fermentation

MDPI

Article

Effects of Total Flavonoids from *Taraxacum mongolicum* Hand.-Mazz. on Fermentation Quality, Antioxidant Status and Microbial Community of *Caragana korshinskii* Kom. silage

Shuai Huang *, Wencan Ke, Qiang Lu, Longfei Gao, Xiaodong Zhou and Chengyan Ma

College of Forestry and Prataculture, Ningxia University, Yinchuan 750021, China; kewencan@nxu.edu.cn (W.K.); luqiang@nxu.edu.cn (Q.L.); g15109540264@163.com (L.G.); 13619593637@163.com (X.Z.); machengyan68@163.com (C.M.)

* Correspondence: shuaihuang010@nxu.edu.cn

Abstract: The present study aims to investigate effects of total flavonoids from *Taraxacum mongolicum* Hand.-Mazz. (FT) on fermentation quality, antioxidant status, and microbial community of *Caragana korshinskii* Kom. (CK) silage. CK was ensiled with no additive (CON), 1% FT, and 2% FT on a fresh weight (FW) basis for 60 days. The results showed that 1% FT and 2% FT groups displayed higher DM content than CON group, and 2% FT group had the best effect on nutrient preservation. Compared with CON and 1% FT groups, 2% FT group exhibited the best silage fermentation quality and the highest antioxidant activity, including increased lactic acid, acetic acid concentrations, and the activities of antioxidant enzymes, as well as decreased pH and the ammonia nitrogen (NH_3-N) concentration. Moreover, the addition of 2% FT significantly affected the microbial community, such as increased abundance of *Lactobacillus* and decreased abundances of *Pseudomonas* and unidentified *Cyanobacteria*. The abundances of *Lactobacillus parafarraginis* and *Lactobacillus brevis* were negatively correlated to pH, while they were positively correlated with T-AOC, GSH-Px, and CAT activities. In conclusion, 2% FT may be used as additives to promote the fermentation quality and antioxidant activity of CK silage.

Keywords: *Caragana korshinskii* Kom.; flavonoids; *Taraxacum mongolicum* Hand.-Mazz.; fermentation quality; antioxidant status; microbial community

Citation: 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. https://doi.org/10.3390/fermentation9110949

Academic Editor: Qing Zhang

Received: 25 September 2023
Revised: 30 October 2023
Accepted: 30 October 2023
Published: 2 November 2023

1. Introduction

Caragana korshinskii Kom. (CK), a woody legume shrub, is grown in arid and semi-arid regions to help stop desertification and provide environmental protection [1]. However, the overgrowth of CK can result in reduced plant species diversity and regeneration of fresh CK branches [2]. Consequently, routine pruning of older branches is essential, a practice that generates approximately 4 million tons of stubble annually in China [3]. In addition, the expansion of livestock production and the shortage of traditional forage resources necessitate the development of new feed sources. CK, characterized by its high nutritional value, presents a promising option to support livestock production [4]. Cai [5] reported that the preservation of nutrients in woody forage through silage could help address the scarcity of forage resources in arid regions. In general, converting woody plants into naturally high-quality silage proves challenging due to the limited presence of fermentation substrates in legumes [5]. Several studies have indicated that the incorporation of rice bran, *Lactobacillus plantarum*, and molasses to facilitate lactic acid fermentation in *Caragana* silage, creating a valuable high-quality forage resource for livestock production [6,7].

In the context of food safety, one promising dietary approach involves incorporating natural compounds extracted from plants into the diets of ruminants [8]. The usage of metabolites derived from medicinal plants in domestic animals has gained traction in animal research and applications throughout the food production chain [9,10]. As plant

secondary metabolites, flavonoids are known for their antioxidant properties and other bioactivities, such as antimicrobial and anti-inflammatory effects, which contribute to reducing the risk of diseases [11]. Despite their potential benefits, there has been limited research on the utilization of flavonoids from plants in forage silage. Flavonoids and polysaccharides that exist in astragalus and hawthorn residues have been shown to inhibit the growth of various spoilage microorganisms, including *Enterobacter*, and enhance the quality of alfalfa silage [12–14]. Apple pomace and grape pomace are rich in flavonoids and other polyphenols, which inhibited proteolysis and altered the fatty acid composition of ensiled alfalfa [15]. Sea buckthorn pomace also has high flavonoids and other bioactive substances [16], and *L. plantarum* and *L. brevis* could be enriched during alfalfa silage [17]. Thus, flavonoids derived from plants may be beneficial to lactic acid fermentation and the improvement of silage quality in forage.

Taraxacum mongolicum Hand.-Mazz., widely distributed in the warmer temperate zones of the northern hemisphere [18], has a long history of traditional medicinal and dietary use in China [19]. This plant is recognized for its anti-inflammatory, choleretic, antibacterial, anti-allergic, and antioxidant properties, attributed to its bioactive metabolites such as phenolic compounds, sesquiterpene lactones, polysaccharides, and flavonoids [20]. Flavonoids from *Taraxacum mongolicum* Hand.-Mazz. (FT) have antibacterial and antioxidant capabilities [21], and results have shown that FT after fermentation exhibited stronger DPPH free radical scavenging ability and reducing power than that before fermentation [22]. Antioxidants can effectively neutralize excessive free radical production, mitigating oxidative stress when the animal's antioxidant system balance is disrupted due to metabolic disturbances [23,24]. Given this background, we hypothesized that FT could serve as a valuable silage additive to enhance the fermentation quality and antioxidant capacity of CK silage.

To the best of our knowledge, there is limited information available on the application of FT in the ensiling process of CK. Therefore, the objective of this study was to investigate the effects of FT on the fermentation quality, antioxidant status, and microbial community of CK silage.

2. Materials and Methods

2.1. Materials and Silage Preparation

The branch of CK (no inflorescence and leaf) was harvested from Yanchi county, Ningxia, China, in May 2022. After the cutting, the CK branches were broken down and processed into slices less than 1 cm in size using a kneading machine (RC-400, QuFuZhiZao Conveyor Co., Ltd., Qufu, China). The freshly prepared CK material was adjusted to attain a dry matter (DM) content of approximately 40%. Three treatments were applied based on fresh weight (FW): no additive (CON), 1% FT, and 2% FT. The FT (98% total flavonoids; Shaanxi Guanchen Biotech Co., Ltd, Xian, China) used in this experiment was extracted from *Taraxacum mongolicum* Hand.-Mazz. and kept as a fine dry power. The company used ethanol extraction method to extract total flavonoids in *Taraxacum mongolicum* Hand.-Mazz. The whole *Taraxacum mongolicum* Hand.-Mazz. was soaked in 95% ethanol, and the solid–liquid ratio was 1:6 (g:mL). The extraction temperature was set at 75 °C and the extraction time was 2 h.

Subsequently, 500 g of the CK material was uniformly mixed with the respective additives and manually packed into polyethylene bags measuring 27 cm × 30 cm (Embossed Food Saver Bag Co., Ltd., Chengdu, China). These bags were tightly vacuum-sealed (DZ-400 Vacuum packaging machine, Yizhong Machinery Co., Zhucheng, China). A total of 9 bags (comprising 3 treatments with 3 replicates each) were prepared and stored at ambient temperature (24–26 °C) for 60 days.

2.2. Analysis of Chemical Composition, Fermentation Characteristics, and Microbial Composition

To determine fermentation parameters, 10 g samples of silage were diluted with 90 mL of distilled water, filtered through four layers of cheesecloth, and refrigerated at 4 °C for 24 h.

The resulting supernatant was used for pH measurement, conducted using a calibrated pH meter (PHS-3G, Mettler Toledo, Zurich, Switzerland). Before analyzing ammonia nitrogen (NH_3-N) and organic acids, a subsample of the supernatant was subjected to centrifugation at 2500 rpm for 10 min and then filtered through a 0.22 μm microporous filter (LFW-JCD-100/150, Lefilter Co., Ltd., Xinxiang, China). NH_3-N concentration was determined using the phenol-sodium hypochlorite colorimetric method [25]. Organic acids were quantified using high-performance liquid chromatography (HPLC) with a KC-811 column (Shodex; Shimadzu, Japan) at an oven temperature of 50 °C, a flow rate of 1 mL/min, and detection at 210 nm, following the method outlined by [26].

For dry matter (DM) determination, samples were dried in a forced-air oven (GZX-DH 202-4-S, Botai Co., Ltd., Shanghai, China) at 65 °C for 72 h and ground to pass through a 1.0 mm screen for subsequent chemical analysis. Total nitrogen (TN) content was assessed using a Kjeldahl apparatus (K-360, BUCHI Laboratory Equipment Trade Co., Ltd., Shanghai, China), and crude protein (CP) was calculated by multiplying the TN content by 6.25. Neutral detergent fiber (NDF) was determined using heat-stable α-amylase as the method of Van Soest [27], while acid detergent fiber (ADF) was measured in accordance with the method described by Robertson and Van Soest (1981) using an ANKOM A2000i fiber analyzer (A2000i, ANKOM Technology, New York, NY, USA). Water-soluble carbohydrates (WSC) were quantified via anthrone-sulfuric acid colorimetry [28], and ether extract (EE) was analyzed using the procedures outlined by the Association of Official Analytical Chemists (AOAC) [29].

The plate count method was employed to enumerate the number of lactic acid bacteria (LAB), yeasts, and molds in both the fresh CK materials and silage samples. To achieve this, 5 g samples were homogenized in 50 mL of sterilized physiological saline (8.5 g/L) for 45 min in an incubator shaker (HZQ-F160A, Jingda Co., Ltd., Changzhou, China) (25 ± 2 °C; shaker rate: 120 rpm). The resulting solution was then serially diluted (10-fold). LAB were enumerated by inoculating MRS broth (HB0384, Hope Bio-Technology Co., Ltd., Qingdao, China) and incubating at 37 °C in an anaerobic incubator (Anaerobic box; TEHER Hard Anaerobox, ANX-1; Hirosawa Ltd., Tokyo, Japan) for 48–72 h. Yeasts and molds were enumerated by inoculating potato medium (PDA, containing 0.1% chloramphenicol, HB0233-12, Hope Bio-Technology Co., Ltd., Qingdao, China) and incubating at 30 °C for 7 d. Yeasts were distinguished from molds by colony appearance and observation of cell morphology. LAB, yeasts, and molds were counted by a serial dilution method. Microbial numbers were expressed in colony-forming units (cfu), converted into logarithmic form, and reported on a fresh matter (FM) basis.

2.3. Antioxidant Capacity Analysis

Following the thawing of the silage sample filtrates, which had been stored at −20 °C for antioxidant analysis, the filtrates were subsequently subjected to centrifugation at 12,000× *g* for 10 min at 4 °C. The resulting liquid supernatant was employed for the assessment of various antioxidant parameters, including total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT). These antioxidant parameters were detected by a colorimetric method (Victor Nivo Multimode Plate Reader, PerkinElmer Life & Analytical Sciences Ltd., Funlyclun, UK). The specific determination steps were operated in accordance with the manufacturer's instructions of commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.4. Microbial Community Analysis

For the DNA extraction of silage samples, a DNA isolation kit (Tiangen, DP302-02, Beijing, China) was employed in accordance with the manufacturer's instructions. Subsequently, full-length 16S rRNA gene amplification for SMRT sequencing was carried out through PCR.

The analysis of full-length 16S rRNA amplicon sequencing data, including the construction of the 16S rRNA library, quality control for PCR amplifications, sequence pre-

processing, species annotation, and assessment of alpha diversity, followed the procedures outlined in Xu [30]. Furthermore, to assign taxonomy to the sequences, a comparison was made with the NCBI 16S ribosomal RNA database, with classification achieved at a bootstrap threshold of 0.8, using RDP classifier software. The sample ordination, based on beta diversity, was explored using principal coordinate analysis (PCoA).

2.5. Statistical Analysis

To assess the effects of FT, a one-way analysis of variance (ANOVA) was conducted utilizing the general linear model procedure within the Statistical Package for Social Science (SPSS 21.0, SPSS, Inc., Chicago, IL, United States). To discern differences among treatment means, Duncan's tests were employed. The significance was declared at $p < 0.05$. An online platform (http://www.omicshare.com/tools, accessed on 12 June 2023) was used to analyze the sequencing data of the microbial community.

3. Results

3.1. Chemical and Microbial Compositions of CK before Ensiled

Table 1 shows the chemical and microbial compositions of CK before being ensiled. The DM content was 426 g/kg FM, and the contents of CP, EE, WSC, NDF, and ADF were 64.8, 25.2, 10.1, 802, and 665 g/kg DM, respectively. The number of LAB, yeasts, and molds in fresh CK was 3.19, 0.00, and 3.86 log10 cfu/g FM, respectively.

Table 1. Chemical and Microbial Compositions of CK before being Ensiled.

Items [1]	CK
DM (g/kg FM)	426
CP (g/kg DM)	64.8
EE (g/kg DM)	25.2
WSC (g/kg DM)	10.1
NDF (g/kg DM)	802
ADF (g/kg DM)	665
LAB (log_{10} cfu/g FM)	3.19
Yeasts (log_{10} cfu/g FM)	0.00
Molds (log_{10} cfu/g FM)	3.86

[1] DM, dry matter; CP, crude protein; EE, ether extract; WSC, water-soluble carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; cfu, colony forming unit; LAB, lactic acid bacteria; FM, fresh matter.

3.2. Effect of FT on the Fermentation Quality of CK Silage

Table 2 illustrates the fermentation quality of CK silage after 60 days of ensiling. The pH and the NH_3-N concentration of 2% FT group were lower compared with CON and 1% FT groups ($p < 0.01$). The lactic acid and acetic acid concentrations of 2% FT group were higher compared with CON and 1% FT groups ($p < 0.01$). Meanwhile, the highest and lowest LAB number were presented in 2% FT and CON groups ($p < 0.01$), respectively. Propanoic acid, butyric acid, yeasts, and molds were not detected in all groups.

Table 2. Effects of FT on the Fermentation Quality of CK Silage.

Items [1]	Treatments [2]			SEM [3]	*p*-Value
	CON	1% FT	2% FT		
pH	3.78 a	3.77 a	3.72 b	0.0	<0.01
Lactic acid (g/kg DM)	37.0 b	37.3 b	44.7 a	1	<0.01
Acetic acid (g/kg DM)	2.90 b	4.02 b	12.3 a	1.1	<0.01
Propanoic acid (g/kg DM)	ND	ND	ND	ND	-
Butyrate (g/kg DM)	ND	ND	ND	ND	-
NH_3-N (g/kg TN^{-1})	14.7 a	14.4 a	11.7 b	0	<0.01

Table 2. *Cont.*

Items [1]	Treatments [2]			SEM [3]	*p*-Value
	CON	1% FT	2% FT		
LAB (log 10 cfu/g FM)	2.49 c	3.72 b	4.50 a	0.2	<0.01
Yeasts (log 10 cfu/g FM)	ND	ND	ND	ND	-
Molds (log 10 cfu/g FM)	ND	ND	ND	ND	-

Different letters indicate significant differences in the same row ($p < 0.05$). [1] DM, dry matter; NH_3-N, ammonia nitrogen; TN, total nitrogen; LAB, lactic acid bacteria; cfu, colony forming unit; FM, fresh matter. ND, not detected. [2] CON, control group; 1% FT, silage treated with 1% FT; 2% FT, silage treated with 2% FT. [3] SEM, standard error of the mean.

3.3. Effect of FT on the Chemical Composition of CK Silage

Table 3 shows the chemical composition of CK silage after 60 days of ensiling. The DM content of CON group was the lowest compared with 1% FT and 2% FT groups ($p < 0.01$). The CP content of 2% FT group was higher than that of CON and 1% FT groups ($p < 0.01$). The contents of NDF and ADF in 2% FT group were lower compared with CON and 1% FT groups ($p < 0.01$). Furthermore, there were no significant differences in WSC and EE contents among three groups ($p > 0.05$).

Table 3. Effects of FT on the Chemical Composition of CK silage.

Items [1]	Treatments [2]			SEM [3]	*p*-Value
	CON	1% FT	2% FT		
DM (g/kg FM)	417 c	424 b	428 a	1	<0.01
CP (g/kg DM)	65.9 b	66.1 b	67.4 a	0	<0.01
WSC (g/kg DM)	3.50	3.62	3.57	0.1	0.73
EE (g/kg DM)	44.4	43.6	42.3	1	0.28
NDF (g/kg DM)	761 a	758 a	738 b	4	<0.05
ADF (g/kg DM)	647 a	644 a	604 b	5	<0.01

Different letters indicate significant differences in the same row ($p < 0.05$). [1] DM, dry matter; FM, fresh matter; CP, crude protein; WSC, water-soluble carbohydrate; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber. [2] CON, control group; 1% FT, silage treated with 1% FT; 2% FT, silage treated with 2% FT. [3] SEM, standard error of the mean.

3.4. Effect of FT on the Antioxidant Capacity of CK Silage

Table 4 illustrates the antioxidant capacity of CK silage after 60 days of ensiling. The T-AOC, SOD, and CAT activity of 2% FT group were higher than that of CON and 1% FT groups ($p < 0.01$), and the T-AOC and CAT activity of 1% FT groups were higher than that of the CON group ($p < 0.01$). The GSH-Px activities of 1% FT and 2% FT groups were higher than that of the CON group ($p < 0.01$).

Table 4. Effects of FT on the Antioxidant Capacity of CK Silage.

Items [1]	Treatments [2]			SEM [3]	*p*-Value
	CON	1% FT	2% FT		
T-AOC (mmol/g Prot)	72.7 c	84.5 b	90.5 a	3	<0.01
SOD (U/g FW)	173 b	173 b	215 a	8	<0.01
GSH-Px (U/g FW)	210 b	320 a	334 a	20	<0.01
CAT (U/g FW)	8.08 c	16.2 b	21.7 a	2.0	<0.01

Different letters indicate significant differences in the same row ($p < 0.05$). [1] T-AOC, total anti-oxidation competence; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; Prot, protein of the homogenate; FW, fresh weight. [2] CON, control group; 1% FT, silage treated with 1% FT; 2% FT, silage treated with 2% FT. [3] SEM, standard error of the mean.

3.5. Effect of FT on the Microbial Communities of CK Silage

Table 5 shows the alpha-diversity of the microbial communities of CK silage after 60 days of ensiling. The coverage depth for all treatments was above 99%. Compared with

the CON group, 1% FT and 2% FT groups decreased the richness indices (Ace and Chao 1), however, there were no significant differences among three groups ($p > 0.05$). There were no significant differences in the Simpson and Shannon indexes between three groups ($p > 0.05$).

Table 5. Effects of FT on the Alpha-Diversity of Microbial Communities of CK Silage.

Items	Treatments [1]			SEM [2]	*p*-Value
	CON	1% FT	2% FT		
Ace	379	215	199	80	0.66
Chao1	364	198	189	77	0.64
Simpson	0.61	0.72	0.37	0.1	0.52
Shannon	3.89	3.26	1.85	0.8	0.59
Coverage	0.99	0.99	0.99	0.0	0.66

[1] CON, control group; 1% FT, silage treated with 1% FT; 2% FT, silage treated with 2% FT. [2] SEM, standard error of the mean.

The principal coordinate analysis was used to study the correlations of microbial community structure in CK silage after 60 days of ensiling. There were clear separations and differences in bacterial communities among the three groups (Figure 1).

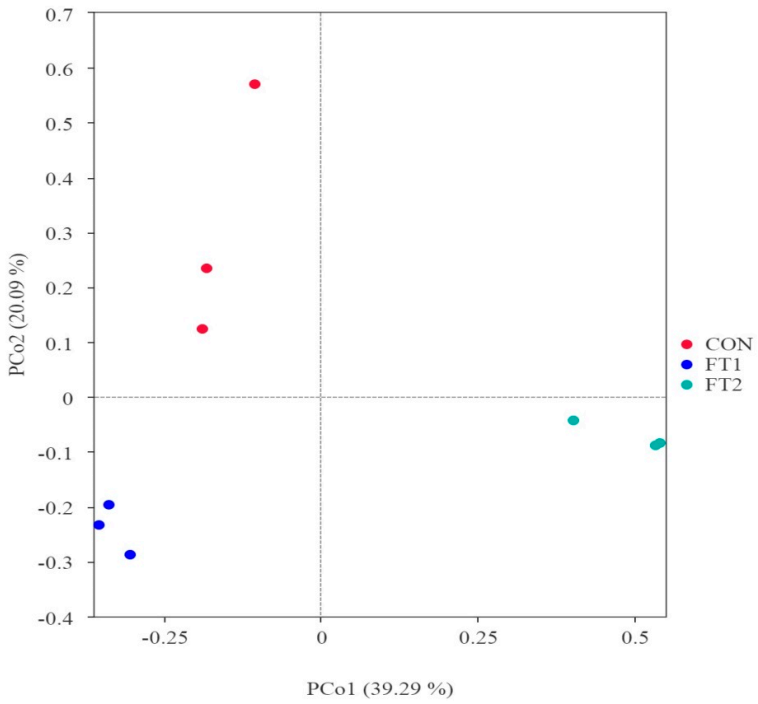

Figure 1. Principal coordinate analysis of the microbial communities of silage. CON, control group; FT1, silage treated with 1% FT; FT2, silage treated with 2% FT.

Figure 2A describes the microbial communities at the phylum level. *Firmicutes* and *Proteobacteria* were the dominant phyla in three groups. The abundance of *Firmicutes* was the highest in 2% FT group (84.7%), followed by the 1% FT group (62.3%) and the CON group (17.8%). The FT addition obviously decreased the abundance of *Proteobacteria*, which was the highest in CON group (56.1%), followed by that in 1% FT group, and 2% FT group (30.7%, and 8.1%, respectively). In addition, 2% FT and 1% FT groups also decreased the abundances of *Cyanobacteria* and *Bacteroidetes* compared with CON group (0.1%, 5.6% and 14.7%; 0.3%, 0.8%, and 4.3%).

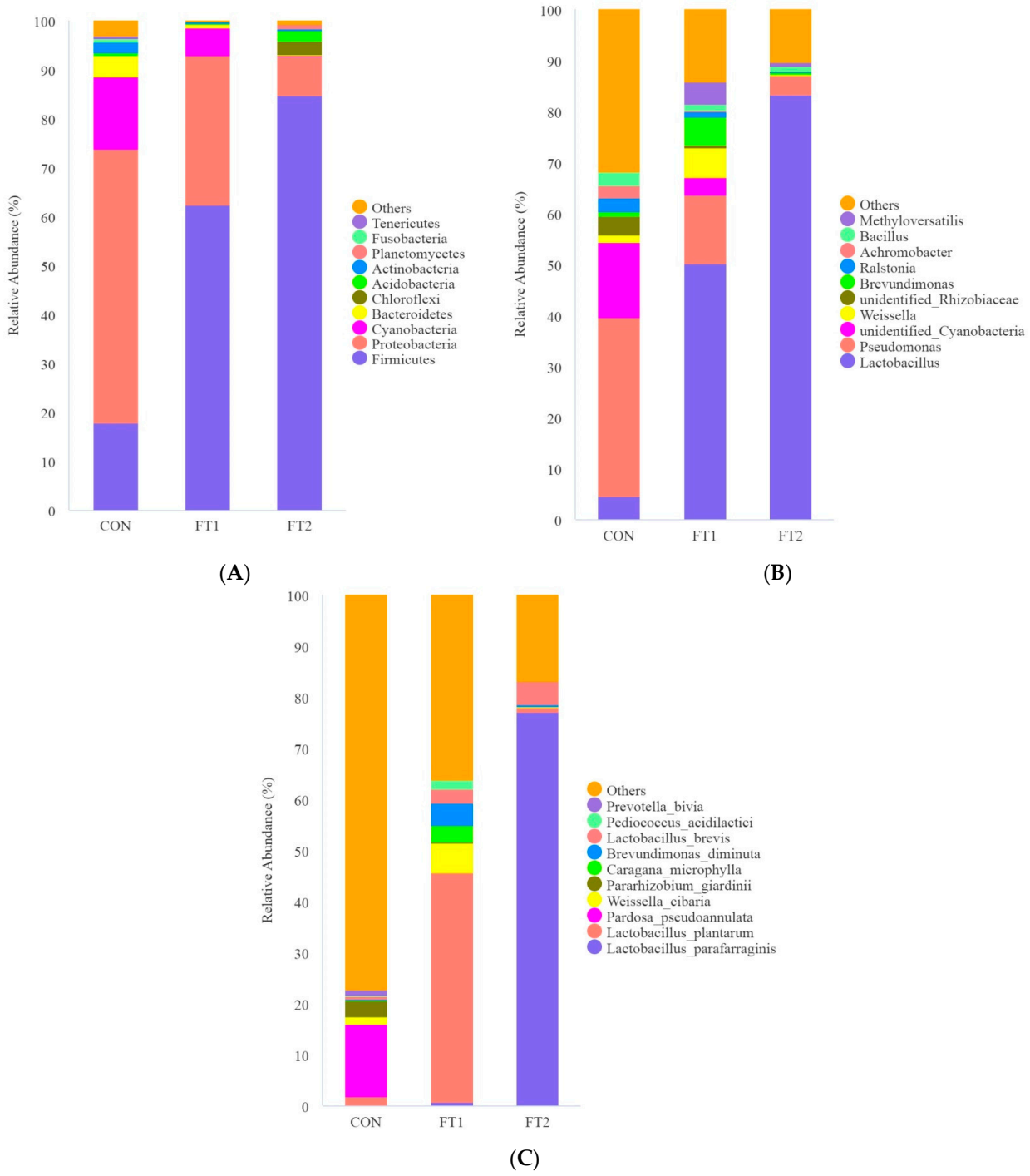

Figure 2. The microbial abundance at the phylum (**A**), genus (**B**), and species (**C**) levels in silage. CON, control group; FT1, silage treated with 1% FT; FT2, silage treated with 2% FT.

Figure 2B describes the microbial communities at the genus level. *Lactobacillus*, the main microbes in 2% FT group (83.2%) and 1% FT group (50.1%), and that in CON group was 4.5%. The relative abundance of *Pseudomonas* and unidentified *Cyanobacteria* in 2% FT and 1% FT groups were higher than that in CON group (3.9%, 13.5%, and 35.2%; 0.0%, 3.5%, and 14.7%).

Figure 2C describes the microbial communities at the species level. *L. parafarraginis* was the most dominant in 2% FT group (77.1%) and *L. plantarum* was the most dominant in 1% FT group (45.0%). The relative abundances of *Pardosa pseudoannulata* in 2% FT and 1% FT groups were lower than that in the CON group (0.0%, 0.0% and 14.2%). Moreover,

the relative abundance of *L. brevis* in 2% FT was higher than that in 1% FT and CON groups (4.5%, 2.7%, and 0.5%).

The taxonomic differences between the different treatments are shown in the clade representation generated by LEfSe (Figure 3). The 1% FT and 2% FT additions significantly affected the microbial composition of CK silage (LAD > 4). The 1% FT group increased the abundance of *Pigmentiphaga*. The 2% FT group increased the abundances of *Lactobacillaceae*, *Lactobacillales*, *Lactobacillus*, *Oceanobacillus profundus*, *Oceanobacillus*, and *L. paralimentarius*.

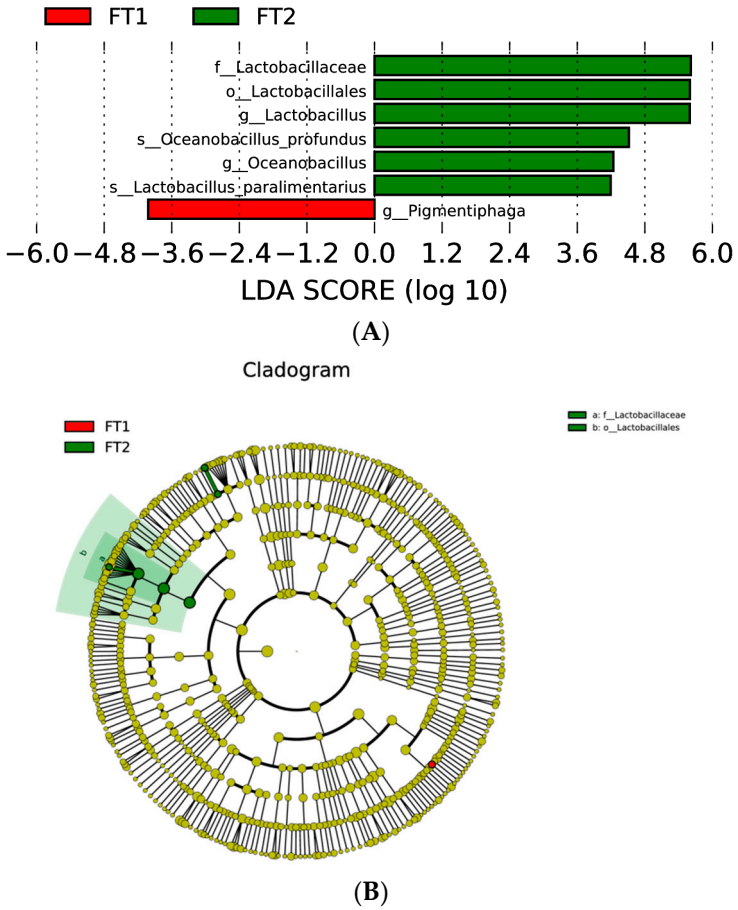

Figure 3. LDA value distribution and evolutionary branch of different species in silage treated with different additives. (**A**) LDA value distribution of different species. (**B**) Evolutionary branch diagram of various species. FT1, silage treated with 1% FT; FT2, silage treated with 2% FT.

3.6. The Correlation between Microorganisms and Fermentation Parameters, Antioxidant Capacity of CK Silage

Figure 4 illustrates the correlation between microorganisms (species level) and fermentation parameters, antioxidant capacity of CK silage. The abundance of *L. parafarraginis* exerted positive correlations with lactic acid and acetic acid concentrations, LAB number ($p < 0.01$), T-AOC, GSH-Px, and CAT activities ($p < 0.05$), while it showed negative correlations with pH and NH_3-N concentration ($p < 0.05$). The abundance of *L. brevis* showed positive correlations with acetic acid concentration, LAB number ($p < 0.01$), T-AOC, GSH-Px, and CAT activities ($p < 0.05$), while it showed negative correlations with pH ($p < 0.05$). The abundances of *Weissella cibaria* and *Pararhizobium giardinii* showed negative correlations with SOD activity ($p < 0.05$). The abundance of *P. pseudoannulata* showed negative correlations with acetic acid concentration and GSH-Px activity ($p < 0.05$). The abundance of *Caragana microphylla* showed positive correlations with NH_3-N concentration ($p < 0.05$).

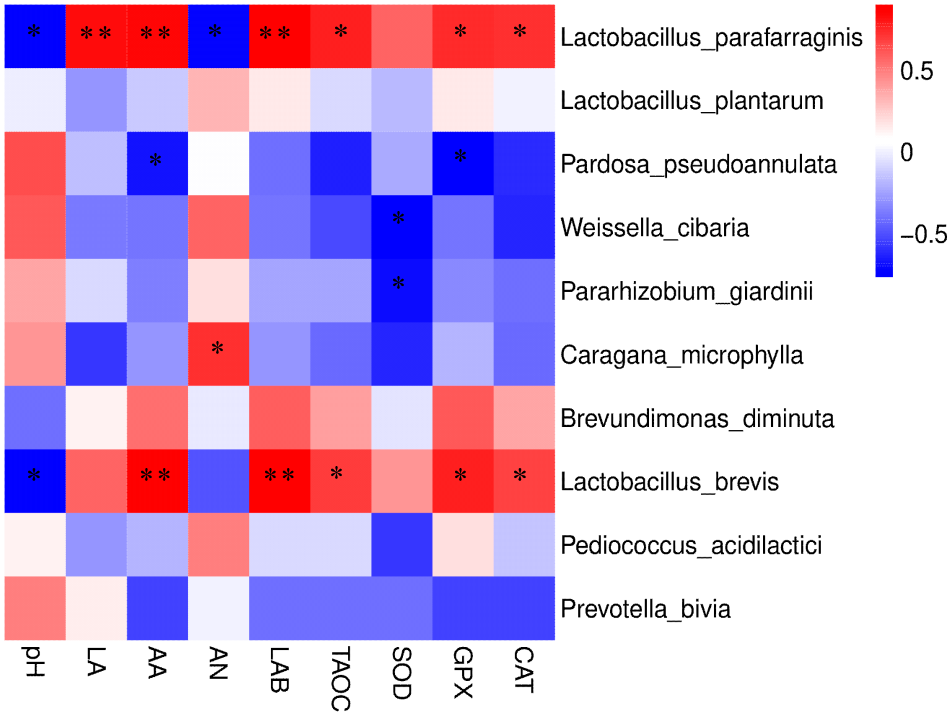

Figure 4. The correlation between microorganisms and fermentation parameters, antioxidant capacity at the species level using Spearman's correlation analysis. LA, lactic acid; AA, acetic acid; AN, NH_3-N; LAB, lactic acid bacteria; TAOC, total anti-oxidation competence; SOD, superoxide dismutase; GPX, glutathione peroxidase; CAT, catalase. * Represents $p < 0.05$ and ** represent $p < 0.01$.

4. Discussion

4.1. Effect of FT on the Chemical Composition of Fresh CK and Silage

The WSC content plays a pivotal role in determining the quality of silage fermentation. Ensuring satisfactory fermentation quality requires WSC values exceeding 5% DM [31]. Additionally, the presence of LAB at levels surpassing 5.0 log 10 cfu/g during ensiling is crucial for achieving well-preserved silage [32]. In this study, instances of lower WSC content and LAB number, coupled with elevated mold counts, suggested a requirement for additives in the preparation of CK silage. It was observed that FT-treated silage exhibited a higher DM content compared to the CON group. This outcome suggested that FT effectively mitigated DM loss during the ensiling process. Furthermore, in comparison to freshly harvested CK, silage treated with FT preserved protein content remarkably well. Notably, 2% FT group displayed significantly higher CP levels than both 1% FT and CON groups. This preservation of protein content could be attributed to two potential mechanisms. Firstly, the active inhibition of proteases in the low-pH environment directly reduced proteolysis. Secondly, the formation of protein–polyphenol complexes in the silage treated with 2% FT, facilitated by the presence of flavonoids, which are a subgroup of polyphenols [33]. Fresh CK exhibited substantial levels of NDF and ADF, while 2% FT group exhibited lower fiber contents compared to the CON group in silage. This reduction in NDF and ADF contents within 2% FT group could be attributed to processes such as acidic hydrolysis and potential fibrinolytic enzyme production by microorganisms during the fermentation of silage [17]. The findings of this study collectively suggest that the application of 2% FT yields the most favorable results in terms of nutrient preservation in CK silage, highlighting FT's potential as a valuable silage additive.

4.2. Effect of FT on the Fermentation Quality of CK Silage

The organic acids (mainly lactic acid and acetic acid), NH_3-N concentrations, and pH are vital indicators of well-preserved forage silage quality [34]. The accumulation

of NH_3-N during ensiling is a recognized marker of protein degradation [35]. 2% FT group exhibited lower pH and NH_3-N concentration than CON. Flavonoids are part of polyphenols, and the decreased NH_3-N content in 2% FT-treated silage might be due to the formation of complexes of polyphenols and proteins, or the inhibition of plant and/or bacterial proteolytic enzymes [36]. Meanwhile, the highest lactic acid concentration and LAB number was found in 2% FT group. Similarly, apple pomace and grape pomace contain polyphenol, such as phenolic acids, flavonoids, and so on [37,38], and the additions resulted in an elevated lactic acid concentration coupled with a decrease in pH and nonprotein nitrogen concentrations in the ensiled alfalfa [15]. In the present study, the acetic acid concentration of 2% FT group was the highest among the three groups. The reason might be due to *L. parafarraginis* being the most dominant in 2% FT group (77.1%). *L. parafarraginis*, belonging to the *Lactobacillus buchneri* group [39], is known for its capability to enhance acetic acid concentration and bolster the aerobic stability of silage across a wide spectrum of temperatures [40]. It is worth noting that propanoic acid, butyric acid, yeasts, and molds were not detected in any of the groups, possibly due to the inhibitory effects of the low-pH environment on undesirable bacteria and fungi, including *Clostridia* [41]. Prior research has illuminated that *Lactobacillus* spp. showcases a higher degree of resistance to phenolic compounds when compared to other bacterial groups such as *Clostridium* spp., *Bacteroides* spp., *Escherichia coli*, and *Bacillus subtilis* [33].

Therefore, the addition of 2% FT may foster a microenvironment characterized by low pH value and elevated levels of bioactive components under the conditions of this experiment, thereby facilitating the rapid proliferation of desirable microorganisms such as LAB under anaerobic conditions. To delve deeper into these findings, microbial community analysis was conducted.

4.3. Effect of FT on the Antioxidant Capacity of CK Silage

Oxidative stress has been associated with various health disorders in cattle [42], and it can significantly impair animal production outcomes [43]. Numerous studies have highlighted the role of dietary antioxidants in enhancing both the antioxidant capacity of animals and the antioxidant properties of animal-derived products [24,44,45]. Therefore, augmenting the antioxidant capacity of silage is of paramount importance and is highly desirable in the context of animal nutrition. Prior investigations by Zhang [46,47] have underscored that alfalfa silage inoculated with LAB exhibiting robust antioxidant activity displayed the highest T-AOC and CAT activity. Generally, endogenous antioxidants such as SOD, CAT, and GSH-Px serve as the initial line of defense against oxidative stress within cells. These enzymes perform functions such as dismutating superoxide radicals (O_2^-), breaking down hydrogen peroxide (H_2O_2), and converting hydroperoxides into harmless molecules (H_2O/alcohol and O_2), respectively [48]. Compared with CON group, 1% FT and 2% FT groups showed better antioxidant capacity. Furthermore, 2% FT-treated CK silage exhibited the highest T-AOC, SOD, GSH-Px, and CAT activities, which was probably due to the substantial antioxidant capacity inherent in FT. As an important subgroup of plant phenols, flavonoids endow high antioxidant activity, attributable to their polyhydroxy structure [49,50]. In vitro assays have revealed that flavonoids extracted from fermented dandelion exhibit superior antioxidant activity compared to those from unfermented dandelion, as evidenced by their effectiveness in scavenging DPPH radicals and their reducing power [22]. Consequently, these findings lend support to the hypothesis that FT functions analogously to chemical antioxidants, bolstering the antioxidant capacity of silage.

4.4. Effect of FT on the Microbial Communities of CK Silage

In this study, the high coverage value of approximately 0.99 for each sample indicated the comprehensive sequencing coverage achieved, signifying that the majority of microbial species were successfully detected. The beta-diversity analysis unveiled distinct separation and variations in bacterial communities within the CK silage, strongly suggesting that

the microbial composition underwent significant changes due to FT treatment during the ensiling process.

Specific alterations in bacterial composition were observed at various taxonomic levels among the three experimental groups. *Firmicutes* and *Proteobacteria* emerged as the top two predominant phyla in CK silage, consistent with prior research findings [51–53]. *Lactobacillus*, recognized for its pivotal role and beneficial attributes in ensiled forage, is typically the dominant bacterial strain responsible for driving lactic fermentation during ensiling [31]. Intriguingly, FT-treatment led to a notable increase in the abundance of *Lactobacillus*, particularly in 2% FT group, where it constituted as much as 83% of the total microbial population. Within them, *L. parafarraginis* emerged as the most dominant species in 2% FT group, comprising a substantial 77.1% of the population. This might be due to the addition of 2% FT, which could provide substrates and energy supplementation for the propagation of *L. parafarraginis*, and/or due to the fact that *L. parafarraginis* is highly resistant to phenolic compounds. Previous studies have demonstrated that *L. parafarraginis* ZH1 contributes to the aerobic stability and safety of silage by producing anti-yeast compounds such as acetic acid, hexadecanoic acid, benzoic acid, tetradecanoic acid, and octadecanoic acid [54]. *L. parafarraginis* is classified as a heterofermentative LAB, endowed with the ability to convert lactic acid into acetic acid and 1, 2-propylene glycol [51]. This capability might elucidate the observed high acetic acid content in 2% FT group. These findings collectively suggest that the addition of 2% FT might enhance the aerobic stability and safety of CK silage by fostering the proliferation of *L. parafarraginis*. However, it is essential to note that this hypothesis warrants further experimental validation. *L. plantarum* is a common dominant bacterium in silage, and that was the most dominant in 1% FT group (45.0%). However, the abundance of *L. plantarum* in 2% FT group was considerably lower, and this might be due to the replacement of *L. plantarum* by *L. parafarraginis* in 2% FT group during later silage. As photosynthetic bacterial phylum, *Cyanobacteria* exist in a variety of growing environments and a wide variety of generated products [55]. Li [56] reported that *Cyanobacteria* was the dominant phylum in four fresh tropical forages, and *Cyanobacteria* decreased to marginal levels in the silage of paspalum and stylo. Another research also reported that *Cyanobacteria* was the dominant phylum in the control *Neolamarckia cadamba* leaf silage, and intrinsic tannins significantly decreased the abundance of *Cyanobacteria* [57]. Similar to the above studies, FT-treatment markedly decreased the abundances of unidentified *Cyanobacteria*/*P. pseudoannulata*, especially for 2% FT group. *Pseudomonas*, considered an undesirable bacterial strain in silage due to its potential for biogenic amine production and the reduction of protein content and nutritional value [58], experienced a marked reduction in abundance following FT treatment, especially in 2% FT group. These observations align with findings from previous studies involving various forage types, including corn stover, red clover, alfalfa, and *Moringa oleifera* leaves [51,52,59,60]. Therefore, the incorporation of 2% FT appears to be an effective strategy for improving silage conditions, leading to alterations in both the silage environment and microbial dynamics.

4.5. The Correlation between Microorganisms and Fermentation Parameters, Antioxidant Capacity of CK Silage

Silage fermentation is a highly intricate biological process in which *Lactobacillus* assumes a central role. *Lactobacillus* has been observed to exert a positive influence on lactic acid production, while concurrently affecting pH and NH_3-N levels [17,61]. Similar to the above research, there existed a negative correlation between pH and the abundance of *Lactobacillus*, particularly *L. parafarraginis* and *L. brevis*. Furthermore, it was evident that the abundance of *L. parafarraginis* exhibited a negative correlation with NH_3-N concentration in the present study. Research by Cai [32] has elucidated that lactic acid-producing cocci initiate lactic fermentation during the early stages of ensiling; however, their viability diminishes under acidic conditions. Consequently, lactic acid-producing rods, exemplified by *Lactobacillus*, assume a pivotal role in pH reduction during the latter stages of ensiling. *Lactobacillus's* sensitivity to reduced pH level underscores the notable correlation coeffi-

cients between NH_3-N and Lactobacillus [53]. It was worth noting that the abundances of *L. parafarraginis* and *L. brevis* were positively correlated with acetic acid concentration. It was probably because of both species were heterofermentative LAB that could convert lactic acid to acetic acid [62]. The abundance of *C. microphylla* (belonging to *Cyanobacteria*) was positively correlated with NH_3-N concentration. The explanation was that *C. microphylla* could lead to proteolysis.

The abundances of *L. parafarraginis* and *L. brevis* exhibited positive correlations with T-AOC, GSH-Px, and CAT activities, and results showed that *L. parafarraginis* and *L. brevis* might promote the improvement of antioxidant capacity. The precise mechanisms underlying this phenomenon warrants further investigation to elucidate the specific pathways or compounds through which *L. parafarraginis* and *L. brevis* exert their influence on antioxidant activity. The abundances of *W. cibaria* and *P. giardinii* were negatively correlated with SOD activity, *W. cibaria* and *P. giardinii* potentially reduced antioxidant capacity in CK silage. To better understand this phenomenon, it is essential to delve into the mechanisms by which these microorganisms impact SOD activity and to explore whether they produce metabolites or compounds that interfere with the antioxidant defense systems in silage.

5. Conclusions

Significant changes in fermentation quality, antioxidant status, and microbial community occurred at FT-treatment CK silage. In particular, 2% FT addition had the best effect on nutrient preservation in CK silage, and 2% FT addition could improve the silage quality and antioxidant activity. 2% FT addition also increased the abundances of desirable LAB but decreased the abundances of undesirable microorganisms in CK silage. The results of this research contribute to the application of FT as a potential functional feed additive in CK silage. In the future, it could be possible to reveal the functional microbial with FT through metagenomics and develop other similar medicinal herbs in forage silage.

Author Contributions: Conceptualization, S.H.; methodology, S.H. and L.G.; software, L.G.; validation, L.G.; formal analysis, W.K.; investigation, L.G., X.Z. and C.M.; resources, S.H.; data curation, L.G.; writing—original draft preparation, S.H.; writing—review and editing, S.H. and W.K.; visualization, Q.L.; supervision, S.H.; project administration, S.H.; funding acquisition, S.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Natural Science Foundation of Ningxia (2023AAC03094).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We sincerely thank the Key Laboratory of High-efficiency Production Model Innovation of Forage of the Ministry of Agriculture for their technical support for this research.

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

References

1. Zhang, S.J.; Chaudhry, A.S.; Osman, A.; Shi, C.Q.; Edwards, G.R.; Dewhurst, R.J.; Cheng, L. Associative effects of ensiling mixtures of sweet sorghum and alfalfa on nutritive value, fermentation and methane characteristics. *Anim. Feed Sci. Technol.* **2015**, *206*, 29–38. [CrossRef]
2. Yang, Y.; Liu, B.R. Effects of planting *Caragana* shrubs on soil nutrients and stoichiometries in desert steppe of Northwest China. *Catena* **2019**, *183*, 104213. [CrossRef]
3. Li, W.C.; Zhang, S.J.; Zhang, T.Z.; Shen, Y.Q.; Han, L.J.; Peng, Z.J.; Xie, Z.X.; Zhong, C.; Jia, S.R. Bacterial cellulose production from ethylenedi-amine pretreated *Caragana korshinskii* Kom. *Ind. Crop. Prod.* **2021**, *164*, 113340. [CrossRef]
4. Wang, G.H.; Chen, Z.X.; Shen, Y.Y.; Yang, X.L. Efficient prediction of profile mean soil water content for hillslope-scale *Caragana korshinskii* plantation using temporal stability analysis. *Catena* **2021**, *206*, 105491. [CrossRef]
5. Cai, Y.M.; Du, Z.M.; Yamasaki, S.; Nguluve, D.; Tinga, B.; Macome, F.; Oya, T. Community of natural lactic acid bacteria and silage fermentation of cornstover and sugarcane tops in Africa. *Asian-Australas. J. Anim. Sci.* **2020**, *33*, 1252–1264. [CrossRef] [PubMed]

6. You, J.T.; Zhang, H.; Zhu, H.F.; Xue, Y.L.; Cai, Y.M.; Zhang, G.J. Microbial community, fermentation quality, and in vitro degradability of ensiling *Caragana* with lactic acid bacteria and rice bran. *Front. Microbiol.* **2022**, *13*, 804429. [CrossRef] [PubMed]
7. Ke, W.C.; Wang, Y.; Rinne, M.; Franco, O.M.; Li, F.H.; Lin, Y.F.; Zhang, Q.; Cai, Y.M.; Zhang, G.J. Effects of lactic acid bacteria and molasses on the fermentation quality, in vitro dry matter digestibility, and microbial community of Korshinsk peashrub (*Caragana korshinskii* Kom.) silages harvested at two growth stages. *Grass Forage Sci.* **2023**, *78*, 1–13. [CrossRef]
8. Chen, L.; Bao, X.Y.; Guo, G.; Huo, W.J.; Xu, Q.F.; Wang, C.; Liu, Q. Treatment of alfalfa silage with tannin acid at different levels modulates ensiling characteristics, methane mitigation, ruminal fermentation patterns and microbiota. *Animal. Feed. Sci. Technol.* **2021**, *278*, 114997. [CrossRef]
9. Hristov, A.N.; Oh, J.; Firkins, J.L.; Dijkstra, J.; Kebreab, E.; Waghorn, G.; Makkar, H.P.S.; Adesogan, A.T.; Yang, W.; Lee, C.; et al. Special topics-Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. *J. Anim. Sci.* **2013**, *91*, 5045–5069. [CrossRef]
10. Mertenat, D.; Cero, M.D.; Vogl, C.R.; Ivemeyer, S.; Meier, B.; Maeschli, A.; Hamburger, M.; Walkenhorst, M. Ethnoveterinary knowledge of farmers in bilingual regions of Switzerland—Is there potential to extend veterinary options to reduce antimicrobial use? *J. Ethnopharmacol.* **2019**, *246*, 112184. [CrossRef]
11. Shen, N.; Wang, T.F.; Gan, Q.; Liu, S.A.; Wang, L.; Jin, B. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. *Food. Chem.* **2022**, *383*, 132531. [CrossRef] [PubMed]
12. Hashemi, S.; Davoodi, H. Herbal plants and their derivatives as growth and health promoters in animal nutrition. *Vet. Res. Commun.* **2011**, *35*, 169–180. [CrossRef] [PubMed]
13. Bouaziz, F.; Helbert, C.B.; Romdhane, M.B.; Koubaa, M.; Bhiri, F.; Kallel, F.; Chaari, F.; Driss, D.; Buon, L.; Chaabouni, S.E. Structural data and biological properties of almond gum oligosaccharide: Application to beef meat preservation. *Int. J. Biol. Macromol.* **2015**, *72*, 472–479. [CrossRef] [PubMed]
14. Ni, K.K.; Wang, X.K.; Lu, Y.; Guo, L.N.; Li, X.M.; Yang, F.Y. Exploring the silage quality of alfalfa ensiled with the residues of astragalus and hawthorn. *Bioresour. Technol.* **2020**, *297*, 122249. [CrossRef]
15. 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. Technol.* **2015**, *202*, 12–19. [CrossRef]
16. Kitrytė, V.; Povilaitis, D.; Kraujalienė, V.; Šulniūtė, V.; Pukalskas, A.; Venskutonis, P.R. Fractionation of sea buckthorn pomace and seeds into valuable components by using high pressure and enzyme-assisted extraction methods. *LWT-Food Sci. Technol.* **2017**, *85*, 534–538. [CrossRef]
17. Chen, L.Y.; Qu, H.; Bai, S.Q.; Yan, L.J.; You, M.H.; Gou, W.L.; Li, P.; Gao, F.Q. Effect of wet sea buckthorn pomace utilized as an additive on silage fermentation profile and bacterial community composition of alfalfa. *Bioresour. Technol.* **2020**, *314*, 123773. [CrossRef]
18. González-Castejón, M.; Visioli, F.; Rodriguez-Casado, A. Diverse biological activities of dandelion. *Nutr. Rev.* **2012**, *70*, 534–547. [CrossRef]
19. Duan, L.; Zhang, C.M.; Zhao, Y.; Chang, Y.Z.; Guo, L.; Donato, P.D.; Silvestri, B. Comparison of bioactive phenolic compounds and antioxidant activities of different parts of *Taraxacum mongolicum*. *Molecules* **2020**, *25*, 3260. [CrossRef] [PubMed]
20. Schütz, K.; Carle, R.; Schieber, A. Taraxacum-A review on its phytochemical and pharmacological profile. *J. Ethnopharmacol.* **2006**, *107*, 313–323. [CrossRef]
21. Park, S.; Kim, D.S.; Wu, X.G.; Yi, Q.J. Mulberry and dandelion water extracts prevent alcohol-induced steatosis with alleviating gut microbiome dysbiosis. *Exp. Biol. Med.* **2018**, *243*, 882–894. [CrossRef] [PubMed]
22. Liu, N.; Song, M.; Wang, N.F.; Wang, Y.; Wang, R.F.; An, X.P.; Qi, J.W. The effects of solid-state fermentation on the content, composition and in vitro antioxidant activity of flavonoids from dandelion. *PLoS ONE* **2020**, *15*, e0239076. [CrossRef] [PubMed]
23. Ding, W.R.; Wang, L.N.; Zhang, J.; Ke, W.C.; Zhou, J.W.; Zhu, J.X.; Guo, X.S.; Long, R.J. Characterization of antioxidant properties of lactic acid bacteria isolated from spontaneously fermented yak milk in the Tibetan Plateau. *J. Funct. Foods* **2017**, *35*, 481–488. [CrossRef]
24. Tian, X.Z.; Paengkoum, P.; Paengkoum, S.; Chumpawadee, S.; Ban, C.; Thongpea, S. Short communication: Purple corn (*Zea mays* L.) stover silage with abundant anthocyanins transferring anthocyanin composition to the milk and increasing antioxidant status of lactating dairy goats. *J. Dairy Sci.* **2019**, *102*, 413–418. [CrossRef]
25. 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]
26. Tian, J.P.; Yu, Y.D.; Yu, Z.; Shao, T.; Na, R.S.; Zhao, M.M. Effects of lactic acid bacteria inoculants and cellulase on fermentation quality and in vitro digestibility of *Leymus chinensis* silage. *Grassl. Sci.* **2014**, *60*, 199–205. [CrossRef]
27. 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]
28. Murphy, R.P. A method for the extraction of plant samples and the determination of total soluble carbohydrates. *J. Sci. Food Agr.* **1958**, *9*, 714–717. [CrossRef]
29. AOAC. *Official Methods of Analysis of AOAC International*; Association of Analytical Communities: Gaithersburg, MD, USA, 2000.
30. Xu, D.M.; Ding, W.R.; Ke, W.C.; Li, F.H.; Zhang, P.; Guo, X.S. Modulation of metabolome and bacterial community in whole crop corn silage by inoculating homofermentative *Lactobacillus plantarum* and heterofermentative *Lactobacillus buchneri*. *Front. Microbiol.* **2019**, *9*, 3299. [CrossRef]

31. Ni, K.K.; Wang, F.F.; Zhu, B.G.; Yang, J.X.; 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. *Bioresoure Technol.* **2017**, *238*, 706–715. [CrossRef]
32. Cai, Y.M.; 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. Microbiol.* **1998**, *64*, 2982–2987. [CrossRef] [PubMed]
33. Lee, M.R.F.; Scott, M.B.; Tweed, J.K.S.; Minchin, F.R.; Davies, D.R. Effects of polyphenol oxidase on lipolysis and proteolysis of red clover silage with and without a silage inoculant (*Lactobacillus plantarum* L54). *Anim. Feed Sci. Technol.* **2008**, *144*, 125–136. [CrossRef]
34. Hashemzadeh-Cigari, F.; Khorvash, M.; Ghorbani, G.R.; Ghasemi, E.; Taghizadeh, A.; Kargar, S.; Yang, W.Z. Interactive effects of molasses by homofermentative and heterofermentative inoculants on fermentation quality, nitrogen fractionation, nutritive value and aerobic stability of wilted alfalfa (*Medicago sativa* L.) silage. *J. Anim. Physiol. Anim. Nutr.* **2014**, *98*, 290–299. [CrossRef]
35. Nkosi, B.D.; Meeske, R.; van der Merwe, H.J.; Groenewald, I.B. Effects of homofermentative and heterofermentative bacterial silage inoculants on potato hash silage fermentation and digestibility in rams. *Anim. Feed Sci. Technol.* **2010**, *157*, 195–200. [CrossRef]
36. Albrecht, K.A.; Muck, R.E. Proteolysis in ensiled forage legumes that vary in tannin concentration. *Crop Sci.* **1991**, *31*, 464–469. [CrossRef]
37. Lu, Y.R.; Foo, L.Y. The polyphenol constituents of grape pomace. *Food Chem.* **1999**, *65*, 1–8. [CrossRef]
38. Garcia, Y.D.; Valles, B.S.; Lobo, A.P. Phenolic and antioxidant composition of by-products from the cider industry: Apple pomace. *Food. Chem.* **2009**, *117*, 731–738. [CrossRef]
39. Endo, A.; Okada, S. *Lactobacillus farraginis* sp. *nov*. and *Lactobacillus parafarraginis* sp. *nov*., heterofermentative lactobacilli isolated from a compost of distilled shochu residue. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 708–712. [CrossRef]
40. Liu, Q.H.; Yang, F.Y.; Zhang, J.G.; Shao, T. Characteristics of *Lactobacillus parafarraginis* ZH1 and its role in improving the aerobic stability of silages. *J. Appl. Microbiol.* **2014**, *117*, 405–416. [CrossRef]
41. 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]
42. Miller, J.K.; Brzezinska-Slebodzinska, E.; Madsen, F.C. Oxidative stress, antioxidants, and animal function. *J. Dairy Sci.* **1993**, *76*, 2812–2823. [CrossRef] [PubMed]
43. Liu, Q.H.; Shao, T.; Bai, Y.F. The effect of fibrolytic enzyme, *Lactobacillus plantarum* and two food antioxidants on the fermentation quality, alpha-tocopherol and beta-carotene of high moisture napier grass silage ensiled at different temperatures. *Animal. Feed. Sci. Technol.* **2016**, *221*, 1–11. [CrossRef]
44. Kotsampasi, B.; Christodoulou, C.; Tsiplakou, E.; Mavrommatis, A.; Mitsiopoulou, C.; Karaiskou, C.; Dotas, V.; Robinson, P.H.; Bampidis, V.A.; Christodoulou, V.; et al. Effects of dietary pomegranate pulp silage supplementation on milk yield and composition, milk fatty acid profile and blood plasma antioxidant status of lactating dairy cows. *Anim. Feed Sci. Technol.* **2017**, *234*, 228–236. [CrossRef]
45. Khosravi, M.; Rouzbehan, Y.; Rezaei, M.; Rezaei, J. Total replacement of corn silage with sorghum silage improves milk fatty acid profile and antioxidant capacity of Holstein dairy cows. *J. Dairy Sci.* **2018**, *101*, 10953–10961. [CrossRef]
46. Zhang, Y.X.; Ke, W.C.; Bai, J.; Li, F.H.; Xu, D.M.; Ding, Z.T.; Guo, X.S. The effect of *Pediococcus acidilactici* J17 with high-antioxidant activity on antioxidant, α-tocopherol, β-carotene, fatty acids, and fermentation profiles of alfalfa silage ensiled at two different dry matter contents. *Anim. Feed Sci. Technol.* **2020**, *268*, 114614. [CrossRef]
47. Zhang, Y.X.; Huang, Z.P.; Vyas, D.; Adesogan, A.T.; Franco, M.; Ke, W.C.; Li, F.H.; Bai, J.; Ding, Z.T.; Guo, X.S. Antioxidant status, chemical composition and fermentation profile of alfalfa silage ensiled at two dry matter contents with a novel *Lactobacillus plantarum* strain with high-antioxidant activity. *Anim. Feed Sci. Technol.* **2021**, *272*, 114751. [CrossRef]
48. Ighodaro, O.M.; Akinloye, O.A. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alex. J. Med.* **2018**, *54*, 287–293. [CrossRef]
49. Bao, T.; Xu, Y.; Gowd, V.; Zhao, J.C.; Xie, J.H.; Liang, W.K.; Chen, W. Systematic study on phytochemicals and antioxidant activity of some new and common mulberry cultivars in China. *J. Funct. Foods.* **2016**, *25*, 537–547. [CrossRef]
50. Jakub, T.; Karel, Š. Flavonoids as potent scavengers of hydroxyl radicals. *Compr. Rev. Food Sci. Food Saf.* **2016**, *15*, 720–738. [CrossRef]
51. Xu, Z.S.; He, H.Y.; Zhang, S.S.; Kong, J. Effects of inoculants *Lactobacillus brevis* and *Lactobacillus parafarraginis* on the fermentation characteristics and microbial communities of corn stover silage. *Sci. Rep.* **2017**, *7*, 13614. [CrossRef]
52. Dong, Z.H.; Li, J.F.; Chen, L.; Wang, S.R.; Shao, T. Effects of freeze-thaw event on microbial community dynamics during red clover ensiling. *Front. Microbiol.* **2019**, *10*, 1559. [CrossRef] [PubMed]
53. Zi, X.J.; Li, M.; Chen, Y.Y.; Lv, R.L.; Zhou, H.L.; Tang, J. Effects of citric acid and *Lactobacillus plantarum* on silage quality and bacterial diversity of king grass silage. *Front. Microbiol.* **2021**, *12*, 631096. [CrossRef] [PubMed]
54. Liu, Q.H.; Lindow, S.E.; Zhang, J.G. *Lactobacillus parafarraginis* ZH1 producing anti-yeast substances to improve the aerobic stability of silage. *Anim. Sci. J.* **2018**, *89*, 1302–1309. [CrossRef]
55. Heberline, J.A. Cyanobacteria: Omics and manipulation. *Environ. Prog. Sustain.* **2017**, *36*, 980. [CrossRef]
56. Li, D.X.; Ni, K.K.; Zhang, Y.C.; Lin, Y.L.; Yang, F.Y. Fermentation characteristics, chemical composition and microbial community of tropical forage silage under different temperatures. *Asian-Australas J. Anim. Sci.* **2019**, *32*, 665–674. [CrossRef]

57. He, L.W.; Lv, H.J.; Xing, Y.Q.; Chen, X.Y.; Zhang, Q. Intrinsic tannins affect ensiling characteristics and proteolysis of *Neolamarckia cadamba* leaf silage by largely altering bacterial community. *Bioresour. Technol.* **2020**, *311*, 123496. [CrossRef]
58. Dunière, L.; Sindou, J.; Chaucheyras-Durand, F.; Chevallier, I.; Sergentet, D. Silage processing and strategies to prevent persistence of undesirable microorganisms. *Animal. Feed. Sci. Technol.* **2013**, *182*, 1–15. [CrossRef]
59. 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]
60. Wang, Y.; He, L.W.; Xing, Y.Q.; Zhou, W.; Pian, R.Q.; Yang, F.Y.; Chen, X.Y.; Zhang, Q. Bacterial diversity and fermentation quality of *Moringa oleifera* leaves silage prepared with lactic acid bacteria inoculants and stored at different temperatures. *Bioresour. Technol.* **2019**, *284*, 349–358. [CrossRef]
61. Yang, L.L.; Yuan, X.J.; Li, J.F.; Dong, Z.H.; Shao, T. Dynamics of microbial community and fermentation quality during ensiling of sterile and nonsterile alfalfa with or without *Lactobacillus plantarum* inoculant. *Bioresour. Technol.* **2019**, *275*, 280–287. [CrossRef]
62. Danner, H.; Holzer, M.; Mayrhuber, E.; Braun, R. Acetic acid increases stability of silage under aerobic conditions. *Appl. Environ. Microb.* **2003**, *69*, 562–567. [CrossRef]