ENDOGENOUS *LACTOCOCCUS LACTIS* AND *ENTEROCOCCUS FAECIUM* IMPROVE THE SILAGE QUALITY AND AEROBIC STABILITY OF *CYPERUS ESCULENTUS* STEM AND LEAF

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ABSTRACT

This study aimed to investigate the effect of dominant endogenous lactic acid bacteria isolated from natural *Cyperus esculentus* stems and leaves silage on the quality of the silage and to provide a basis for improving the quality of *Cyperus esculentus* stems and leaves silage in South Xinjiang. Five treatment groups were established in this experiment: CK (control group), PA (adding 5×10^7 CFU/g FM *Enterococcus faecium*), EF (adding 5×10^7 CFU/g FM *Pediococcus lactis*), LP (adding 5×10^7 CFU/g FM *Lactiplantibacillus plantarum Lactobacillus plantarumas*), and PE (adding 5×10^7 CFU/g FM *Pediococcus lactis+Enterococcus faecium*,1:1). Each treatment was performed in three replicates. After 60 days of fermentation, sensory evaluation was performed to detect nutritional components, fermentation indicators, microbial content, and aerobic stability time. At 3d, 7d, 15d, 30d and 60d of fermentation, the water-soluble carbohydrate (WSC) content in PE group was significantly higher than that in CK group. The pH value and ammonia nitrogen content (NH_3-N) of all treatment groups were significantly lower than those of the CK group at 3d, 7d, 15d, 30d and 60d of fermentation. The number of lactic acid bacteria (LAB) in the PE, PA, and EF groups was significantly higher than that in the CK group ($P < 0.05$). The number of molds in all treatment groups was significantly lower than that in the CK group ($P < 0.05$). The number of aerobic bacteria (AB) in the PA, EF, and PE groups was significantly lower than that in the LP group ($P < 0.05$). The 72-hour in vitro gas production of *Cyperus esculentus* silage is higher than that of *Cyperus esculentus* stems and leaves. In summary, adding different lactic acid bacteria, especially endogenous ones, can effectively improve the quality of pea stem and leaf silage and improve the feeding value.

Keywords: *Cyperus esculentus* L., Silage, Lactobacillus, Fermentation quality, Subordinate function

INTRODUCTION

Currently, the primary approach used by researchers both domestically and internationally to improve silage quality is the addition of exogenous lactic acid bacteria (LAB) inoculants (Queirozo *et al*., 2013; Zhang *et al*., 2009). *Cyperus esculentus* stems and leaves represent high-quality silage raw materials: they are rich in soluble sugars, crude protein, and other nutrients that can meet the fermentation requirements of LAB, thereby ensuring silage quality. At the same time, their utilization enables the recycling of agricultural waste, providing low-cost feed for the livestock industry and alleviating environmental pressure. In practical applications, the specific method and dosage of inoculant addition must be comprehensively determined based on factors such as differences in silage raw material types and storage environmental conditions, and further adjusted and optimized according to actual silage outcomes to ensure that the final silage quality meets the expected goals (Li *et al*., 2020). Commonly used LAB inoculants at present are mainly composed of homofermentative LAB, often combined with sugar sources or cellulase to ensure the fermentation quality of *C. esculentus* stems and leaves (Jin *et al.,* 2020; He *et al*., 2022). However, research on the addition of excellent LAB naturally attached to the surface of *C. esculentus* stems and leaves remains relatively scarce, and systematic comparisons of the fermentation effects of endogenous bacteria are lacking. From a theoretical perspective, fermentation raw materials themselves carry a certain number of native LAB strains, which are more adapted to the plant's growth environment and thus more likely to survive stably therein (Amer *et al*., 2024). During the silage fermentation process, these naturally occurring LAB strains participate in the construction and dynamic evolution of the microbial community structure, a process that may have positive effects on the quality and functional characteristics of the final fermentation products (Wang *et al*., 2018). In contrast, exogenous LAB inoculants refer to microbial preparations obtained by artificially screening and culturing target LAB strains before adding them to the fermentation raw material. Although such exogenous inoculants can also exert the physiological functions of LAB during fermentation, their adaptability to the plant's own living environment is often somewhat inferior compared with LAB strains naturally attached to the raw material surface. In addition, the natural antimicrobial substances contained in *C. esculentus* itself can inhibit the growth and reproduction of some LAB strains, thereby making it difficult for the silage system to decrease to a suitable pH level and affecting the silage outcome (Chou *et al*., 2023).

Therefore, in the present study, two types of lactic acid bacteria tolerant to the antimicrobial substances of *C. esculentus*, namely Pediococcus acidilactici and Enterococcus faecium, were first isolated and screened from the natural silage system of *C. esculentus* stems and leaves. These two strains were then used as a specialized lactic acid bacteria inoculant and applied to the silage material of *C. esculentus* stems and leaves. Subsequently, by systematically analyzing the nutritional quality indices, fermentation characteristic parameters, and aerobic stability of the resulting silage products, the specific mechanisms through which these two lactic acid bacteria affect the silage performance of *C. esculentus* stems and leaves were further investigated, thereby providing a theoretical basis for improving the silage quality of this raw material.

MATERIALS AND METHODS

Experimental Materials

The *Cyperus esculentus* used in this study were sourced from the No. 6 planting area of the 54th Regiment, 3rd Division of the Xinjiang Production and Construction Corps, located at 38°60' N, 77°09' E, with an altitude of 1 231 m. The growing period in this region was from May 27 to October 7, 2024. On October 4, the stems and leaves of *C. esculentus* in this planting area were mown at a height of 3–4 cm above the ground, and then chopped to a length of 2–3 cm using a grinder with a kneading function. The processed stems and leaves were subsequently packed into transparent polyethylene bags measuring 30 cm × 40 cm × 0.24 mm, with each bag containing

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500 g ± 10 g of material. After sealing with a vacuum sealer, 10 silage bags were prepared for each treatment. All silage samples were fermented at 18–23 °C for 60 days, after which the bags were opened for aerobic stability analysis and sensory evaluation. The detailed nutritional composition of the silage raw material used in this experiment is shown in Tab. 1.

Two types of biological additives were used in this study. The first type consisted of two bacterial strains with good tolerance to the antimicrobial substances of *Cyperus esculentus*, which were isolated, identified, and screened from natural silage samples of *C. esculentus* stems and leaves, namely *Pediococcus acidilactici* (GenBank Accession No. OQ630001) and *Enterococcus faecium* (GenBank Accession No. OQ629997). The second type was a commercially available *Lactiplantibacillus* plantarum preparation purchased from Xinjiang Tiankang Biotechnology Co., Ltd., with a viable count of 3×10^9 colony-forming units (CFU)/g.

Experimental Design

A total of five treatment groups were established in this experiment: a control group (CK, sprayed with an equal volume of sterile water), EF (*Enterococcus faecium*, 5×10^7 CFU/g fresh matter, FM), PA (*Pediococcus acidilactici*, 5×10^7 CFU/g FM), LP (*Lactiplantibacillus plantarum*, 5×10^7 CFU/g FM), and PE (*P. acidilactici* + *E. faecium*, 1 : 1, 5×10^7 CFU/g FM), with five replicates per treatment. L. plantarum and E. faecium were cultured in MRS liquid medium, while *P. acidilactici* was cultured in M17 liquid medium. The viable cell count of each bacterial suspension was determined using the plate count method (Gao *et al*., 2022), and the required volume of each suspension was calculated based on the application rate per unit fresh weight for subsequent silage inoculation.For inoculation, the respective bacterial suspensions were uniformly sprayed onto the chopped C. esculentus stem and leaf material using a sterile sprayer. After thorough mixing for each treatment, 500 g of the inoculated material was weighed and packed into polyethylene bags (40 cm × 30 cm × 0.24 mm) equipped with a one-way exhaust valve, followed by vacuum sealing. All silage bags were then incubated at room temperature (20 ± 3 °C) for natural fermentation until the end of the fermentation period (Yang *et al*., 2024). Sampling was conducted on days 3, 7, 15, 30, and 60 of ensiling. At each sampling time point, three silage bags were randomly selected from each treatment group. The contents of each bag were thoroughly mixed, and analytical samples were collected to determine nutrient composition, fermentation quality parameters, and microbial community counts (Wang, 2024).

The experimental animals used in the in vitro gas production test were three healthy fistulated sheep from the Teaching and Experimental Station of the College of Animal Science and Technology, Shihezi University. This experiment was approved by the Animal Welfare Committee of Shihezi University, with the ethical approval number A2023-226. The sheep were fed three times daily at 08:00, 14:00, and 20:00, and had free access to water.

Within 2 hours after the morning feeding (08:00) of the experimental sheep, rumen fluid samples were collected from each of the three sheep via rumen fistula. After collection, all samples were thoroughly mixed and immediately transferred to a thermally insulated container for transport back to the laboratory. In the laboratory, the mixed rumen fluid was preprocessed by filtration through four layers of gauze. During the entire filtration process, CO_2 gas was continuously bubbled through the system to maintain a strictly anaerobic environment. To minimize the impact of handling on the samples, all the above procedures were performed rapidly in a constant-temperature water bath maintained at 39 °C.

Exactly 200 mg of *Cyperus esculentus* stem and leaf raw material and 200 mg of the same material after 60 d of ensiling were weighed separately using a precision balance. The two types of samples were then loaded into in vitro fermentation syringes, with six replicate syringes prepared for each sample. During each batch of incubation, six blank samples (containing culture medium only) were also set up in parallel. A small amount of medical-grade petroleum jelly was applied to the front one-third of the plunger of each syringe. The samples were then gently loaded into the syringes while the rubber tubing of each syringe was clamped to prevent sample overflow from the tip. Finally, the loaded syringes were placed in a constant-temperature water bath at 39 °C and preheated for 30 min (Liu *et al*., 2025).

Determination Indicators and Methods

To ensure the standardization and reliability of sensory evaluation, the scoring method proposed by the German Agricultural Society was adopted in this study. After opening the silage bags, the quality grade of the silage was comprehensively evaluated based on three dimensions of the *Cyperus esculentus* stem and leaf silage: odor characteristics, physical structure, and appearance color.

For the determination of nutritional components, classic and widely recognized methods were used for each index: dry matter (DM) content was measured by oven drying at 105 °C; crude protein (CP) content was determined using the Kjeldahl method; water-soluble carbohydrate (WSC) content was analyzed by the anthrone colorimetric method; neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were measured using the Van Soest method; and ash content was determined by ignition at 550 °C (GB/T 20806-2022).

The key fermentation parameters were determined using the following methods: the pH of the silage material was measured directly with a pH meter (Model pHs-3c, Leici); the contents of organic acids produced during fermentation, including lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA), were quantitatively analyzed by high-performance liquid chromatography (HPLC); and the change in ammonia nitrogen (NH_3-N) content in the silage system was determined by the phenol-hypochlorite colorimetric method (DB15/T 1458-2018).

The microbial community counts in the *Cyperus esculentus* stem and leaf silage were determined using the plate count method. For different types of microorganisms, specific media and culture conditions were used: aerobic bacteria (AB) were cultured on nutrient agar at 30 °C under aerobic conditions; lactic acid bacteria (LAB) were cultured on MRS medium at 37 °C under anaerobic conditions; yeasts were cultured on malt extract agar at 27 °C under aerobic conditions; and

Tab. 1: Nutrient composition of *cyperus esculentus* 1. silage raw material (DM/%)

Items	pH	DM/%	CP/% DM	EE/% DM	Ash/% DM	WSC/% DM	NDF/% DM	ADF/% DM
Content	6.75	37.16	11.98	3.13	13.27	17.96	49.37	27.61

molds were cultured on high-salt Czapek–Dox agar at 27 °C under aerobic conditions (General Administration of Quality Supervision, 2002).

During counting, intact single colonies were used as the identification standard, and plates with colony numbers in the range of 10–100 were selected for statistical analysis to ensure the accuracy of the results. The number of target

microorganisms per gram of fresh silage (FM), expressed as colony-forming units (CFU), was calculated using the following formula (DENG *et al*., 2023):

(1)

$$\text{Microbial count}(\text{CFU/g FM}) = \\ = \frac{\text{number of colonies} \times \text{dilution factor} \times 1000(\mu\text{L})}{\text{inoculation volume}(\mu\text{L})}$$

To accurately evaluate the aerobic stability of the silage, the relevant measurements were conducted on the 60th day of fermentation. First, the opening of each silage bag was carefully cleaned to reduce the risk of microbial and contaminant introduction and to minimize rapid moisture loss from the silage material (Wang *et al*., 2019). A multi-point temperature recorder was used to monitor temperature changes in the samples: multiple temperature probes were placed at the center of the material within each silage bag, while three additional probes were placed in the surrounding environment to track ambient temperature in real time. All probes were programmed to record temperatures at 10-minute intervals, and three sample probes were assigned to each treatment group. The aerobic stability time was defined as the time required for the temperature of the silage sample to rise 2 °C above the ambient temperature, which indicates the onset of spoilage and deterioration (Muck, 2013).

In Vitro Gas Production

The in vitro gas production method described by Menke *et al.* (Menke *et al.*, 1997) was used in this experiment to simulate the rumen fermentation environment. At different time points after the start of incubation, the gas production of each sample was recorded based on the readings from the glass syringes.

Data Analysis

The data were initially processed using Excel 2024. One-way analysis of variance (ANOVA) was performed using SPSS 26.0, and Duncan's multiple range test was applied for mean comparisons. Graphs were generated using Origin 2024.

For the comprehensive evaluation of silage quality and aerobic stability, the membership function method was used for quantitative analysis in this study. Among the indicators, those positively correlated with silage quality (including dry matter, crude protein, water-soluble carbohydrates, lactic acid, acetic acid, and aerobic stability time) were calculated using formula (2). In contrast, indicators negatively correlated with silage quality (including pH, ash, neutral detergent fiber, acid detergent fiber, and ammonia nitrogen) were calculated using formula (3) (Sun *et al*., 2021). After obtaining the membership function values for all indicators, the weighted average was calculated based on the corresponding weight of each indicator. The silage performance of different treatment groups was then ranked according to the magnitude of the weighted average (a higher average indicates better silage quality, and vice versa).

$$\mathrm{F_{ij}}^{+} = \frac{(\mathrm{X_{ij}} - \mathrm{X_{min}})}{(\mathrm{X_{max}} - \mathrm{X_{min}})} \quad \textbf{(2)}$$

$$\mathrm{F_{ij}}^{-} = 1 - \frac{(\mathrm{X_{ij}} - \mathrm{X_{min}})}{(\mathrm{X_{max}} - \mathrm{X_{min}})} \quad \textbf{(3)}$$

In the above formulas, F_{ij} represents the membership value of the *j*-th index for the *i*-th treatment; X_{ij} is the measured value of the *j*-th index for the *i*-th treatment; and X_{max} and X_{min} denote the maximum and minimum values, respectively, of the *j*-th index across all experimental treatment groups.

RESULTS AND ANALYSIS

Sensory Evaluation of *Cyperus esculentus* Stem and Leaf Silage During Fermentation Under Different Treatments

Tab. 2 presents the sensory evaluation results of the silage from each treatment group after 60 days of fermentation. The control group was predominantly yellowish-green with only a small amount of brown coloration, and its odor was a mixture of a weak sour smell and a faint grassy aroma. The material texture was relatively loose and not sticky to the touch. The LP group exhibited a yellowish-brown color, with a slightly stronger sour odor than the CK group, and the texture was soft and also non-sticky. The PA group maintained a yellowish-green appearance, with an odor combining a faint aroma and a distinct sourness, and the texture was similarly soft and non-sticky. The EF group showed a yellowish-brown color mixed with some green, and its sour aroma was less intense than that of the PA group; however, the material remained soft, only slightly moist, and not sticky. The PE group was yellowish-green with a clear sour aroma, and the texture was moderately soft and moist, also without stickiness. No mold was observed in any of the treatment groups during the sensory evaluation, indicating that all treatments met the basic storage requirements for silage.

Tab. 2: Sensory evaluation of *cyperus esculentus* l. silage

Treatment	Color	Texture	Scent	Aggregate score	Grades
CK	1	2	10	13	Second-class good
LP	2	2	11	15	First-classexcellent
PA	2	2	12	16	First-class good
EF	2	2	11	15	First-class good
PE	2	3	13	18	First-class good

Tab. 3: Changes of nutritional quality of *cyperus esculentus* silage with different additives

Items	Time	Treatments					SEM	*P*-value
		CK	PA	EF	LP	PE		
DM/%	3 d	32.18 ± 0.20^{b}	32.39 ± 0.27^{ab}	32.51 ± 0.18^{ab}	32.20 ± 0.19^{b}	32.69 ± 0.25^{a}	0.051	0.047
	7 d	30.66 ± 0.09^{c}	31.57 ± 0.18^{a}	31.55 ± 0.07^{a}	31.25 ± 0.06^{b}	31.67 ± 0.11^{a}	0.029	0.001
	15 d	30.67 ± 0.11^{c}	31.29 ± 0.16^{ab}	31.41 ± 0.11^{ab}	31.10 ± 0.16^{b}	31.56 ± 0.08^{a}	0.059	0.007
	30 d	29.83 ± 0.32^{c}	31.74 ± 0.16^{ab}	31.94 ± 0.09^{a}	31.34 ± 0.05^{b}	32.02 ± 0.22^{a}	0.060	0.000
	60 d	28.95 ± 0.15^{c}	31.07 ± 0.15^{b}	31.13 ± 0.06^{b}	30.70 ± 0.11^{b}	31.72 ± 0.15^{a}	0.058	0.000
CP/% DM	3 d	11.47 ± 0.06^{b}	11.67 ± 0.05^{a}	11.70 ± 0.03^{a}	11.61 ± 0.04^{ab}	11.74 ± 0.02^{a}	0.020	0.014
	7 d	11.25 ± 0.03^{c}	11.65 ± 0.04^{a}	11.67 ± 0.01^{a}	11.53 ± 0.02^{b}	11.69 ± 0.01^{a}	0..015	0.000
	15 d	10.94 ± 0.02^{c}	11.06 ± 0.04^{ab}	10.99 ± 0.02^{bc}	10.98 ± 0.04^{bc}	11.14 ± 0.04^{a}	0.016	0.022
	30 d	9.68 ± 0.02^{d}	9.82 ± 0.04^{c}	10.19 ± 0.05^{a}	9.96 ± 0.04^{b}	10.25 ± 0.03^{a}	0.018	0.000
	60 d	8.29 ± 0.02^{c}	8.62 ± 0.08^{b}	8.75 ± 0.04^{ab}	8.44 ± 0.01^{c}	8.87 ± 0.04^{a}	0.023	0.001
NDF/% DM	3 d	48.97 ± 0.04^{a}	48.73 ± 0.07^{a}	48.65 ± 0.06^{a}	48.88 ± 0.04^{a}	48.85 ± 0.06^{a}	0.026	0.215
	7 d	48.47 ± 0.19^{a}	47.42 ± 0.29^{a}	47.70 ± 0.15^{ab}	46.97 ± 0.15^{bc}	46.68 ± 0.18^{c}	0.089	0.008
	15 d	47.82 ± 0.24^{a}	46.38 ± 0.23^{abc}	46.30 ± 0.21^{bc}	46.71 ± 0.28^{ab}	45.72 ± 0.20^{c}	0.106	0.020
	30 d	46.80 ± 0.24^{a}	45.73 ± 0.26^{abc}	45.44 ± 0.31^{bc}	46.18 ± 0.15^{ab}	45.10 ± 0.34^{c}	0.123	0.032
	60 d	45.48 ± 0.29^{a}	43.86 ± 0.31^{b}	43.83 ± 0.11^{b}	44.01 ± 0.13^{b}	42.96 ± 0.15^{c}	0.098	0.003
ADF/% DM	3 d	27.51 ± 0.30^{a}	26.44 ± 0.19^{ab}	26.29 ± 0.17^{ab}	26.70 ± 0.47^{ab}	26.87 ± 0.15^{b}	0.131	0.152
	7 d	26.64 ± 0.19	26.54 ± 0.27	26.47 ± 0.22	26.52 ± 0.20	26.44 ± 0.28	0.114	0.586
	15 d	26.50 ± 0.27	26.48 ± 0.18	26.41 ± 0.21	26.48 ± 0.21	26.39 ± 0.14	0.094	0.912
	30 d	26.20 ± 0.29	26.21 ± 0.15	26.04 ± 0.19	26.06 ± 0.22	26.02 ± 0.16	0.095	0.476
	60 d	26.16 ± 0.21	26.05 ± 0.28	26.29 ± 0.33	26.02 ± 0.34	25.97 ± 0.32	0.155	0.961
WSC/% DM	3 d	12.28 ± 0.11^{c}	12.55 ± 0.08^{b}	12.62 ± 0.06^{ab}	12.40 ± 0.07^{bc}	12.85 ± 0.05^{a}	0.036	0.006
	7 d	9.32 ± 0.18^{a}	8.56 ± 0.09^{bc}	8.55 ± 0.14^{bc}	8.83 ± 0.14^{b}	8.31 ± 0.04^{c}	0.059	0.003
	15 d	8.92 ± 0.10^{a}	8.11 ± 0.09^{bc}	7.96 ± 0.07^{c}	8.50 ± 0.25^{ab}	7.51 ± 0.08^{d}	0.063	0.000
	30 d	5.97 ± 0.09^{c}	6.77 ± 0.33^{ab}	6.65 ± 0.28^{bc}	6.21 ± 0.10^{bc}	7.39 ± 0.03^{a}	0.094	0.006
	60 d	4.09 ± 0.05^{d}	5.84 ± 0.04^{b}	5.82 ± 0.08^{b}	5.19 ± 0.08^{c}	6.46 ± 0.15^{a}	0.042	0.000
Ash/% DM	3 d	13.09 ± 0.08^{a}	12.96 ± 0.04^{a}	12.95 ± 0.06^{a}	13.00 ± 0.06^{a}	12.62 ± 0.16^{b}	0.042	0.047
	7 d	13.03 ± 0.06^{a}	12.68 ± 0.11^{ab}	12.80 ± 0.14^{ab}	12.92 ± 0.08^{ab}	12.52 ± 0.16^{b}	0.054	0.095
	15 d	12.87 ± 0.06^{a}	12.59 ± 0.06^{b}	12.60 ± 0.06^{b}	12.78 ± 0.07^{ab}	12.37 ± 0.06^{c}	0.029	0.003
	30 d	12.59 ± 0.08	12.54 ± 0.05	12.51 ± 0.08	12.52 ± 0.12	12.32 ± 0.02	0.036	0.243
	60 d	12.29 ± 0.02	12.27 ± 0.02	12.30 ± 0.02	12.29 ± 0.01	12.24 ± 0.03	0.011	0.497

Note: Different lowercase letters as superscripts in the same row of data indicate significant differences (P<0.05), while the same lowercase letters or no letters indicate no significant differences (P>0.05). Same as below.

Changes in Nutritional Quality of *Cyperus Esculentus* Silage During Fermentation Under Different Treatments

Tab. 3 shows the changes in dry matter, crude protein, neutral detergent fiber, acid detergent fiber, water-soluble carbohydrates, and ash contents in each treatment group during fermentation. After 60 days of fermentation, the DM content in all treatment groups decreased compared with the initial stage of ensiling.Regarding the differences among groups at different fermentation stages: on days 3 and 7 of ensiling, the DM content in the PE group was significantly higher than that in the control group and the LPgroup ($P < 0.05$), while no significant differences were observed among the other treatment groups ($P > 0.05$). At day 30 of fermentation, the DM contents in the EF group and the PE group showed significant advantages and were significantly higher than that in the LP group ($P < 0.05$). By day 60, the DM content in the PE group was significantly higher than that in all other treatment groups ($P < 0.05$), indicating that this mixed-culture treatment was the most effective in reducing DM loss during ensiling of *Cyperus esculentus* stems and leaves.

Throughout the entire ensiling period, the CP content showed an overall trend of gradual decline, and this downward trend began to level off after 30 days of fermentation. On day 7 of ensiling, the CP content in the LP group was significantly lower than that in the PE group, the EF group, and the PA group ($P < 0.05$), while no significant differences were observed among the other groups ($P > 0.05$). At day 15 of fermentation, the CP content in the PE group showed a significant advantage and was significantly higher than that in the EF and LP groups ($P < 0.05$). By day 60, the CP content in the PE group remained significantly higher than that in the PA group ($P < 0.05$).

During the ensiling process, the contents of NDF and ADF showed a consistent trend, both decreasing continuously from the start to the end of fermentation. On day 3 of fermentation, there were no significant differences in NDF content among the treatment groups ($P > 0.05$). For ADF content, however, the PE group was significantly lower than the control group ($P < 0.05$), while the other treatment groups (EF, PA, LP) showed no significant differences compared with either PE or CK ($P > 0.05$).At days 15 and 30 of fermentation, the NDF content in the PE group exhibited a significant advantage and was significantly lower than that in the CK and LP groups ($P < 0.05$). By day 60, the NDF content in the PE group remained significantly lower than that in the CK group and all other single-strain treatment groups

($P < 0.05$).After the entire fermentation period, the PE group had the lowest NDF and ADF contents among all treatments respectively. These results indicate that the mixed-culture treatment was the most effective in degrading the fibrous components of *Cyperus esculentus* stems and leaves during ensiling.

Throughout the entire fermentation period, the WSC content showed a continuous downward trend. By day 7 of ensiling, the WSC content in all lactic acid bacteria treatment groups had decreased significantly compared with day 3, and all treatment groups had significantly lower WSC contents than the control group ($P < 0.05$).At day 15 of fermentation, the WSC content in the PE group was significantly lower than that in the CK group and all other single-strain treatment groups ($P < 0.05$), indicating that the PE group had the highest WSC consumption efficiency at this stage.By day 30, the trend in WSC content had shifted: the PE group exhibited a significantly higher WSC content than the PA, EF, and CK groups ($P < 0.05$).At day 60, the WSC content in the CK group was significantly lower than that in all LAB-treated groups ($P < 0.05$). Among the LAB treatments, the PE group had a significantly higher WSC content than the CK group and all other single-strain groups ($P < 0.05$), while the LP group had a significantly lower WSC content than the PA and EF groups ($P < 0.05$). These results suggest that different LAB strains exhibit significant differences in their efficiency of WSC utilization during ensiling.

Throughout the entire fermentation period, the ash content showed only minor fluctuations. On day 3 of ensiling, the ash content in the PE group was significantly lower than that in the other treatment groups ($P < 0.05$). On day 7, the ash content in the control group was significantly higher than that in the PE group, while no significant differences were observed between the remaining treatment groups and either CK or PE ($P > 0.05$).At day 15 of fermentation, the ash contents in the LP and CK groups were significantly higher than that in the PE group ($P < 0.05$), and the PE group maintained a significantly lower ash content compared with the other treatments ($P < 0.05$).By days 30 and 60 of fermentation, there were no significant differences in ash content between any of the lactic acid bacteria-treated groups and the CK group ($P > 0.05$).

Changes in Fermentation Quality of Cyperus Esculentus Stem and Leaf Silage During Ensiling Under Different Treatments

The changes in pH and NH_3-N content after the addition of different lactic acid bacteria treatments are shown in Tab. 4. With respect to pH, the values in all lactic acid bacteria-treated groups and the control group gradually decreased with increasing fermentation time. Throughout the entire ensiling period, the pH in the LAB-inoculated groups remained consistently lower than that in the CK group.By day 60 of fermentation, the pH of the PE group had dropped to the lowest level among all treatments (4.87), whereas the CK group exhibited the highest pH (6.45).

In terms of NH_3-N content, the PE group showed a significant advantage: throughout the entire fermentation process, the NH_3-N content in the PE group remained significantly lower than that in all other treatment groups ($P < 0.05$). At day 60 of

Tab. 4: Changes changes in fermentation quality of *cyperus esculentus* stem and leaf silage during fermentation under different treatments

Items	Time	Treatments					SEM	*P*-vale
		CK	PA	EF	LP	PE		
pH	3 d	6.70 ± 0.01^{a}	6.59 ± 0.01^{c}	6.58 ± 0.01^{c}	6.63 ± 0.01^{b}	6.49 ± 0.01^{d}	0.002	0.001
	7 d	6.69 ± 0.01^{a}	6.41 ± 0.01^{c}	6.40 ± 0.01^{c}	6.57 ± 0.02^{b}	6.17 ± 0.01^{d}	0.002	0.000
	15 d	6.63 ± 0.01^{a}	6.21 ± 0.01^{c}	6.19 ± 0.01^{c}	6.31 ± 0.01^{b}	6.07 ± 0.01^{d}	0.002	0.000
	30 d	6.53 ± 0.01^{a}	6.10 ± 0.02^{c}	6.11 ± 0.01^{c}	6.22 ± 0.01^{b}	5.53 ± 0.01^{d}	0.002	0.000
	60 d	6.45 ± 0.01^{a}	5.47 ± 0.01^{c}	5.62 ± 0.01^{c}	6.01 ± 0.01^{b}	4.87 ± 0.01^{d}	0.001	0.000
LA/g/kg	3 d	0.31 ± 0.01^{d}	0.41 ± 0.01^{b}	0.41 ± 0.01^{b}	0.37 ± 0.01^{c}	0.48 ± 0.01^{a}	0.004	0.000
	7 d	0.45 ± 0.02^{bc}	0.53 ± 0.02^{ab}	0.53 ± 0.01^{ab}	0.43 ± 0.02^{c}	0.58 ± 0.03^{a}	0.012	0.012
	15 d	0.47 ± 0.01^{d}	0.64 ± 0.01^{b}	0.65 ± 0.02^{b}	0.52 ± 0.02^{c}	0.72 ± 0.02^{a}	0.008	0.000
	30 d	0.50 ± 0.01^{d}	0.86 ± 0.02^{b}	0.86 ± 0.03^{b}	0.63 ± 0.01^{c}	0.98 ± 0.02^{a}	0.015	0.000
	60 d	0.53 ± 0.01^{d}	0.97 ± 0.02^{b}	1.04 ± 0.05^{b}	0.72 ± 0.02^{c}	1.28 ± 0.03^{a}	0.003	0.000
AA/g/kg	3 d	0.17 ± 0.01^{a}	0.14 ± 0.01^{b}	0.07 ± 0.01^{d}	0.12 ± 0.01^{bc}	0.11 ± 0.01^{bc}	0.001	0.008
	7 d	0.22 ± 0.01^{a}	0.23 ± 0.01^{a}	0.17 ± 0.01^{b}	0.21 ± 0.01^{a}	0.23 ± 0.02^{a}	0.005	0.022
	15 d	0.27 ± 0.01^{b}	0.32 ± 0.02^{ab}	0.31 ± 0.01^{ab}	0.26 ± 0.01^{b}	0.34 ± 0.02^{a}	0.007	0.022
	30 d	0.25 ± 0.02^{d}	0.37 ± 0.01^{b}	0.37 ± 0.01^{b}	0.31 ± 0.01^{c}	0.45 ± 0.02^{a}	0.006	0.000
	60 d	0.26 ± 0.01^{d}	0.46 ± 0.01^{b}	0.49 ± 0.01^{ab}	0.38 ± 0.01^{c}	0.52 ± 0.01^{a}	0.006	0.000
NH3-N/g/kg	3 d	0.07 ± 0.01^{a}	0.03 ± 0.00^{b}	0.03 ± 0.01^{b}	0.03 ± 0.01^{b}	0.03 ± 0.00^{b}	0.003	0.006
	7 d	0.11 ± 0.01^{a}	0.07 ± 0.00^{bc}	0.07 ± 0.01^{bc}	0.09 ± 0.01^{ab}	0.06 ± 0.00^{c}	0.004	0.009
	15 d	0.12 ± 0.01^{a}	0.11 ± 0.01^{a}	0.10 ± 0.01^{a}	0.11 ± 0.01^{a}	0.07 ± 0.01^{b}	0.003	0.008
	30 d	0.17 ± 0.01^{a}	0.11 ± 0.01^{b}	0.12 ± 0.01^{b}	0.12 ± 0.01^{b}	0.08 ± 0.01^{c}	0.002	0.000
	60 d	0.23 ± 0.01^{a}	0.18 ± 0.01^{b}	0.18 ± 0.01^{b}	0.19 ± 0.01^{ab}	0.17 ± 0.00^{b}	0.004	0.012

Tab. 6: Changes in the number of microorganisms during silage fermentation of *cyperus esculentus* stems and leaves

Items	Time	Treatments					SEM	P-value
		CK	PA	EF	LP	PE		
LAB (log CFU/g FM)	3 d	8.25^{d}	8.63^{b}	8.65^{b}	8.49^{c}	8.78^{a}	0.014	< 0.001
	7 d	8.28^{d}	8.92^{ab}	8.87^{ab}	8.63^{c}	8.98^{a}	0.010	< 0.001
	15 d	8.42^{b}	9.49^{a}	9.50^{a}	9.47^{a}	9.51^{a}	0.029	< 0.001
	30 d	8.17^{d}	9.11^{bc}	9.20^{ab}	8.97^{c}	9.23^{ab}	0.021	< 0.001
	60 d	7.74^{c}	8.71^{b}	8.77^{b}	8.69^{b}	8.88^{a}	0.012	< 0.001
Yeast (log CFU/g FM)	3 d	5.03^{a}	4.55^{c}	4.78^{b}	4.89^{ab}	4.41^{c}	0.030	< 0.001
	7 d	5.27^{a}	4.93^{bc}	5.07^{ab}	5.22^{a}	4.76^{c}	0.033	0.001
	15 d	4.84^{b}	4.70^{c}	4.58^{d}	4.80^{bc}	7.74^{a}	0.016	0.001
	30 d	4.54^{a}	4.31^{c}	4.27^{c}	4.41^{b}	4.33^{bc}	0.011	< 0.0001
	60 d	3.80^{ab}	3.62^{cd}	3.54^{d}	3.72^{bc}	3.65^{cd}	0.009	0.001
Mold (log CFU/g FM)	3 d	3.80^{c}	3.86^{abc}	3.89^{abc}	3.92^{ab}	3.85^{bc}	0.025	0.062
	7 d	3.83^{a}	3.29^{c}	3.54^{b}	3.30^{c}	3.57^{b}	0.013	< 0.001
	15 d	4.14^{a}	3.53^{c}	3.60^{c}	3.87^{b}	3.51^{d}	0.026	< 0.001
	30 d	3.77^{a}	< 2.00	< 2.00	3.62^{ab}	< 2.00	0.007	0.076
	60 d	4.15^{a}	< 2.00	< 2.00	3.71^{b}	< 2.00	0.053	0.022
AB (log CFU/g FM)	3 d	9.09^{d}	9.21^{c}	9.19^{c}	9.45^{a}	9.33^{b}	0.005	< 0.001
	7 d	8.81^{a}	8.39^{d}	8.39^{d}	8.56^{b}	8.46^{c}	0.003	< 0.001
	15 d	8.38^{b}	8.45^{b}	8.66^{a}	8.30^{b}	8.32^{b}	0.016	< 0.001
	30 d	8.36^{ab}	8.11^{c}	8.13^{c}	8.36^{ab}	8.26^{b}	0.014	< 0.001
	60 d	7.86^{a}	7.53^{bc}	7.40^{c}	7.57^{b}	7.41^{c}	0.016	< 0.001

Tab. 5: Subordinate function analysis of different treatments on silage quality of cyperus esculentus

Index	Treatment				
	CK	PA	EF	LP	PE
pH	0.01	0.81	0.78	0.62	0.98
LA	0.25	0.91	0.93	0.89	0.96
AA	0.04	0.86	0.88	0.77	0.89
DM	0.18	0.85	0.85	0.76	0.92
CP	0.22	0.89	0.89	0.65	0.95
Ash	0.94	0.54	0.56	0.56	0.55
NH_3-N	0.22	0.90	0.91	0.88	0.97
WSC	0.02	0.86	0.84	0.53	0.96
NDF	0.43	0.70	0.73	0.64	0.89
ADF	0.64	0.70	0.43	0.73	0.89
Aerobic stability	0.03	0.86	0.81	0.56	0.97
Average	0.25	0.82	0.81	0.70	0.89

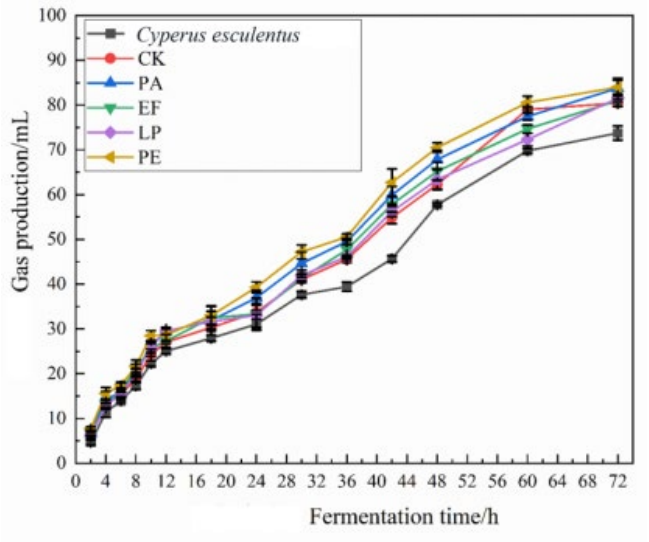

Fig. 1: In vitro cumulative gas production curves of *cyperus esculentus* and its silage

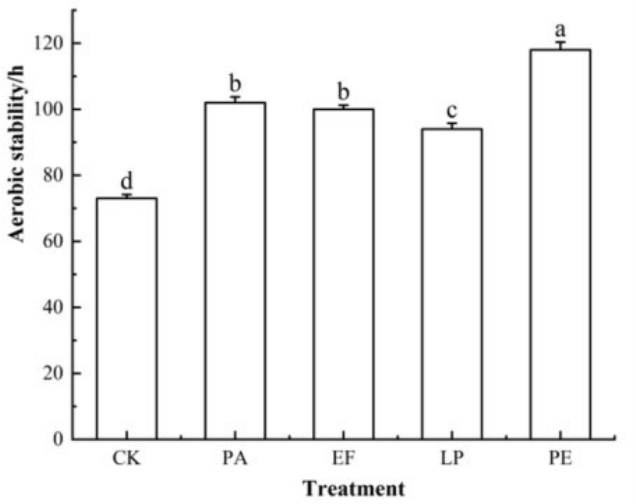

Fig. 2: Aerobic stability of *cyperus esculentus* silage

fermentation, the NH_3-N content in the control group was significantly higher than that in the PA, EF, and PE groups ($P < 0.05$).

With respect to lactic acid content, all treatment groups showed a gradual increase over the course of fermentation. Among these, the PE group maintained a significantly higher lactic acid content than all other treatments throughout the entire ensiling period ($P < 0.05$). The lactic acid contents in the PA and EF groups were significantly higher than those in the LP group and the control group ($P < 0.05$).By day 60 of fermentation, the lactic acid contents of the treatment groups were ranked from highest to lowest as follows: PE > EF > PA > LP > CK. These results further indicate that the PE treatment was the most effective in promoting lactic acid accumulation during ensiling.

In terms of acetic acid content, all treatment groups also exhibited a gradual increase throughout the entire ensiling period. At day 60 of fermentation, the acetic acid content in the PE group showed a significant advantage and was significantly higher than that in all other treatment groups except the EF group, as well as the control group ($P < 0.05$). However, there was no significant difference in acetic acid content between the EF and PE groups ($P > 0.05$), suggesting that the mixed culture and Enterococcus faecium have similar effects in promoting acetic acid synthesis during ensiling.

Changes in Microbial Counts During Fermentation Under Different Treatments

As shown in Tab. 5, the lactic acid bacteria (LAB) counts in all treatment groups increased gradually during the first 30 days of fermentation, but began to decline after day 30. From day 7 to day 60 of ensiling, the LAB counts in all LAB-inoculated groups were significantly higher than those in the control group (CK) ($P < 0.05$).On day 7 of ensiling, the LAB counts in the PE group,PA group,and EF group were significantly higher than those in the LP group ($P < 0.05$). On day 15, there were no significant differences in LAB counts among the treatment groups ($P > 0.05$). By day 60, the PE group had the highest LAB count among all treatments ($P < 0.05$).

The yeast and aerobic bacteria (AB) counts showed a continuous decreasing trend throughout the entire ensiling period. On day 15 of fermentation, except for the PA group, the yeast count in the EF group was significantly lower than that in the control group and the other treatments ($P < 0.05$). On day 30, the yeast counts in all lactic acid bacteria-treated groups were significantly lower than that in the CK group ($P < 0.05$), but differences existed among the LAB groups; the yeast count in the LP group was significantly higher than those in the PA and EF groups ($P < 0.05$). By day 60, the CK group had the highest yeast count among all treatments.

By day 7 of fermentation, the differences among groups had changed: the aerobic bacteria (AB) counts in the PA, EF, and PE groups were significantly lower than that in the LP group ($P < 0.05$), while no significant differences were observed among the PA, EF, and PE groups themselves ($P > 0.05$).At day 15 of ensiling, the AB count in the EF group was significantly higher than those in the other treatment groups ($P < 0.05$).By day 30 of fermentation, the AB counts in the PA and EF groups had become significantly lower than those in the remaining treatment groups ($P < 0.05$).

In vitro Cumulative Gas Production of *Cyperus esculentus* and Its Silage

As shown in Fig. 1, the in vitro gas production of both *Cyperus esculentus* and its silage gradually increased with the extension of fermentation time, reaching the maximum value at 72 h, after which the gas production tended to stabilize. Throughout the entire fermentation process, the gas production of the silage was consistently higher than that of the original *Cyperus esculentus,* and the PE treatment group exhibited the highest gas production.

Effects of Different Treatments on the Aerobic Stability of *Cyperus esculentus* Stem and Leaf Silage

In terms of differences among groups, there was no significant difference in aerobic stability time between the EF and PA groups ($P > 0.05$), whereas significant differences were observed among all other treatment groups($P < 0.05$). Notably, the aerobic stability time of all lactic acid bacteria (LAB)-inoculated groups was extended by 29–46 h compared with the control group. Among these, the PE group, which received both *Pediococcus acidilactici* and *Enterococcus faecium*, exhibited a significantly longer aerobic stability time than all other treatment groups ($P < 0.05$), indicating that this mixed-culture treatment was the most effective in improving the aerobic stability of *Cyperus esculentus* stem and leaf silage.

Comprehensive Evaluation of *Cyperus Esculentus* Stem and Leaf Silage Quality Under Different Treatments

A membership function-based comprehensive analysis method was employed to construct a comprehensive evaluation system using 11 key indicators. The specific evaluation results are presented in Tab. 6. In this method, the comprehensive evaluation score serves as a quantitative criterion for assessing silage quality, with higher scores indicating superior silage quality and lower scores indicating poorer quality.

After 60 days of fermentation, the comprehensive ranking of silage quality for *Cyperus esculentus* stem and leaf silage across the different treatment groups was as follows: PE group > PA group > EF group > LP group > CK group.

DISCUSSION

Effects of Lactic Acid Bacteria on the Nutritional Quality of *Cyperus esculentus* Stem and Leaf Silage

The core objective of adding lactic acid bacteria inoculants during the silage of *Cyperus esculentus* stems and leaves is to facilitate rapid and efficient fermentation. It accelerates lactic acid accumulation and pH decline in the early silage stage, inhibits the proliferation of undesirable microorganisms, thereby extending the effective storage period of silage, maintaining its quality stability, and preventing rapid deterioration (Sharp *et al*., 1994). *Pediococcus acidilactici* and *Enterococcus faecium* used in this study are both homofermentative LAB, capable of normal growth under low pH conditions. Their inoculation can promote the proliferation of beneficial LAB in the silage system, inhibit the activity of harmful microorganisms such as Clostridium and aerobic bacteria, reduce gas production and dry matter loss during silage, and improve the silage quality of *Cyperus esculentus* stems and leaves (Zhao *et al*., 2017). When LAB multiply to a certain population size, they inhibit the metabolism of undesirable bacteria through nutrient competition and acidic environment construction, reducing the inefficient consumption of WSC (Li *et al*., 2022). This explains the significant slowdown in the WSC degradation rate of all treatment groups after 30 days of silage observed in this study. Homofermentative LAB can reduce protein hydrolysis in silage (Zhang *et al*., 2023), which is consistent with the results of this study: the CP content in all LAB-inoculated groups was significantly higher than that in the control group, indicating that inoculation effectively reduces protein degradation loss.NH_3-N is a key indicator of silage quality; higher levels denote more extensive amino acid and protein decomposition, indicating poor fermentation (Zhao *et al*., 2017). This degradation during silage is mainly driven by undesirable microbial metabolism (Niu *et al*., 2018). Thus, regulating the silage microbial community and inhibiting harmful microbes are critical to reducing NH_3-N production. Additionally, standardized silage preparation and controlled storage conditions can further mitigate protein and amino acid degradation, enhancing overall silage efficacy (Jiang *et al*., 2020).

Based on the results obtained in this study, inoculation with LAB was able to reduce the loss of CP in *Cyperus esculentus* stem and leaf silage to a certain extent. From a mechanistic perspective, LAB can convert part of the protein in the silage into lactic acid and other organic acids through their fermentation metabolism (Wang *et al*., 2021). This transformation effectively reduces the production of NH_3-N in the system. Therefore, the relatively lower proportion of NH_3-N in the LAB-inoculated silage groups directly indicates less extensive protein degradation and superior fermentation quality. In addition, the results of the present study also showed that all LAB inoculation treatments decreased the NDF and ADF contents of *Cyperus esculentus* silage. In the feed nutritional evaluation system, NDF and ADF contents are key indicators affecting feed intake and digestibility in livestock (Liu *et al*., 2020). Higher NDF and ADF contents indicate a greater proportion of fibrous material, which may limit feed intake and reduce digestibility. Moreover, factors such as chop length, limitations in the fibrolytic enzyme activity of the strains used, and insufficient fermentation duration can also influence NDF and ADF contents in the final silage. Feed with high fiber content is difficult for the animal digestive system to fully break down and utilize. Particularly for ruminants, the digestion of such feed requires a longer retention time in the rumen and involves more extensive microbial metabolism (Suwignyo *et al*., 2021). In contrast, when NDF and ADF contents are lower, the proportion of fibrous material is reduced, making the feed easier for animals to consume and improving its digestibility and absorption efficiency. This, in turn, can promote animal growth, development, and overall production performance (Hu *et al*., 2020; Xian *et al*., 2022).

Effects of Lactic Acid Bacteria on the Fermentation Quality and Microbial Counts of *Cyperus esculentus* Stem and Leaf Silage

The results of this study showed that after 60 days of fermentation, the pH of the PE group had dropped to the lowest level among all treatments (4.87), although this value still did not meet the pH standard for high-quality silage (Sun *et al.*, 2023). It is worth noting that the rate of pH decline during *Cyperus esculentus* ensiling was slower than that observed with other conventional silage materials, and the overall pH remained relatively high. This observation is consistent with the findings of previous studies (Li *et al.*, 2020). Inoculation with the composite LAB additive significantly reduced the pH of *Cyperus esculentus* silage, which laid a favorable foundation for further improving its silage quality in subsequent research. Based on these results, future experiments could attempt to combine molasses with composite LAB additives(Li *et al*., 2022) to investigate the potential synergistic effects of this combination on the fermentation quality of *Cyperus esculentus* silage.

From the perspective of the effects of different LAB inoculation types, both the single addition of endogenous dominant LAB and the application of composite LAB preparations significantly reduced the pH of *Cyperus esculentus* stem and leaf silage. However, it should be noted that the pH in the treatment group supplemented with commercial LP remained relatively higher compared with the other LAB-treated groups. This result suggests that the secondary metabolites naturally abundant in *Cyperus esculentus*, such as phenolic compounds, flavonoids, and saponins—all of which possess broad-spectrum antimicrobial activity—may have inhibited the normal growth and metabolism of the commercial *L. plantarum* strain to some extent through multiple mechanisms, including membrane disruption and the inhibition of key enzyme activities (Yang *et al.*, 2022). Therefore, to mitigate the inhibitory effects of these endogenous substances on LAB activity and further optimize the silage quality of *Cyperus esculentus* stems and leaves, future studies could consider adopting a combined approach involving both commercial LAB additives and endogenous dominant LAB strains.

The leaves of *Cyperus esculentus* are rich in various antioxidant substances, including flavonoids, carotenoids, coumarins, phenolics, alkaloids, terpenoids, and anthraquinones (Yan *et al*., 2010). Among these, flavonoids and cardiac

glycosides have well-documented effects against certain diseases. Previous studies have confirmed that flavonoids possess strong antimicrobial activity, exerting significant inhibitory effects on most bacteria and molds, with bacteria generally being more susceptible to flavonoid inhibition than molds (Yan *et al*., 2010;.Jing *et al*., 2016). In the present study, two endogenous LAB strains isolated from the natural ensiling system of *Cyperus esculentus* stems and leaves, together with one commercial *Lactiplantibacillus plantarum* strain, were used as silage fermentation additives. The results showed that the endogenous dominant LAB and the mixed LAB additive were better able to adapt to the internal environment of *Cyperus esculentus* stem and leaf silage: compared with the commercial *L. plantarum* treatment and the control group, these two treatment groups exhibited significantly lower pH values and significantly higher LAB counts. Notably, the silage quality of *Cyperus esculentus* stems and leaves treated with commercial *L. plantarum* was significantly inferior to that of the groups treated with endogenous LAB. This difference is presumably related to the structural characteristics and active substances of *Cyperus esculentus* itself—the externally added commercial *L. plantarum* may have been affected by the antimicrobial substances present in the stems and leaves, leading to inhibited growth and reproduction of the strain, which in turn resulted in a slower decline in pH during silage fermentation.

Although the pH values of all treatment groups in this study remained higher than the standard for high-quality silage, no spoilage was observed throughout the fermentation process, and no mold or decay was detected at the end of fermentation. This phenomenon may be closely related to the inherent antimicrobial components of *Cyperus esculentus* itself. Previous studies have found that ethanol extracts of *Cyperus esculentus* leaves exhibit significant inhibitory effects against *Shigella*, *Salmonella*, *Staphylococcus aureus*, and *Escherichia coli* (Jing *et al*., 2016).

In vitro Cumulative Gas Production of Cyperus esculentus and Its Silage

The gas produced during in vitro fermentation mainly consists of carbon dioxide and methane. From a source perspective, gas production is closely related to the carbohydrates and CP present in the forage, as these are the primary substrates for microbial fermentation. The cumulative gas production can reflect the fermentability of the forage, and this indicator is mainly influenced by two factors: the actual content of fermentable organic matter and the activity level of rumen microorganisms. Under relatively consistent conditions, higher-quality forage generally produces a greater volume of gas in the rumen environment. In the present study, the in vitro gas production of *Cyperus esculentus* silage at 72 h was higher than that of the original *Cyperus esculentus* material, indicating that the digestibility of *Cyperus esculentus* was improved after ensiling.

CONCLUSION

The three LAB strains used in this study all improved the quality of *Cyperus esculentus* stem and leaf silage and extended its aerobic stability to varying degrees. Among these, the endogenous LAB strains isolated from the natural ensiling system of *Cyperus esculentus* stems and leaves exhibited better performance than the commercial LAB strain. In terms of strain combinations, the mixed inoculation of *Pediococcus acidilactici* and *Enterococcus faecium* was more effective than singlestrain inoculation. The comprehensive ranking of silage quality after inoculation with different strains was as follows: PE > PA > EF > LP > CK. This further confirms the advantage of using endogenous mixedculture LAB to optimize the ensiling effect of *Cyperus esculentus* stem and leaf silage.

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