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ORIGINAL PAPER

Effect of inoculations with different lactic acid bacteria on the fermentation profile and quality of high-moisture fodder pea (*Pisum sativum* L.) silage

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ABSTRACT. One of the efficient techniques for preserving high moisture forages is ensiling. However, the successful ensiling progress of legumes largely depends on the epiphytic microbial flora, buffering capacity, and the water soluble carbohydrate content of the ensiled forage. In this study, three selected strains of lactic acid bacteria (LAB), were used as microbial additives (at 10^6 CFU/g fresh matter) to fodder pea (*Pisum sativum* L.). These strains included *Lactobacillus bifermentans* (LS-65-2-2) and *Lactobacillus plantarum* (LS-72-2), both isolated from rangelands in Turkiye, along with *Bacillus subtilis*, which is already applied for these purposes. The aim was to assess the effects of these strains on microbial composition and the quality of the resulting silage. Silage opening was conducted at five time points (on days 0, 2, 5, 7 and 45) with three replicates. The effects of LAB inoculations were determined to be statistically different ($P < 0.001$). The study results demonstrated the following values of the parameters tested: pH (4.52–4.86), lactic acid bacteria (5.51–8.46 $\log_{10}$ CFU/g silage), enterobacteria (2.24–3.61 $\log_{10}$ CFU/g silage), yeasts (6.20–7.03 $\log_{10}$ CFU/g silage), neutral detergent fibre (38.85–41.93%), acid detergent fibre (ADF, 32.91–35.75%), and relative feed value (RFV, 135.90–151.73). LAB inoculations caused a significant decrease in pH and an increase in dry matter (DM) recovery ($P < 0.001$) in fodder pea silage compared to the control. The abundance of LAB in the silages increased significantly ($P < 0.001$), while the content of enterobacteria ($P < 0.001$), pH, NH_3-N ($P < 0.01$) and ADF ($P < 0.05$) in inoculated silages decreased. The RFV significantly improved following inoculation with the *L. bifermentans* strain. Overall, the addition of LAB strains improved the fermentation process and silage quality compared to *B. subtilis*, as well as enhanced DM recovery and reduced silage pH.

Introduction

Silage is an important method of preserving highmoisture forage crops, significantly reducing quality and nutrient losses compared to haymaking. Silage quality largely depends on the flora of epiphytic microorganisms present on the forage, such as lactic acid bacteria, enterobacteria, moulds, and yeasts. Lactic acid bacteria convert water-soluble carbohydrates (WSC) into lactic acid, which facilitates a rapid decrease in silage pH. Fodder pea (*Pisum sativum* L.) is valued as a forage legume for its high protein content compared to many other forage crops (Blagojević et al., 2017). However, it is difficult to ensile due to its relatively low WSC content (Canpolat et al., 2019), high buffering

capacity (Fraser et al., 2001), and low DM content at harvest. The use of microbial inoculants has the potential to improve silage quality prepared from fodder pea plant. These inoculants can alter many silage quality parameters, although the magnitude of their effects on fermentation profiles depends heavily on the characteristics of the strains used (Ertekin and Kizilsimsek, 2020; Günaydın et al., 2023; Akbay et al., 2023a,b). Lactic acid bacteria (LAB) strains have been classified into homofermentative and heterofermentative based on their physiological characteristics. Homo-LAB strains, such as *Lactobacillus*, *Enterococcus* and *Pediococcus* are widely used as silage inoculants due to their rapid and efficient production of lactic acid (2 mol) from glucose (1 mol) (Weinberg and Muck, 1996; Muck, 2010; Ellis et al., 2016). *Bacillus subtilis*, traditionally used as a direct feed supplement (Zhang et al., 2016), or as a bacterial inoculant in biological feeds for ruminants, has been classified as a fourth-generation strain silage inoculant (Bai et al., 2022) due to its potential to enhance animal performance (Zhang et al., 2016) and improve fermentation quality (Bai et al., 2021). The aim of this study was to determine the impact of selected LAB strains – *Lactobacillus bifermentans* and *Lactobacillus plantarum*, isolated from grassland flora – on the fermentation process and silage quality of high-moisture fodder pea and compare them to the currently utilised *B. subtilis*.

Material and methods

Silage raw material and LAB strains

The Taskent fodder pea (*Pisum sativum* L.) cultivar was grown in 2022 at the Experimental Farm of the University of Kahramanmaraş Sutcu Imam University in Southern Turkiye under rainfed growing conditions. The plants were harvested in the early morning hours on 25 May during the bottom pod formation stage. The *B. subtilis* KUEN 1581 inoculant, with a density of 2×10^9 CFU/g, was obtained from SIM Silage (Kahramanmaraş, Turkiye). *L. bifermentans* and *L. plantarum* isolated from Turkiye grassland flora under a project supported by the Turkiye Scientific and Technical Research Organization (TUBITAK) were used as microbial inoculants. *L. bifermentans* (LS-65-2-2) and *L. plantarum* (LS-72-2) were regenerated in MRS (De Man, Rogosa ve Sharpe) broth in 400 ml bottles by incubation at 37 °C for 48 h. Cell densities were determined by cultivation on MRS agar medium.

Silage preparation and microbial and chemical analyses

Each *Lactobacillus* strains was added to 4000 g of fresh fodder pea plant material at a theoretical concentration of 10^6 CFU/g ensuring thorough mixing by hand in sterile gloves. All inoculants were diluted with 10 ml of distilled water, and for the control silages, 10 ml of deionised water was used in place of inoculants. The plant material was chopped into 2–4 cm fragments and ensiled in vacuum-sealed plastic bags. Approximately 400 g of fresh forage material was placed into each bag. A total of 60 vacuumed silage packages were prepared, representing four treatment groups (Control, *L. bifermentans*, *L. plantarum* and *B. subtilis*), five silage opening time points (T_{0day}, T_{2day}, T_{5day}, T_{7day} and T_{45day}), and three replicates. The silages were maintained in a cool, shaded area under laboratory conditions. Homogenised samples (20 g) were collected from the silage material at each opening time point (T_0, T_2, T_5, T_7 and T_{45}). The samples were mixed with 180 ml of Ringer solution and blended at high speed for one minute. The pH of the silage extracts was immediately measured after filtration through Whatman 54 filter paper (Whatman, Florham, NJ). Microbial counts were conducted using ten-fold serial dilutions. The number of lactic acid bacteria was determined by pour-plating on MRS agar with a double overlay for anaerobic conditions, followed by incubation at 36 °C for 48 to 72 h. The number of enterobacteria was determined by pour-plating on violet red bile glucose agar (VRBD) with a single overlay, and the plates were incubated at 36 °C for 18 h. Yeast and mould counts were enumerated by pourplanting on malt extract agar (MEA) acidified with lactic acid to pH 4, with a single overlay, and the plates were incubated at 32 °C for 48 h. The DM content of the fresh forage (T_0) and the resulting silage (T_{45}) was determined by drying samples at 70 °C in a forced-air oven for 48 h. The silages were opened after 45 days of ensiling and analysed for pH, NH_3-N, neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP), and crude ash (CA) contents. Ash content was determined by incinerating the dry samples in a muffle furnace at 525 °C for 8 h. Nitrogen (N) content was measured using the Kjeldhal method, and crude protein content was calculated as N × 6.25. Ether extract were analysed using the method of AOAC (1990). Cell wall fibre components, including NDF and ADF were analysed according to the method described by Van Soest et al. (1991). To assess feed quality, the relative feed value (RFV) was calculated using the following formula:

DDM = 88.9 − (0.779 × ADF%);
DMI = 120/(NDF%);
RFV = (DDM × DMI)/1.29;

where: DDM – digestible dry matter; ADF – acid detergent fibre; DMI – dry matter intake; NDF – neutral detergent fibre; RFV – relative feed value.

Dry matter recovery (DMR), which indicates how much DM was retained in the silage compared to its initial content, was calculated using the formula:

$$DMR\ (\%) = DM\ of\ T_{60}\ silage / DM\ of\ T_0\ silage \times 100.$$

Statistical analysis of the data was performed using JMP statistical software (SAS Institute, Cary, NC), and treatment groups were compared using the least significant difference (LSD) test.

Results

The pH of the control treatment was statistically higher compared to all inoculated treatments, reaching a value of 4.86. The pH values of samples inoculated with *L. plantarum* and *B. subtilis* were comparable. The *L. bifermentans* strain was particularly effective in sharply reducing pH ($P < 0.001$) of the silage from the beginning of fermentation compared to other microbial inoculants. This rapid and pronounced reduction in pH provides a significant advantage by preventing proteolysis in legume silages (Table 1). Interactions between opening time points and LAB strains is presented in Figure 1, where it is evident that the pH of fresh material (T_0) was higher compared to silage samples taken at subsequent time points (T_2, T_5, T_7 and T_{45}). After 7 days, the pH in the *L. bifermentans*inoculated silage

Table 1. Effects of different bacterial inoculants on the pH of silages at different opening time points

Bacteria inoculant	T_0	T_2	T_5	T_7	T_{45}	Mean
Control	5.92^{b}	4.84^{e}	4.65^{e}	4.63^{e}	4.27^{hi}	4.86^{A}
Lactobacillus bifermentans	5.86^{c}	4.21^{jk}	4.20^{k}	4.14^{l}	4.18^{kl}	4.52^{C}
Lactobacillus plantarum	6.13^{a}	4.41^{g}	4.26^{ij}	4.26^{ij}	4.26^{ij}	4.66^{B}
Commercial (*Bacillus subtilis*)	5.85^{c}	4.55^{s}	4.32^{h}	4.28^{hi}	4.26^{ij}	4.65^{B}
Mean	5.94^{A}	4.50^{B}	4.36^{C}	4.33^{D}	4.24^{E}	
P-value	T:0.0001 LAB:0.0001 TXLAB:0.0001					
LSD	T:0.03** LAB:0.25** TXLAB: 0.06**					
CV	0.73					

T – silage opening time points (T_0, T_2, T_5, T_7, T_{45} – openings at days 0, 2, 5, 7, 45, respectively), LSD – least significant difference, CV – coefficient of variation, LAB – lactic acid bacteria, TXLAB – effect of interaction between silage opening time points and LAB **$P < 0.001$; $^{a-k, ABC}$ – different letters indicate significant differences between mean values

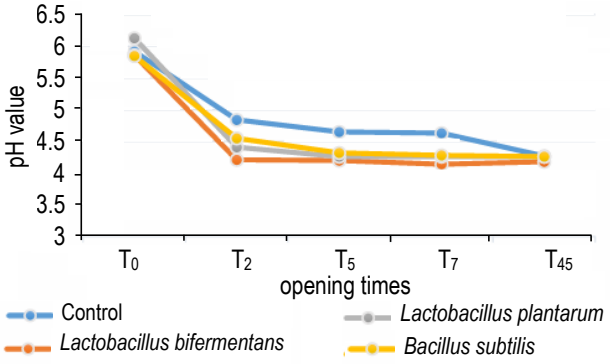

Figure 1. Effect of interaction between lactic acid bacteria strains and silage opening time points on pH value;
T – silage opening time points (T_0, T_2, T_5, T_7, T_{45} – openings at days 0, 2, 5, 7, 45, respectively)

decreased to 4.14 ($P < 0.001$), i.e. it was significantly lower than in the untreated silage. The pH values of both *L. plantarum* and *L. bifermentans*-inoculated silages decreased rapidly during the first 2 days of ensiling; however, after this period, the pH in the *L. plantarum*-inoculated silage stabilised at this level ($P < 0.001$), while it continued to decrease in *L. bifermentans*-inoculated silage throughout the fermentation process.

Table 2 presents the variation in the abundance of LAB, enterobacteria, yeasts, and moulds at individual silage opening time points. Silage inoculated with *L. plantarum* showed higher counts of LAB compared to the untreated silage and other inoculations. The number of LAB in the fresh material was determined at 4.00 $\log_{10}$ CFU/g, which increased during the early fermentation period, reaching 11.98 $\log_{10}$ CFU/g at time point T_7. However, this count dropped to 3.16 $\log_{10}$ CFU/g by the end of the fermentation process, indicating that the silage had stabilised and fermentation was almost complete. During the fermentation period, the count of LAB in both treated and untreated silages exhibited significant variability across opening time points, which suggested the presence of an interaction between opening time and the abundance of LAB (Figure 2a). For example, the number of *L. plantarum* was lower than *B. subtilis* at T_2, equal at T_5, and higher at T_7 and T_{45}. Similarly, *L. bifermentans* counts were higher at T_2, T_5, and T_{45} in comparison to *B. subtilis* abundance, but the values were opposite at T_7. After day 7, both *L. bifermentans* and *B. subtilis* counts decreased, falling below the levels observed in the fresh material (T_0), indicating that these strains were particularly aggressive during fermentation. By day 45 of fermentation, LAB counts decreased by 92.93% for *B. subtilis* compared to T_7, while these values for the control silage,

Table 2. Effects of different bacterial inoculants on the number of lactic acid bacteria, enterobacteria and yeasts in silages at different opening time points

Bacteria inoculant	T_0	T_2	T_5	T_7	T_{45}	Mean
Lactic acid bacteria						
Control	2.91^{l}	7.26^{fgh}	7.13^{fgh}	8.91^{d}	1.32^{m}	5.51^{D}
Lactobacillus bifermentans	4.17^{j}	7.62^{ef}	8.70^{d}	11.31^{c}	3.59^{k}	7.08^{B}
Lactobacillus plantarum	5.75^{i}	7.05^{gh}	8.00^{e}	14.69^{a}	6.82^{h}	8.46^{A}
Bacillus subtilis	3.19^{kl}	7.50^{efg}	8.00^{e}	13.02^{b}	0.92^{m}	6.53^{C}
Mean	4.00^{D}	7.36^{C}	7.96^{B}	11.98^{A}	3.16^{E}	
P-value	T:0.0001 LAB:0.0001 TXLAB:0.0001					
LSD	T:0.27** LAB:0.24** TXLAB: 0.53**					
CV, %	4.67					
Enterobacteria						
Control	5.55^{bc}	5.83^{b}	3.37^{f}	1.61^{gh}	1.68^{g}	3.61^{A}
Lactobacillus bifermentans	5.33^{cd}	3.54^{f}	1.10^{ijk}	nd I	1.22^{hij}	2.24^{D}
Lactobacillus plantarum	7.89^{a}	4.71^{e}	1.40^{ghi}	0.85^{jk}	1.32^{ghi}	3.23^{B}
Bacillus subtilis	5.00^{de}	4.82^{e}	1.69^{gh}	0.66^{k}	1.72^{g}	2.78^{C}
Mean	5.94^{A}	4.72^{B}	1.89^{C}	0.78^{E}	1.48^{D}	
P-value	nd T:0.0001 LAB:0.0001 TXLAB:0.0001					
LSD	T:0.23*** LAB:0.21*** TXLAB: 0.46***					
CV, %	9.43					
Yeast						
Control	3.59^{h}	7.20^{cde}	6.72^{ef}	8.21^{ab}	7.62^{c}	6.67^{B}
Lactobacillus bifermentans	4.36^{g}	7.52^{cd}	8.41^{a}	8.43^{a}	4.47^{g}	6.64^{B}
Lactobacillus plantarum	6.79^{ef}	6.77^{ef}	7.48^{cd}	7.49^{cd}	6.59^{f}	7.03^{A}
Bacillus subtilis	3.10^{h}	7.01^{def}	8.54^{a}	7.66^{bc}	4.70^{g}	6.20^{C}
Mean	4.46^{D}	7.13^{B}	7.79^{A}	7.95^{A}	5.84^{C}	
P value	T:0.0001 LAB:0.0001 TXLAB:0.0001					
LSD	T:0.28** LAB:0.25** TXLAB: 0.56**					
CV, %	5.32					

T – silage opening time points (T_0, T_2, T_5, T_7, T_{45} – openings at days 0, 2, 5, 7, 45, respectively), LSD – least significant difference, CV – coefficient of variation, LAB – lactic acid bacteria, TXLAB – effect of interaction between silage opening time points and LAB; ns – non-significant, $^{**}P < 0.001$; $^{a\text{-}k,\ ABC}$ – different letters indicate significant differences between mean values

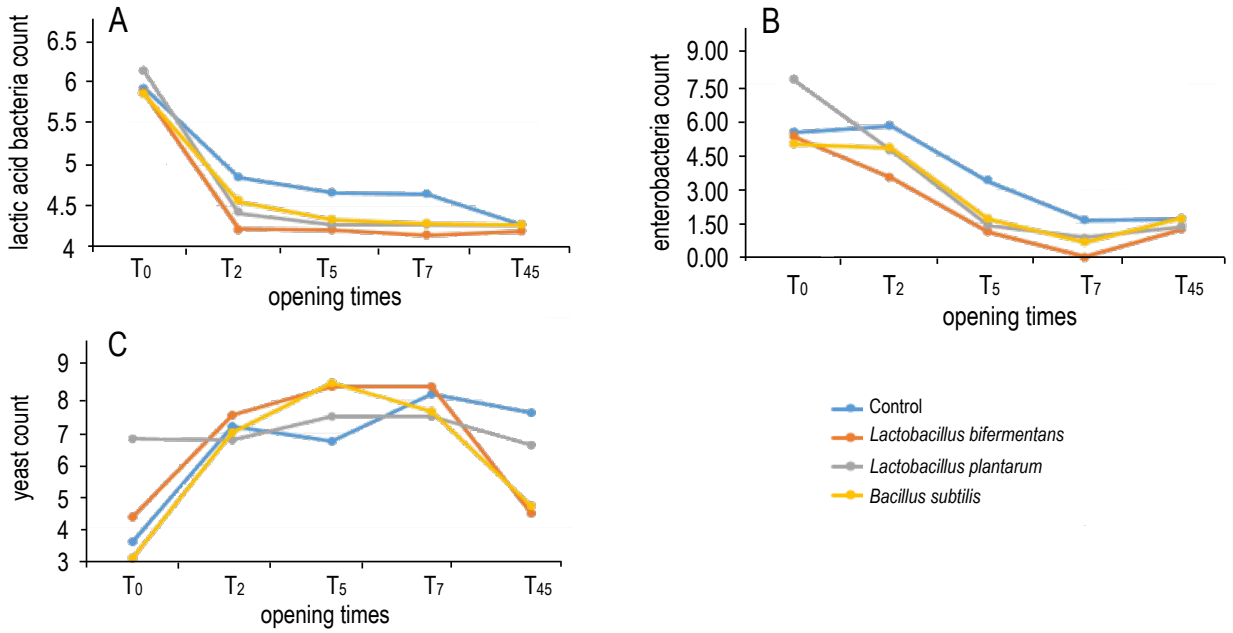

Figure 2. A. Effect of interaction between lactic acid bacteria strains and silage opening time points on the count of lactic acid bacteria; B. Effect of interaction between lactic acid bacteria strains and silage opening times points on the count of enterobacteria; C. Effect of interaction between lactic acid bacteria strains and silage opening time points on the count on yeast count

T – silage opening time points (T_0, T_2, T_5, T_7, T_{45} – openings at days 0, 2, 5, 7, 45, respectively)

and silages supplemented with *L. bifermantans*, and *L. plantarum* strains were 85.19, 68.26 and 53.57%, respectively.

The count of enterobacteria in the untreated silages was 3.61 $\log_{10}$ CFU/g silage, while this value in silages inoculated with *L. bifermentans* significantly decreased to 2.24 $\log_{10}$ CFU/g, showing that this strain was more effective than *L. plantarum* and *B. subtilis* ($P < 0.001$). The abundance of enterobacteria decreased during the fermentation period and a slight increase was observed on day 45 of ensiling ($P < 0.001$). However, even at this stage, enterobacteria levels remained much lower than those recorded at T_0, T_2 and T_5 (Table 2). Figure 2b shows that the number of enterobacteria was low in silages inoculated with *L. bifermentans* (0.00 $\log_{10}$ CFU/g fresh material) at T_7 ($P < 0.001$). At all sampling points, untreated silages had consistently higher enterobacteria counts compared to inoculated silages.

The yeast count (Figure 2c) increased from the beginning of the ensiling process to day 7 but then decreased by day 45. The yeast count in untreated silages was 6.67 $\log_{10}$ CFU/g silage, while this value significantly decreased to 6.20 $\log_{10}$ CFU/g in silages treated with *B. subtilis*, showing that *B. subtilis* was more effective at restricting yeast growth than both *L. plantarum* and *L. bifermentans* ($P < 0.001$). Although the highest number of yeasts in the early fermentation period was found in *L. bifermentans*inoculated silages, their abundance decreased at the end of ensiling (T_{45}) in all treated silages compared to the control treatment.

Treatment with LAB strains did not statistically alter the DM content of the fresh material (T_0). However, a statistically significant difference in DM content was observed in the resulting silage (T_{45}), indicating that the DMR of the silage was improved by inoculation. Fodder pea silages inoculated with both *L. plantarum* (25.81%) and *L. bifermentans* (24.76%) had higher DM values compared to the untreated silages (24.15%) and those treated with *B. subtilis* (23.36%) ($P < 0.05$). Although LAB inoculation did not significantly affect the overall DMR, the highest recovery values were found in silages treated with *L. plantarum* (99.05%), followed by *L. bifermentans* (98.85%), *B. subtilis* (96.23%), and the control (92.03%) (Table 3).

Table 3. Effects of different bacterial inoculants on the number of dry matter ratio of silages at different opening time points

Bacteria inoculant	DM (T_0)	DM (T_{45})	DMR
Control	26.39	24.15^{B}	92.03
Lactobacillus bifermentans	25.04	24.76^{AB}	98.85
Lactobacillus plantarum	26.06	25.81^{A}	99.05
Bacillus subtilis	24.03	23.36^{B}	96.23
Mean	25.10	24.52	97.94
P-value	0.3807	0.0256	0.3775
LSD	ns	1.40*	ns
CV, %	5.89	2.86	5.28

DM – dry matter, DMR – dry matter recovery, T – silage opening time points (T_0, T_{45} – openings at days 0, 45, respectively), LSD – least significant difference, CV – coefficient of variation; *$P < 0.05$; ns – non-significant; ^{AB} – different letters indicate significant differences between mean values

The mean values of NH_3-N, CP, CA, NDF, ADF, and RFV content of fodder pea silages at T_{45} are given in Table 4. The NH_3-N concentration in the mature silage, which reflects the extent of proteolysis in the silage, was significantly lower in the silage treated with LAB, with *L. bifermentans* showing the greatest effect (15.25 g/50 ml) ($P < 0.01$). The CP content ranged from 16.32 to 17.84%, with *L. plantarum* yielding the highest protein levels.

Table 4. Chemical compositions of *Pisum sativum* L. silages at day 45 (T_{45})

Bacteria inoculant	NH_3-N	CP	CA	NDF	ADF	RFV
Control	20.55^{a}	16.32^{c}	7.43	41.83	35.75^{a}	135.90^{b}
Lactobacillus bifermentans	15.25^{c}	17.01^{b}	8.02	38.85	32.91^{b}	151.73^{a}
Lactobacillus plantarum	18.53^{b}	17.84^{a}	7.37	41.93	35.53^{a}	136.13^{b}
Bacillus subtilis	19.88^{ab}	16.95^{b}	6.99	40.00	32.79^{b}	147.30^{ab}
Mean	18.55	17.03	7.45	40.65	34.24	142.77
P-value	0.002[6]	0.0017	0.6251	0.0764	0.0183	0.0502
LSD	1.99**	0.49**	ns	ns	2.03*	12.70*
CV	5.39	1.41	12.54	3.25	2.97	4.45

LSD – least significant difference, CV – coefficient of variation, CP – crude protein, CA – crude ash, NDF – neutral detergent fibre, ADF – acid detergent fibre, RFV – relative feed value, **$P < 0.01$; *$P < 0.05$; ns – non-significant; ^{abc}– different letters indicate significant differences between mean values

Silages treated with *L. bifermentans* and *B. subtilis* contained similar protein levels, while the lowest protein content was determined in the untreated silages ($P < 0.01$). The CA content ranged from 6.99 to 8.02%, and differences between the treatments were not statistically significant. The NDF content varied between 38.85 and 41.93%, and the differences in NDF values were also not statistically significant. ADF values ranged from 32.79% in *L. plantarum*-inoculated silages to 35.75% in the control silages. *L. bifermentans* and *B. subtilis* were more effective in reducing the ADF value than *L. plantarum* ($P < 0.05$). LAB inoculation significantly increased ($P < 0.05$) the RFV content, with the highest RFV value obtained in the *L. bifermentans*inoculated silage (151.73), and the lowest (153.90) in the control treatment.

Discussion

The number of epiphytic LAB in the microbial composition of the ensiling material is one of the important factors determining the direction of silage fermentation. Silage is generally well preserved if the number of epiphytic LAB exceeds the value of 10^5 CFU/g fresh material (Cai et al., 1999). According to our results, the number of LAB in the fresh material of *Pisum sativum* L. was very low, and their abundance increased following LAB inoculations before ensiling. The inoculants improved the microbial profile, notably increasing lactic acid bacteria and reducing yeast and mould counts in the resulting fodder pea silage. Muck (1988) has observed that silage fermentation is largely influenced by the number and type of epiphytic microorganisms on the plants, and a higher ratio of LAB in the silage can lead to more efficient fermentation, resulting in a lower pH and the inhibition of growth of undesirable microorganisms. Higher yeast counts were detected in the forage pea plants inoculated with *L. plantarum* compared to the control, both in the fresh material and at the end of fermentation. LAB-inoculated silages had a lower pH than the untreated (control) silages, and *L. bifermentans* strain (a homofermentative LAB) was the most effective strain in reducing the pH value. *L. bifermentans* sharply lowered silage pH from the beginning of fermentation compared to *B. subtilis* and *L. plantarum* inoculants. These results are consistent with those of Fraser et al. (2001), who obtained a relatively low pH at the end of fermentation using homofermentative LAB strains. Moreover, under anaerobic conditions, LAB were shown to cause a rapid drop in the pH during ensiling (Muck, 2013). Similarly, in the present study, the fastest pH reduction throughout fermentation was observed in the silage treated with *L. bifermentans*. It is well established that the final pH of silage is a key indicator of fermentation quality (Wang et al., 2019; Peng et al., 2021). Silage with a pH value of 4.20 or lower is typically considered to be well-fermented (Kung et al., 2018). The pH of the ensiled mixture is affected by various factors, such as anaerobic conditions, WSC concentration, microorganisms in the epiphytic flora, DM content, and the buffering capacity of forage crops (Muck, 1988). In legume plants, it is particularly difficult to obtain a pH of 4.20 or below due to their high buffering capacity, low WSC content, and lower DM at harvest. On the other hand, Scherer et al. (2019) suggested that amino acid deamination and decarboxylation, indicated by NH_3-N levels, could decrease the nutritional quality of silage. In the current study, the control silage showed signs of deterioration, including an increase in pH and NH_3-N concentration, as well as a reduction in DM by day 45. However, inoculation with *L. bifermentans* helped prevent spoilage to some extent, as indicated by relatively lower pH and NH_3-N levels. This suggested that a lower silage pH could inhibit the hydrolysis of protein fractions in fodder pea silage. Similar findings were reported in highmoisture alfalfa silage by Yang et al. (2020). The present results demonstrated that the NH_3-N concentrations in all inoculant treatments were significantly lower compared to the control, suggesting that the inoculants were effective in preserving protein. In particular, *L. plantarum* was the most effective strain, as evidenced by the highest CP content in the resulting silage.

In this experiment, LAB inoculants had no significant effect on the NDF content of the fodder pea silage; however, in some studies, the addition of these bacteria caused a decrease in the ADF content of silage (Okuyucu et al., 2018). Additionally, some studies, such as those by Koç et al. (2017), reported a reduction in the NDF content following LAB inoculation. These discrepancies observed between studies may be primarily attributed to differences in the DM content of the plants. In the present study, the DM content was initially determined to be 25.10%, and during the fermentation process, it decreased to 24.52%, indicating some DM losses. DM recovery was low in the control silages compared to those treated with inoculants. Our results align with the findings of Kizilsimsek et al. (2020) and Ren et al. (2021). In addition, DM content may be better preserved through inoculation with homofermentative LAB, as Bai et al. (2021) noted that such inoculants are particularly advantageous for legume silages. Homofermentative LAB can produce higher levels of lactic acid, thereby minimising DM losses. Reduced DM is undesirable because it signifies the depletion of valuable nutrients that could otherwise be utilised by animals (Robinson et al., 2016). Inoculant application appears to have a significant inhibitory effect on the growth and activity of unwanted microorganisms, which helps minimise nutrient losses during silage fermentation.

Conclusions

Pisum sativum L. is a valuable legume forage for ruminants and is widely cultivated worldwide. However, lower concentrations of water-soluble

carbohydrates, reduced dry matter content at harvest, and high buffering capacity in legumes present challenges in producing high-quality silage. The inoculation of lactic acid bacteria (LAB) can have a significant impact on the composition of microbial communities during the ensiling process of *Pisum sativum* L. The present study demonstrated that the use of LAB inoculants could improve silage fermentation, preserve forage nutrients, and enhance animal performance. Notably, inoculations with *Lactobacillus bifermentans* reduced pH and NH_3-H levels while inhibiting the growth of enterobacteria.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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