. Microbiome dynamics during ensiling of corn with and without *Lactobacillus plantarum* inoculant. *Appl. Microbiol. Biot.* **2018**, *102*, 4025–4037. [CrossRef] [PubMed]

59. Ridwan, R.; Rusmana, I.; Widyastuti, Y.; Wiryawan, K.G.; Prasetya, B.; Sakamoto, M.; Ohkuma, M. Fermentation Characteristics and Microbial Diversity of Tropical Grass-legumes Silages. *Asian-Australas. J. Anim. Sci.* **2015**, *28*, 511–518. [CrossRef] [PubMed]
60. Huang, S.; Ke, W.; Lu, Q.; Gao, L.; Zhou, X.; Ma, C. Effects of Total Flavonoids from *Taraxacum mongolicum* Hand.-Mazz. on Fermentation Quality, Antioxidant Status and Microbial Community of *Caragana korshinskii* Kom. silage. *Fermentation* **2023**, *9*, 949. [CrossRef]
61. Ke, W.C.; Yang, F.Y.; Undersander, D.J.; Guo, X.S. Fermentation characteristics, aerobic stability, proteolysis and lipid composition of alfalfa silage ensiled with apple or grape pomace. *Anim. Feed. Sci. Tech.* **2015**, *202*, 12–19. [CrossRef]
62. Graf, K.; Ulrich, A.; Idler, C.; Klocke, M. Bacterial community dynamics during ensiling of perennial ryegrass at two compaction levels monitored by terminal restriction fragment length polymorphism. *J. Appl. Microbiol.* **2016**, *120*, 1479–1491. [CrossRef] [PubMed]
63. Wang, C.; He, L.; Xing, Y.; Zhou, W.; Yang, F.; Chen, X.; Zhang, Q. Effects of mixing *Neolamarckia cadamba* leaves on fermentation quality, microbial community of high moisture alfalfa and stylo silage. *Microb. Biotechnol.* **2019**, *12*, 869–878. [CrossRef] [PubMed]
64. Noori, F.; Etesami, H.; Najafi, Z.H.; Khoshkholgh-Sima, N.A.; Hosseini, S.G.; Alishahi, F. Mining alfalfa (*Medicago sativa* L.) nodules for salinity tolerant non-rhizobial bacteria to improve growth of alfalfa under salinity stress. *Ecotoxicol. Environ. Saf.* **2018**, *162*, 129–138. [CrossRef] [PubMed]
65. Dunière, L.; Sindou, J.; Chaucheyras-Durand, F.; Chevallier, I.; Thévenot-Sergentet, D. Silage processing and strategies to prevent persistence of undesirable microorganisms. *Anim. Feed. Sci. Tech.* **2013**, *182*, 1–15. [CrossRef]
66. Chen, L.; Wang, Y.; Li, X.; MacAdam, J.W.; Zhang, Y. Interaction between plants and epiphytic lactic acid bacteria that affect plant silage fermentation. *Front. Microbiol.* **2023**, *14*, 1164904. [CrossRef] [PubMed]
67. Okoye, C.O.; Wang, Y.; Gao, L.; Wu, Y.; Li, X.; Sun, J.; Jiang, J. The performance of lactic acid bacteria in silage production: A review of modern biotechnology for silage improvement. *Microbiol. Res.* **2023**, *266*, 127212. [CrossRef] [PubMed]
68. Wang, X.; Du H; Xu, Y. Source tracking of prokaryotic communities in fermented grain of Chinese strong-flavor liquor. *Int. J. Food Microbiol.* **2017**, *244*, 27–35. [CrossRef]
69. Wang, N.; Wang, Y.; Lin, Y.; Xu, G.; Ni, K.; Yang, F. Effect of lactic acid bacteria and wheat bran on the fermentation quality and bacterial community of Broussonetia papyrifera silage. *Chem. Biol. Technol. Agric.* **2023**, *10*, 130. [CrossRef]