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ORIGINAL ARTICLE

Efficacy of six lactic acid bacteria strains as silage inoculants in forages with different dry matter and water-soluble carbohydrate content

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Abstract

The dry matter (DM), water-soluble carbohydrate (WSC) content, and epiphytic microbiota of forage during ensiling are critical for the production of high-quality preserved forage. This study tested the efficacy of six additive treatments (10^6 CFU/g FM *Lacticaseibacillus rhamnosus* IMI 507023, *Lactiplantibacillus plantarum* [IMI 507026, IMI 507027, and IMI 507028] or *Pediococcus pentosaceus* [IMI 507024 and IMI 507025]) as ensiling agents for grass-clover preservation. Treated and untreated forages were ensiled in 1.75 L glass jars and stored for 90 days at 20 ± 2°C. The effects of treatments on silage fermentation and aerobic stability were tested using grass-clover forage at low and high levels of DM (24.0%–40.1%) and WSC (1.78%–5.27%). Data analysis using a mixed-effects model and principal component analysis revealed improved silage fermentation in treated forages compared to that in the control. The fermentation-related analytes in the treated silages (low pH, ethanol, acetic acid, and high lactic acid) represented a typical homofermentative metabolic pathway. The silage inoculants significantly lowered DM losses and ammonia-N, % of total nitrogen content, ranging between 30.4%–52.5% and 30.5%–63.1% respectively, compared to the control. Additionally, forage type interacted with treatment, indicating that forage management is vital for ensiling and should be considered alongside inoculant use. The improvement in aerobic stability by lactic acid bacteria (LAB) was inconsistent. The principal component analysis of all analytes showed that aerobic stability was most closely correlated with acetic acid and butyric acid concentrations. In conclusion, all LAB strains successfully improved the preservation of forage materials.

KEYWORDS
forage preservation, lactic acid bacteria, *Lacticaseibacillus rhamnosus*, *Lactiplantibacillus plantarum*, *Pediococcus pentosaceus*, silage

1 | INTRODUCTION

The agriculture, forestry, and other land use sectors, accounted for an estimated 13%–21% of global net anthropogenic greenhouse gas emissions from 2010 to 2019. The same sector aims to provide 20%–30% of the 2050 reduction in emissions through near-term mitigation strategies such as improved land, livestock and nutrient management (IPCC, 2022). For example, improved livestock feeding

efficiency can be achieved through efficient forage harvesting and silage conservation (Borreani et al., 2018; Tabacco et al., 2018).

Forage conservation is an ongoing challenge, and the demand for high-quality silage is constantly increasing (Wilkinson & Muck, 2019). Factors such as the time of harvest, plant maturity stage, weather conditions (luminosity, temperature, and relative humidity), pre-ensiling processing (e.g., wilting impacts the dry matter (DM) and water-soluble carbohydrate (WSC) content), and silo type (e.g., bales and bunkers) can affect the DM and WSC content of the same forage type (Downing et al., 2008; Pauly & Wyss, 2019; Windle et al., 2014). The forage epiphytic microbiota comprises a diverse range of microorganisms that are either desirable or undesirable during ensiling. The production of lactic acid (LA) as a fermentation end product by lactic acid bacteria (LAB) is essential for preserving forage of sufficient quality for livestock production. However, their concentration per gram of fresh forage, as well as variation in the performance of different strains, are limiting factors for ensuring high hygienic and nutritive quality silage production. Lactobacilli, *Pediococcus* spp., and *Lactococcus* spp. are among the dominant LAB in spontaneously fermented forage. Therefore, the members of this family are commonly used as single- or mixed-strain inoculants for controlled silage production (Carvalho et al., 2021; Fabiszewska et al., 2019; Wang et al., 2018).

LAB used as silage additives are differentiated based on carbohydrate metabolism, mainly into homofermentative/facultative heterofermentative and obligate heterofermentative groups. Among the homofermentative/facultative heterofermentative species, *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus*, *Pediococcus acidilactici* and *Pediococcus pentosaceus* are commonly used as inoculants, whereas *Lentilactobacillus buchneri* is used among the obligate heterofermentative species (Carvalho et al., 2021; Muck et al., 2018). Homofermentative/facultative heterofermentative LAB generally increase the lactic acid concentration and lower pH, acetic acid, butyric acid, ammonia-N, % total N, and DM losses compared with spontaneously fermented silages. In contrast, heterofermentative LAB are widely favoured for improving aerobic stability as they convert lactic acid to acetic acid and propionic acid, but they are also linked to higher DM loss due to the production of H_2O and CO_2 (Borreani et al., 2018; Gänzle, 2015; McDonald et al., 1991; Muck et al., 2018). Pre-ensiling treatment of forage with LAB shifts the microbial community dynamics at different stages of the ensiling process, which is crucial for reducing the proliferation of hazardous microorganisms and the production of undesirable metabolites (Guo et al., 2023) as well as reaching preservation characteristic targets for maximized nutritive value. Under anaerobic conditions, LAB rapidly ferment the WSC contained in forage, leading to exponential growth and high organic acid production in the first few days of silage production, inhibiting deleterious spoilage, and reducing nutrient loss (McDonald et al., 1991).

In a recent meta-analysis of 130 peer-reviewed studies published since 1996, homofermentative and facultative heterofermentative LAB inoculants showed improved fermentation quality, defined by the following characteristics: low pH, high lactic acid content, and reduced ammonia-N and acetic acid production (Oliveira et al., 2017). However, the same study determined that forage type was the most consistent factor affecting the end-quality of silage following LAB inoculation. Forage WSC content and DM are key drivers of forage fermentation and, therefore, the efficacy of silage inoculants (Pauly & Wyss, 2019).

Therefore, the objective of this study was to evaluate the effects of six individual homofermentative lactic acid bacteria on silage quality when applied to forage with different DM (L and H; low-high range from 24.0% to 40.1%) and WSC content (Ls and Hs; low-high range from 1.78% to 5.27%), obtaining four different forage types: LLs, LHs, HLs, and HHs. The strains in this study were selected because of their safety features, such as a lack of antimicrobial resistance genes (Nikodinoska, Makkonen, et al., 2022c, 2022d, 2022e, 2022f; 2022g; 2022h), and efficacy as shown in other studies (Franco, Nikodinoska, et al., 2022; Gonda et al., 2022; Nikodinoska, Gonda, & Moran, 2022a, 2022b; Apajalahti et al., 2022; Ferrero et al., 2022; Wambecq et al., 2022). In addition, to the best of our knowledge, no studies to date have evaluated the efficacy of these strains using forages with different DM and WSC content in the same experimental design, representing the range of ensiling conditions typically encountered within grassland systems.

2 | MATERIALS AND METHODS

2.1 | Ensiling process and inoculants

A grass-clover dominated ley was selected at the Swedish Livestock Research Centre, Lövsta, Uppsala, Sweden (59°50′ N, 17°46′ E) and harvested in mid-July (the common time for the 2nd cut; 6 weeks after the first cut) for Experiment 1 and in mid-September (the common time for the 3rd–4th cut) for Experiment 2. The grasses present in the ley (in the pre-heading stage) were timothy (*Phleum pratense*), perennial ryegrass (*Lolium perenne*), and meadow fescue (*Festuca pratensis*); the legumes present were red (*Trifolium pratense*) and white clover (*Trifolium repens*). The grass–clover mixture had an average grass: legume ratio of 54:46 (+/−5) (DM basis). Before and after harvesting, the forage was treated to achieve two levels of DM (H = high and L = low) and WSC (Hs = high and Ls = low) content, as described below. Because WSC concentration decreases with decreased exposure to sunlight, and WSC content tends to be lower in the morning than in the afternoon (due to a balance between respiration and photosynthesis), the two different WSC contents were achieved by combining the effects of shade and cutting time (Kagan et al., 2020). Before ensiling, a given area of the ley (sufficient to provide at least 20 kg of fresh forage; approximately 40 m^2) was shaded by using a "tailor-made tent" put in place 24 h before cutting. The harvest times were 08:00 h (shaded area, low WSC content) and 13:00 h (unshaded area, high WSC content). During Experiment 1, the average maximum temperature was 23.8°C (±0.7), with a rainfall of 1.35 mm, and during Experiment 2, the average maximum temperature was 12.6°C (±0.1), with a rainfall of 0.912 mm. The weather data time frame represents the mean of the 5 days during which the grass was harvested.

Both experiments were conducted in a 2 × 2 factorial design with five replicates (complete blocks). For both experiments, blocks (B) were defined as five harvesting days: Wednesday (B1), Thursday (B2), and Friday (B3) for 1 week, and Monday (B4) and Tuesday

TABLE 1 Treatments used in Experiments 1 and 2.

Treatments used in this study	
Strains	**Abbreviation**
Lacticaseibacillus rhamnosus IMI 507023	LR
Pediococcus pentosaceus IMI 507024	PP1
Pediococcus pentosaceus IMI 507025	PP2
Lactiplantibacillus plantarum IMI 507026	LP1
Lactiplantibacillus plantarum IMI 507027	LP2
Lactiplantibacillus plantarum IMI 507028	LP3

(B5) of the following week. Each block represents a new area of ley harvested on each of the five ensiling days. At both harvesting times (morning and afternoon for Ls and Hs WSC content, respectively), the harvested forage was immediately brought to the lab to be divided into two batches: one batch was kept as it was for low DM content (L, as harvested; approximately 25%), and the other batch was dried at 35°C in an oven with forced air circulation until reaching ~35%–40% DM (high DM; H). The same procedure was repeated when ley with high WSC content was harvested in the afternoon. The resulting forage types were labelled as LLs, HLs, LHs, and HHs.

Before ensiling, the forage was chopped to approximately a 30-mm chop length using a stationary forage cutter before being thoroughly mixed on a fresh sheet of plastic film. After mixing, the chopped forage was weighed into equal quantities of 1 kg on a fresh basis and placed into separate clean plastic bags (60 L) for treatment. The treatments used in Experiment 1 were the control, LR, PP1, LP1, and LP2, whereas those used in Experiment 2 were the control, LR, PP2, and LP3. The microbial strains used in this study are listed in Table 1. The application rate of each inoculant was 1×10^6 CFU/g FM, and the inoculant suspension was applied at a rate of 5 mL/kg fresh forage using sterile spray bottles. The controls were sprayed with 5 mL of distilled water. Following the application of treatments, to guarantee an even mix of the forage with the inoculum, treated samples were thoroughly manually mixed within their own plastic bags and then packed in autoclaved 1.75 L glass silos at a density of 479–490 g fresh material/L. Silos were designed with water-filled airlocks in their lids to release fermentation gases during storage without air ingress. All forage handling at the lab was performed while using powder-free disposable nitrile gloves, and surfaces and packing equipment were continuously sterilized by spraying with 70% ethanol. A summary of the treatments and forage types is presented in Table 1. The taxonomic identity and antimicrobial resistance of the microbial strains used in this study have been described previously (Nikodinoska, Makkonen, et al., 2022c, 2022d, 2022e, 2022f; 2022g; 2022h). The silos were then stored at 20°C in a temperature-controlled room.

2.2 | Sample collection and analyses

Fresh forage and silage samples were collected during silo filling at the beginning of the study, and after 90 days of ensiling, respectively. Samples were homogenized, and the chemical analyses described below were carried out. The DM concentration of the fresh plant material was determined in two steps. First, fresh samples were dried for 18 h in a ventilated oven at 60°C (DM-1), followed by milling through a 1.0 mm sieve. A small portion of the sample was then dried a second time at 103°C for 5 h (DM-2). The DM content was determined as DM (%) = DM-1 × DM-2/100. For ash residue determination, dried and milled samples were mineralised through burning for 5 h at 550°C. pH was measured using a pH meter (Metrohm 654, Metrohm AG, Herisau Switzerland). Neutral detergent fibre (NDF) was determined according to the method of van Soest et al. (1991), including an amylase treatment. The WSC content was determined as described by Larsson and Bengtsson (1983).

Nitrogen content was analysed as described by Kjeldahl using a Kjeltec Tecator 103 (Foss Analytical, Hillerød, Denmark) and crude protein (CP) was calculated as Kjeldahl-N/0.16 (or N × 6.25) (Nordic Comittee in Food Analysis, 1976). Ammonium-Nitrogen (NH_4-N) content in liquid silage extracts/juice (extracted by pressing a sample of silage using a hydraulic powered cylinder) was analysed by the phenol-hypochlorite assay using flow-injection analysis (FIAstar™ 5012, Foss Analytical, Hillerød, Denmark). Values are expressed as grams of NH_4-N per 100 g N (i.e., % of total N). The amount of total nitrogen (TN) was obtained from the N analysis of fresh samples, as it was assumed that no N loss occurred during silage fermentation. Nitrate and nitrite were analysed in silage juice with a computerized flow injection analysis (FIA) system (FIAstar™ 5000 Analyser with 'soFIA' software from FOSS Analytical, Hillerød, Denmark) applying colorimetric methods.

Organic acids and alcohols were analysed using high-performance liquid chromatography (HPLC-system Alliance 2795 separations module with temperature control module II range 0–150°C and a 2414 refractive index detector (Waters Assoc., USA); mobile phase: 0.005 M sulphuric acid, flow-rate 0.8 mL/min, injection volume 20 µL; pre-column: ReproGel H 9 µm 30 × 8 mm (Dr. A. Maisch, Ammerbuch, Germany); column packet: ReproGel H 9 µm 300 × 8 mm (Dr. A. Maisch, Ammerbuch, Germany), and a column temperature of 60°C) as described by Ericson and André (2010). Buffering capacity (BC) was analysed as described by McDonald and Henderson (1962) and expressed in grams of lactic acid per 100 g of DM to reach pH 4.00. Dried and milled samples (1.0 g) were used in this study. Losses were measured by weighing the silos after they were filled and during storage. Weight loss was assumed to originate from the silage DM (loss of CO_2 mainly from carbohydrates; only marginal amounts of moisture escaped the silo). Losses were expressed as a percentage of the initial DM weight in the silo after silo filling. No correction was made for CO_2 bound to the silage juice at the silo opening.

An aerobic stability test (ASTA) was performed as described by Honig (1990). After opening the silo, fresh silage (300/350 g of silage on a wet basis) was loosely filled into a PVC drainage tube (1320 mL volume). The lower ends of the tubes were covered with an autoclaved piece of woven fabric (geotextile) and a rubber band. The tubes were inserted into a styrofoam block and an aseptic thermocouple (wire) was inserted into the centre of each tube to record the

TABLE 2 Experiment 1: Mean characteristics (±SEM) of the forage with different DM (low DM, L; and high DM, H), and WSC content (low WSC, Ls; and high WSC, Hs) before ensiling in lab-scale silos.

	Forage type			
Parameter	**LLs**	**LHs**	**HLs**	**HHs**
Dry matter (%)	24.0 (0.63)	27.1 (0.60)	39.8 (1.97)	40.1 (0.89)
WSC (% fresh matter)	2.09 (0.18)	3.57 (0.25)	3.44 (0.38)	5.27 (0.36)
WSC (% DM)	8.7 (0.53)	13.1 (0.71)	8.6 (0.58)	13.1 (0.69)
Crude protein (% DM)	17.9 (0.45)	16.8 (0.28)	17.8 (0.49)	17.0 (0.36)
Nitrate-N (mg/kg DM)	429 (52)	322 (43)	450 (49)	334 (19)
NDF (% DM)	50.0 (0.54)	46.3 (0.91)	49.5 (1.09)	45.4 (1.21)
Ash (% DM)	10.6 (0.12)	10.2 (0.14)	10.7 (0.11)	10.1 (0.18)
pH	5.77 (0.03)	5.70 (0.02)	5.76 (0.05)	5.74 (0.03)
Buffer capacity (g Hla/kg DM)	57.9 (0.89)	55.6 (0.55)	58.0 (0.70)	57.1 (0.69)

Abbreviations: DM, Dry matter; HLs, High dry matter-low water-soluble carbohydrates; HHs, High dry matter-high water-soluble carbohydrates; LLs, Low dry matter-low water-soluble carbohydrates; LHs, Low dry matter-high water-soluble carbohydrates; NDF, Neutral detergent fibre; SEM, Standard error of the mean; WSC, Water-soluble carbohydrates.

temperature. The top and bottom of the tubes were covered with 10 mm-thick polystyrene boards with holes (Ø 10 mm) above and below each tube. ASTA was conducted at 20°C in a temperature-controlled room.

The temperature of each silage sample was recorded every 2 h for a period of up to 10 days (Honig, 1990). The maximum silage temperature and the time required for reaching +3°C above ambient temperature (i.e., 23°C) were taken as a measure of the aerobic stability of the samples.

2.3 | Statistical analysis

Statistical analyses were performed separately for Experiments 1 and 2 using Minitab Software version 21. The treatment, forage type, and their interaction were examined using a mixed-effect model (Minitab), which included treatment and the forage type as fixed effects and block as a random effect. Data are presented as mean values. The factor effect with $p < .05$ was considered statistically significant, and differences between individual groups were established according to Tukey's test. In the supplementary materials, two additional tables related to Experiments 1 and 2, where data are presented as the mean value and standard error of the mean (SEM). Principal Component Analysis (PCA) was performed using the R software (R Core Team, 2021).

3 | RESULTS

3.1 | Experiment I

3.1.1 | Fresh forage composition

The DM and WSC content of forage prior to ensiling, together with the pH, buffer capacity, and chemical composition, are presented in Table 2. The mean DM values were 25.5% and 40.0% for the L and H herbage, respectively. The mean WSC values ranged from 2.1% to 3.4% for the Ls group and from 3.6% to 5.3% for the Hs group. The crude protein concentration was in the range of 16.8%–17.9%. Ash content varied between 10.1% and 10.7%. The mean pH value of the different forages was 5.7.

3.1.2 | Fermented forage composition

The outcomes of Experiment 1 are summarized in Tables 3 and S1. The blocking effect was not significant for any test parameter. The fermentation-related parameters, pH, ammonia-N, lactic acid, acetic acid, and ethanol were all significantly ($p < .001$) affected by the treatment and the forage type. With regard to forage type, the high dry matter forages (HL and HH) showed a higher pH than the low dry matter forages (LL and LH). All treatments showed significantly ($p < .001$) lower pH values than the control, ranging from 3.85 in the LR treatment to 4.18 in the PP1 treatment (Table 3). The highest content of ammonia-N was found in the forage types with low WSC content (LLs and HLs), whereas the lowest values were found in the high WSC forage type. Ammonia-N was lower in the treated forages than in the control forages. Lower values ($p < .001$) were observed in the LR, LP2, and LP3 treatments than in the PP1 treatment. The lactic acid content was significantly lower in the LLs forage compared to the other forage types, with the lactic acid parameter being 12%–50% higher ($p < .001$) in all treated forages than the untreated. The forages treated with PP1 showed lower lactic acid content than those treated with the other inoculants. Higher acetic acid content ($p < .001$) was observed in the low-WSC forage types (LLs and LHs) than in the high-WSC forages. Compared with the control, the acetic acid content was higher in the LP2 and LP3 treatments, and conversely, lower in the LR and PP1 treatments ($p < .001$). The propionic acid and butyric acid did not significantly ($p > .05$) differ among the four forage types, whereas ~84%–96% lower propionic acid content was achieved with the addition of any treatment. Between 39% and 48% lower ethanol content was obtained in treated forages compared to control, except the PP1 treatment significance was not obtained.

TABLE 3 Experiment 1: Composition of four different silage types ensiled with four different microbial treatments.

	Parameter											
	pH	Ammonia-N (% DM)	Lactic acid (% DM)	Acetic acid (% DM)	Propionic acid (% DM)	Butyric acid (% DM)	Ethanol (% DM)	DM (%)	WSC (% DM)	DM loss (% DM)	ASTA (h)	NH_4-N % TN
Treatment												
Control	4.48^{a}	676^{a}	5.29^{a}	0.68^{ab}	0.37^{a}	0.09	0.62^{a}	30.67	3.17	0.92^{a}	204.5^{b}	10.73^{a}
LR	3.85^{c}	309^{c}	7.91^{c}	0.45^{b}	0.05^{b}	0.05	0.38^{bc}	30.62	2.41	0.46^{c}	225.6^{ab}	4.70^{c}
PP1	4.18^{b}	501^{b}	5.94^{ab}	0.44^{b}	0.06^{b}	0.07	0.49^{ab}	30.82	3.48	0.47^{bc}	117.1^{c}	6.99^{b}
LP2	3.97^{bc}	281^{c}	7.16^{abc}	0.86^{a}	0.02^{b}	0.06	0.32^{c}	30.50	2.97	0.60^{bc}	249.6^{a}	3.96^{c}
LP3	3.99^{bc}	341^{bc}	7.22^{bc}	0.84^{a}	0.02^{b}	0.05	0.36^{bc}	30.69	2.14	0.62^{b}	227.9^{ab}	4.15^{c}
Forage type												
LLs	4.03^{b}	528^{a}	5.33^{b}	0.69^{a}	0.06	0.08	0.39^{a}	22.58^{d}	1.54^{a}	0.53	196.56	8.25^{a}
LHs	3.97^{b}	396^{ab}	6.83^{ab}	0.48^{a}	0.11	0.06	0.53^{b}	24.80^{b}	2.61^{a}	0.62	218.08	6.19^{b}
HLs	4.21^{a}	508^{a}	7.89^{b}	0.94^{b}	0.14	0.08	0.44^{ab}	36.88^{c}	2.24^{a}	0.65	189.04	6.08^{b}
HHs	4.16^{ab}	255^{b}	6.57^{ab}	0.50^{a}	0.11	0.04	0.39^{a}	38.38^{a}	4.95^{b}	0.66	216.08	3.89^{c}
SEM	0.04	27.10	0.26	0.04	0.02	0.01	0.02	0.74	0.23	0.03	7.36	0.36
p-values												
Treatment (T)	0.001	0.001	0.002	0.001	0.001	0.769	0.001	0.990	0.127	0.001	0.001	0.001
Forage (F)	0.016	0.001	0.004	0.001	0.583	0.381	0.003	0.001	0.001	0.060	0.111	0.001
T × F	0.362	0.554	0.936	0.176	0.936	0.947	0.304	0.998	0.127	0.130	0.043	0.006
Block	1.000	0.151	0.126	0.119	1.000	0.398	0.114	0.101	0.165	0.115	0.116	0.104
Percentage change from control												
Treatment	**pH**	**Ammonia-N (% DM)**	**Lactic Acid (% DM)**	**Acetic Acid (% DM)**	**Propionic Acid (% DM)**	**Butyric Acid (% DM)**	**Ethanol (% DM)**	**DM (%)**	**WSC (% DM)**	**DM Loss (% DM)**	**ASTA (h)**	**NH_4-N % TN**
LR	−14.0	−54.2	49.5	−33.8	−85.5	−38.3	−38.8	−0.2	−24.1	−49.2	10.32	−56.2
PP1	−6.7	−25.8	12.4	−34.8	−83.8	−22.4	−21.2	0.5	9.6	−48.8	−42.74	−34.8
LP2	−11.3	−58.4	35.3	26.4	−94.3	−32.7	−48.1	−0.6	−6.3	−34.6	22.05	−63.1
LP3	−10.9	−49.6	36.5	23.3	−94.2	−41.9	−42.8	0.1	−32.7	−32.0	11.44	−61.3

Note: a–c: Means with different letters in the same column differ significantly ($p < .05$). Percentage change from control = (Control − Treated)/Control × 100 (negative and positive values indicate reduction and increase, respectively).

Abbreviations: ASTA, Aerobic stability; DM, Dry matter; HLs, High dry matter-low water-soluble carbohydrates; HHs, High dry matter-high water-soluble carbohydrates; LR, *Lacticaseibacillus rhamnosus* IMI 507023; LLs, Low dry matter-low water-soluble carbohydrates; LHs, Low dry matter-high water-soluble carbohydrates; LP2, *Lactiplantibacillus plantarum* IMI 507027; LP3, *Lactiplantibacillus plantarum* IMI 507028; PP1, *Pediococcus pentosaceus* IMI 507024; SEM, Standard error of the mean; s.j, silage juice; WSC, Water soluble carbohydrates.

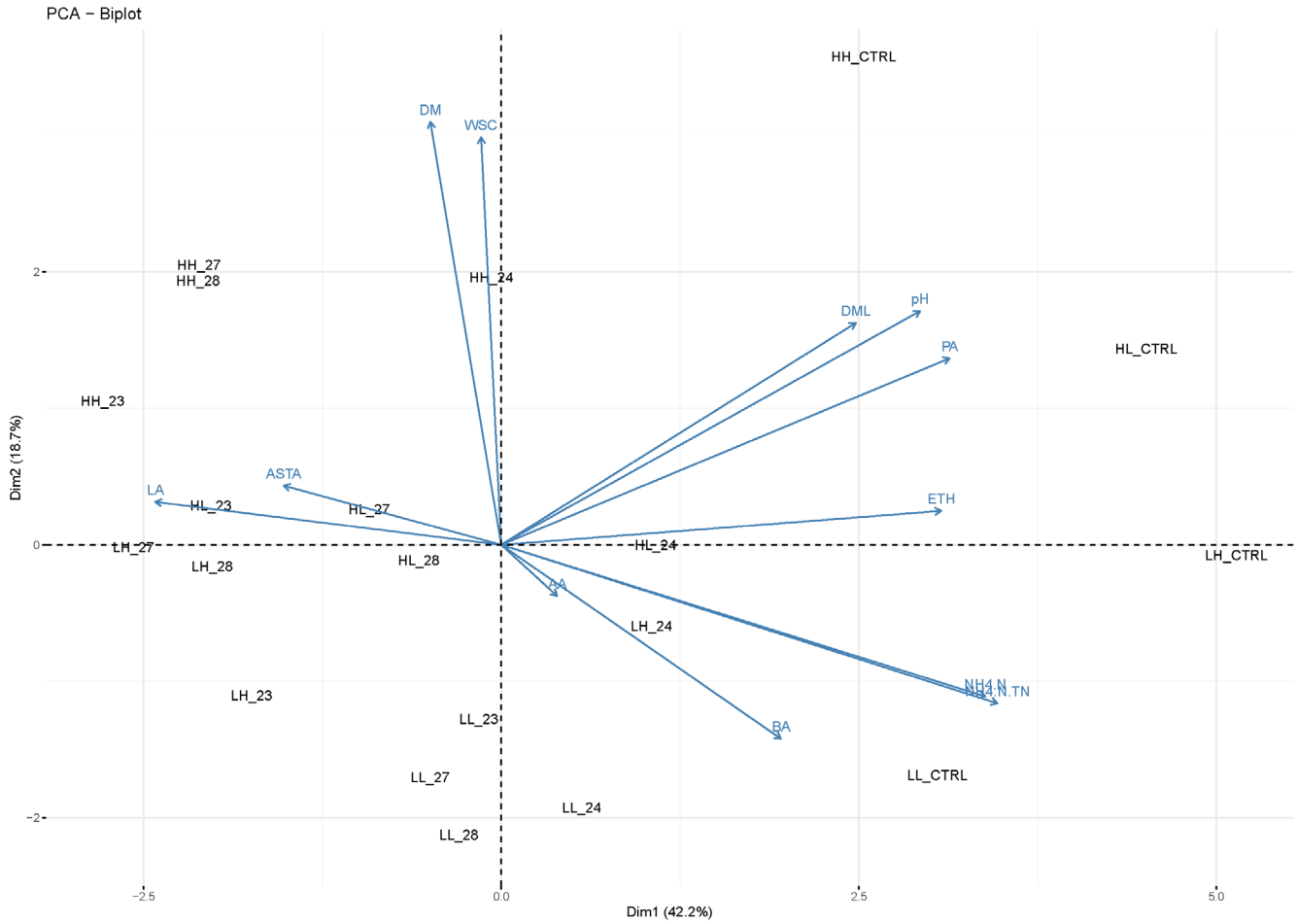

FIGURE 1 Principal Component Analysis (PCA) describing correlations between chemical parameters of ensiled forages with different dry matter (DM) and water-soluble carbohydrates (WSC) content treated with different microbial inoculants Chemical parameters: LA, Lactic Acid; AA, Acetic Acid; BA, Butyric Acid; PA, Propionic Acid; ETH, Ethanol; NH_4-N, Ammonia Nitrogen; NH4.N.Tot, Ammonia Nitrogen, of Total Nitrogen; ASTA, Aerobic Stability. Forage types: HH, high DM and high WSC; HL, high DM and low WSC; LH, low DM and high WSC; LL, low DM and low WSC. Microbial inoculants: 23, *L. rhamnosus* IMI 507023; 24, *P. pentosaceus* IMI 507024; 25, *P. pentosaceus* IMI 507025; 26, *L. plantarum* IMI 507026; 27, *L. plantarum* IMI 507027; and 28, *L. plantarum* IMI 507028.

For the forage-related characteristics, namely DM and WSC content, the forage type had a significant impact but not the treatment or their interaction. In particular, the highest DM content in the silages was observed when forage with high DM content was used, namely HHs, followed by LHs. Dry matter loss was significantly lowered from 32% to 49% with any treatment addition compared to control. Irrespective of the treatment, WSC was the highest ($p < .001$) in the high-DM forage HHs compared to the other forage types. Both forage type, treatment, and their interaction significantly affected NH_4-N % TN, which is an indicator of proteolysis. The highest proteolysis was observed in the LLs forage and the lowest in the HHs forage. All treatments significantly reduced these proteolysis indicators, from 35% to 63% compared with the unsupplemented forage.

A PCA was conducted to determine the relationships between the additive treatments and forage types (Figure 1).

In Experiment 1, the two principal components explained 60.9% of the cumulative percentage of variance. The combination of PC1 and PC2 clearly separated the treatment groups from the controls, except for three out of four forages treated with the PP1. The inoculants LR, LP3, and LP2, when added to forage types containing high WSC content (HHs and LHs), and the inoculant LR when added to the HLs forage, were bound to higher LA and ASTA values than the control. The LA content separated the different forage types; the HHs forage showed the highest lactic acid content compared to the other forage types. The ASTA of the low DM differed from high DM forages. The positively correlated parameters (ASTA, LA, and DM) were negatively correlated with pH, propionic acid, butyric acid, ethanol, DM loss, and NH_4-N. NH_4-N % TN was highly and negatively correlated with the lactic acid.

3.2 | Experiment II

3.2.1 | Fresh forage composition

The DM and WSC contents of forage prior to ensiling, together with the pH, buffer capacity, and chemical composition, are presented in

Parameter	Forage type			
	LLs	LHs	HLs	HHs
Dry matter (%)	24.98 (0.79)	27.64 (0.96)	38.58 (0.84)	38.78 (1.32)
WSC (% fresh matter)	1.78 (0.15)	2.84 (0.22)	2.83 (0.28)	3.96 (0.35)
WSC (%)	7.15 (0.57)	10.21 (0.53)	7.29 (0.63)	10.14 (0.65)
Crude protein (%)	17.57 (0.69)	17.30 (0.56)	17.67 (0.81)	17.47 (0.67)
Nitrate-N (mg/kg DM)	703 (110)	810 (145)	676 (111)	827 (184)
NDF (%)	46.93 (0.88)	44.75 (0.74)	47.12 (0.71)	44.75 (0.29)
Ash (%)	12.38 (0.06)	11.92 (0.20)	12.03 (0.10)	11.87 (0.22)
pH (dry sample)	5.95 (0.02)	5.94 (0.02)	5.97 (0.01)	5.95 (0.02)
Buffer capacity (g Hla/kg DM)	73.12 (1.62)	71.90 (1.57)	72.56 (2.59)	72.05 (1.29)

TABLE 4 Experiment 2: Mean characteristics (± SEM) of the forage with different DM (low DM, L; and high DM, H) and WSC content (low WSC, Ls; and high WSC, Hs) before ensiling in lab-scale silos.

Abbreviations: DM, Dry matter; HLs, High dry matter-low water-soluble carbohydrates; HHs, High dry matter-high water-soluble carbohydrates; LLs, Low dry matter-low water-soluble carbohydrates; LHs, Low dry matter-high water-soluble carbohydrates; NDF, Neutral detergent fibre; SEM, Standard error of the mean; WSC, Water soluble carbohydrates.

Table 4. The mean DM values were 25.0% and 38.8% for the L and H herbage, respectively. The WSC mean values ranged from 1.8% to 2.8% for the Ls and 2.8%–3.7% for the Hs. The crude protein concentration was 17.3%–17.7%. Ash content varied from 11.9% to 12.4%. The mean pH value of the different forages was 5.9.

3.2.2 | Fermented forage composition

The results of Experiment 2 are summarized in Tables 5 and S2. The blocking effect did not show statistically significant differences in the tested parameters.

The fermentation-related parameters pH, lactic acid, acetic acid, ethanol, ammonia-N and NH_4-N % TN, were all significantly ($p < .001$) affected by treatment and the forage type. Moreover, the interaction between treatment and forage was statistically significant for the NH_4-N, % TN and DM losses. The highest pH was observed in HH type, and the lowest was in the LH forage types, ranging from 4.26 to 4.06, respectively. All treatments showed a significantly ($p < .001$) lower pH value than the control (Table 5). Significantly higher ammonia-N content was found in the low-WSC forages (LLs and HLs), compared to the high-WSC forage types (LHs and HHs), being 772%–787% DM and 620%–671% DM, respectively. The same parameter was lower in the treated forages than in the control, with lower values ($p < .001$) following LP1 treatment. Significantly lower lactic acid content was observed in the LL forage type than in the HL forage types. When the microbial inoculant was used, a significantly higher lactic acid content was observed in comparison with the control; the lowest ($p < .001$) lactic acid production among the treated forages was observed with PP2 treatment.

A statistically significant ($p < .001$) higher acetic acid content was observed in the LL forage types, whereas a lowest was observed in the HH forage types. Treatment addition significantly impacted the acetic acid content, being 18%–63% lower compared to control.

Similarly, microbial addition maintained low propionic acid relative to the control, whereas the highest content was observed in the low DM compared to the high DM forages. In addition, the ethanol content was reduced from 38% to 45% upon treatment with any of the additives compared to control, and it was found to be significantly higher in low DM compared to high DM forages.

When the forage-related characteristics, such as DM content, were considered, there was no significant difference in the effects of the treatments. However, significantly higher ($p < .001$) DM content was observed in the high DM forage types (HLs and HHs), being ~36%–37%, compared to 23%–25% in the low DM forage types (LLs and LHs). A significant improvement ($p < .001$) in the DM loss was obtained with the addition of the inoculants, with 0.99% DM loss in the control sample versus 0.5%–0.6% DM loss in the treated silage.

The WSC content was significantly ($p < .001$) higher in the HHs forage type and significantly lower than the control when LR and LP1 were used as additives. In addition, the proteolytic indicator, NH_4-N % TN, was significantly ($p < .001$) affected by both the forage and treatment types. Among the different forage types, a higher NH_4-N % TN was observed in the low DM compared to high DM forages. Moreover, all treatments significantly reduced this parameter by 31%–48% compared to the control.

Silage aerobic stability was not significantly affected by the type of forage, and among the silage additives, only the PP2 treatment showed a significantly lower aerobic stability (66.8 h) compared to the control (221.6 h).

Figure 2 shows the PCA results of Experiment 2. The first two principal components accounted for 79.4% of the cumulative percentage variance. The combination of PC1 and PC2 distinctly separated the treatment groups from the controls, except the LL forage treated with LP1 and LL forage with LR. Concerning the.

In addition, the partially positively correlated parameters lactic acid and ASTA were negatively correlated with the DM and WSC, whereas the remaining fermentation parameters, e.g. ethanol, butyric acid, were positively correlated.

TABLE 5 Experiment 2: Composition of four different silage types ensiled with four different microbial treatments.

	pH	Ammonia-N (% DM)	Lactic acid (% DM)	Acetic acid (% DM)	Propionic acid (% DM)	Butyric acid (% DM)	Ethanol (% DM)	DM (% DM)	WSC (% DM)	DM loss (% DM)	ASTA (h)	NH4-N, % TN
Treatment												
Control	4.54^{a}	984^{a}	6.90^{a}	1.26^{a}	0.17^{a}	0.26	0.63^{a}	30.30	2.50^{a}	0.99^{a}	242.8^{a}	8.79^{a}
LR	3.99^{c}	681^{b}	9.92^{c}	0.82^{c}	0.07^{b}	0.03	0.39^{b}	30.37	0.76^{c}	0.56^{c}	230.4^{a}	6.07^{b}
PP2	4.11^{b}	652^{b}	7.88^{b}	0.49^{d}	0.02^{c}	0.03	0.37^{b}	30.80	2.68^{a}	0.47^{c}	137.7^{b}	5.37^{c}
LP1	4.03^{bc}	533^{c}	9.58^{c}	1.03^{a}	0.02^{c}	0.03	0.35^{b}	30.44	1.34^{b}	0.62^{b}	230.6^{a}	4.58^{d}
Forage type												
LLs	4.14^{bc}	787^{a}	10.34^{a}	1.45^{a}	0.14^{a}	0.21	0.51^{a}	23.54^{c}	0.44^{c}	0.74^{a}	211.3^{a}	9.15^{a}
LHs	4.06^{c}	620^{b}	10.56^{a}	0.93^{b}	0.09^{b}	0.09	0.52^{a}	25.35^{b}	1.67^{b}	0.60^{b}	197.3^{a}	6.66^{b}
HLs	4.21^{ab}	772^{a}	6.99^{b}	0.72^{b}	0.03^{c}	0.02	0.33^{b}	36.23^{a}	1.66^{b}	0.66^{ab}	222.9^{a}	4.83^{c}
HHs	4.26^{a}	671^{b}	6.39^{b}	0.49^{c}	0.02^{c}	0.02	0.39^{b}	36.79^{a}	3.53^{a}	0.65^{ab}	210.0^{a}	4.17^{d}
SEM	0.04	23.96	0.30	0.06	0.01	0.04	0.02	0.74	0.23	0.03	7.09	0.31
p-values												
Treatment (T)	0.001	0.001	0.001	0.001	0.001	0.045	0.001	0.766	0.001	0.001	0.001	0.001
Forage (F)	0.001	0.001	0.001	0.001	0.001	0.158	0.001	0.001	0.001	0.042	0.240	0.001
T × F	0.002	0.006	0.032	0.001	0.001	0.237	0.004	0.997	0.001	0.001	0.386	0.001
Block	1.000	0.097	0.085	0.094	1.000	0.265	0.131	0.090	0.093	0.158	0.102	0.093
Percentage change from control												
Treatment	**pH**	**Ammonia N (% DM)**	**Lactic acid (% DM)**	**Acetic acid (% DM)**	**Propionic acid (% DM)**	**Butyric acid (% DM)**	**Ethanol (% DM)**	**DM (% DM)**	**WSC (% DM)**	**DM loss (% DM)**	**ASTA (h)**	**NH_4-N % TN**
LR	−12.0	−30.7	43.9	−34.9	−59.9	−89.1	−37.5	0.23	−69.6	−43.43	−5.11	−30.9
PP2	−9.5	−33.7	14.3	−61.3	−85.9	−89.4	−41.9	1.65	7.2	−52.53	−43.29	−38.9
LP1	−11.2	−45.8	38.9	−17.9	−85.6	−89.9	−44.8	0.46	−46.4	−37.37	−5.02	−47.8

Note: a–c: Means with different letters in the same column differ significantly ($p < .05$). Percentage change from control = (Control − Treated)/Control × 100 (negative and positive values indicate reduction and increase, respectively).

Abbreviations: ASTA, Aerobic stability; DM, Dry matter; HLs, High dry matter-low water-soluble carbohydrates; HHs, High dry matter-high water-soluble carbohydrates; LLs, Low dry matter-low water-soluble carbohydrates; LHs, Low dry matter-high water-soluble carbohydrates; LR, *Lacticaseibacillus rhamnosus* IMI 507023; LP1, *Lactiplantibacillus plantarum* IMI 507026; PP2, *Pediococcus pentosaceus* IMI 507025; s.j., silage juice; SEM, Standard error of the mean; WSC, Water soluble carbohydrates.

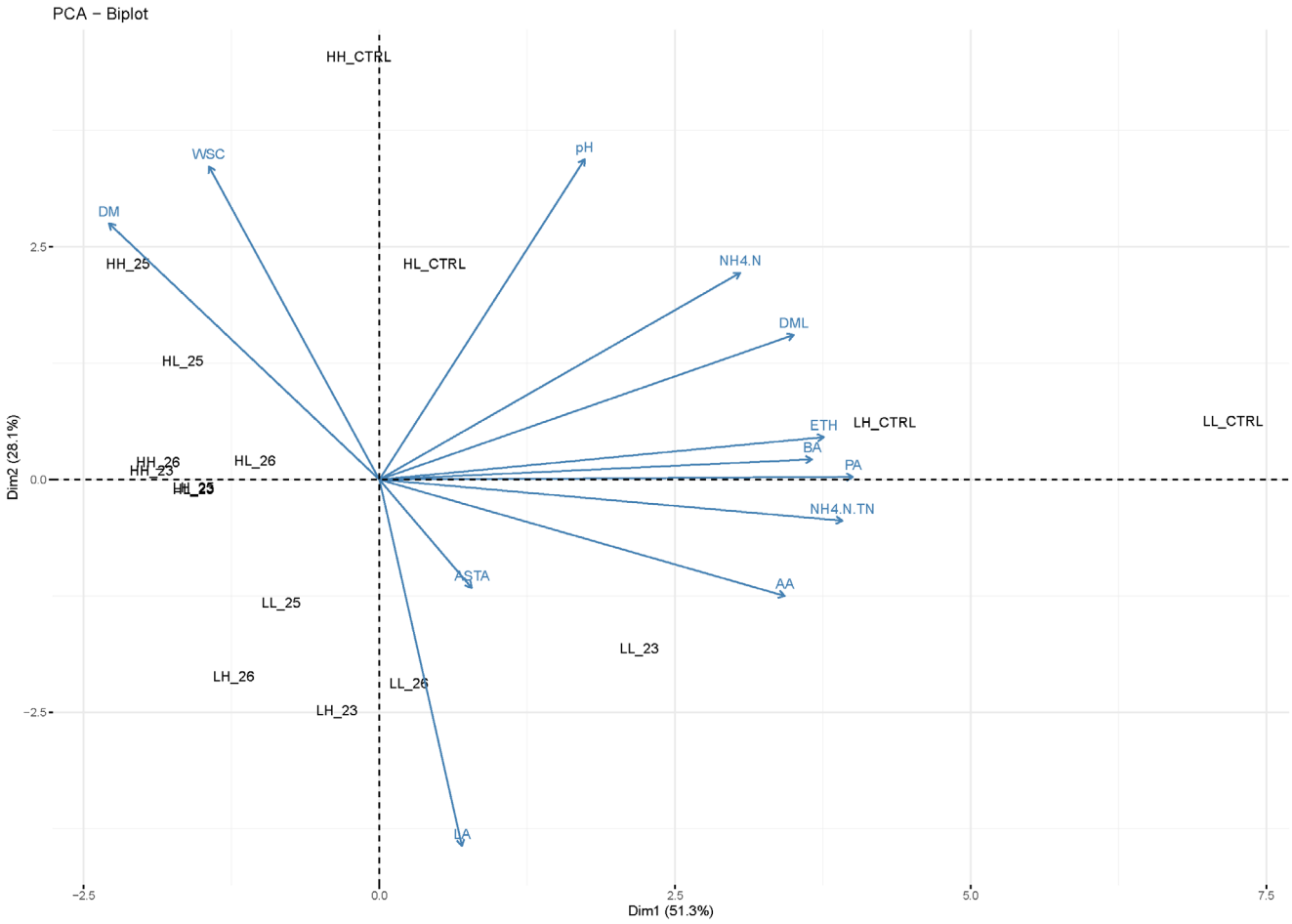

FIGURE 2 Principal Component Analysis (PCA) describing correlations between chemical parameters in different microbial inoculants. Chemical parameters: LA, Lactic Acid; AA, Acetic acid; BA, Butyric Acid; PA, Propionic acid; ETH, Ethanol; NH4-N, Ammonia Nitrogen; NH4.N. Tot, Ammonia Nitrogen, of Total Nitrogen; WSC, water-soluble Carbohydrates; DM, Dry Matter; ASTA, aerobic stability. Forage types: HH, High DM and High WSC; HL, High DM and Low WSC; LH, Low DM and High WSC; LL, Low DM and Low WSC. Microbial inoculants: 23, *L. rhamnosus* IMI 507023; 25, *P. pentosaceus* IMI 507025; 26, *L. plantarum* IMI 50702.

4 | DISCUSSION

4.1 | Composition of fresh forage

Silage can be made from a wide range of purpose grown forage or the by-products of other crops. The chemical characteristics of a crop are one factor that determines the extent to which fermentation drives the preservation of nutrients and a good hygienic quality for animal health. McDonald et al. (1991) summarized the key criteria for a crop that is meant to be preserved as silage as follows: (i) an adequate fermentable source of WSC, influenced by many factors such as species, stage of growth, diurnal variations, and climate; (ii) DM content above 200 g/kg, which tends to increase in grass as the stage of growth advances; and (iii) low buffering capacity, which depends on different anions present in the forage (e.g., organic acids). However, it was highlighted that many crops do not fulfil these requirements, and the management of forage pre-ensiling and/or the use of additives may be necessary to maximize nutrient preservation.

The evaluation of forage for the determination of ensiling difficulty can be based on the WSC content in the fresh material, namely: forage is (i) easy to ensile when WSC is >3%; (ii) moderately difficult to ensile when WSC is 1.5%–3.0%; and (iii) difficult to ensile at <1.5% WSC (EFSA FEEDAP Panel et al., 2018; Pauly & Wyss, 2019). Based on this classification, in this study, the efficacy of microbial additives was tested in forages with different WSC contents, ranging from 1.8% up to 5.3% FM, and different DM contents, ranging from 24.0% up to 40.1%. Our work shows that, in addition to forage type alone, the interaction between microbial additives and forage type plays an important role in final silage quality, regardless of crop maturity (Experiment 1 vs. Experiment 2) confirming previous observations (McDonald et al., 1991; Oliveira et al., 2017).

4.2 | Composition of fermented forage

The successful conservation of forage as silage largely depends on fast acidification to a pH of ~4.2 and the establishment of

anaerobiosis (McDonald et al., 1991). This inhibits endogenous plant enzymes and undesirable spoilage microorganisms and stimulates the growth of LAB, leading to reduced proteolysis and DM loss and, therefore, increased nutrient preservation (Borreani et al., 2018; Kung Jr. et al., 2018). Complex microbial communities are present in the ensiling material and during the different phases of silage production. Studying the metabolites formed during ensiling enables an assessment of the predominant fermentation type, which is driven by the different microbial groups (Carvalho et al., 2021). A recent study reported a change in relative microbial groups through the ensiling process, observing that the most dominant genera in the first few days were *Enterobacteriaceae*, *Leuconostocaceae*, and *Pseudomonadaceae*, which were largely replaced by *Lactobacillaceae* during the first 3 days, with *Lactobacillus* and *Pediococcus* species as the main protagonists, suggesting that LAB are key from the early stages of ensiling (Wang et al., 2020). LAB with homofermentative metabolism reduce glucose to lactic acid with negligible DM loss, whereas LAB with obligate heterofermentative metabolism, produce lactic acid, acetic acid, ethanol, H_2O, and CO_2 with a higher DM loss (Borreani et al., 2018; Carvalho et al., 2021). In both experiments performed in this study, the addition of homofermentative/facultative heterofermentative inoculants significantly reduced the pH to below 4.2. In addition, they increased the lactic acid production, with a 12%–49% increase compared to the control in Experiment 1 and a 14%–44% increase in Experiment 2, with a consequently high lactic acid: acetic acid ratio. Additionally, reduced DM loss of 32%–49% and 37%–53% in the first and second experiments was observed, respectively, suggesting successful homofermentative fermentation in the inoculated silages (Kung Jr. et al., 2018; McDonald et al., 1991). Microbial groups associated with spoilage and fermentative metabolism that can lead to altered silage fermentation can also be identified based on their metabolites. The presence of *Enterobacteria* is linked to high acetic acid, ethanol, and CO_2 production; clostridia are linked with high butyrate, CO_2, and H_2 from glucose and lactic acid, together with increased proteolytic indicators, such as NH_4-N%TN; and yeast reduces glucose and lactate to ethanol and CO_2 (Kung Jr. et al., 2018; McDonald et al., 1991). The two PCA analyses representing multiple parameters for untreated and treated forages in two different periods (July and September) separated these highly abundant metabolites in spontaneously fermented forages from the treated forages, highlighting that more efficient fermentation was driven by the inoculant strains in forages harvested and ensiled at different time periods. This observation was also confirmed by the LR treatment used in both experiments. It is important to emphasize that the inoculants used in this study effectively improved silage quality, regardless of DM and WSC content. In contrast, the preservation of crimped ensiled barley grains with low, medium, and high moisture contents was dependent on the additive type, that is microbial- and salt-based inoculants required high moisture content. In contrast, formic and propionic acid-based additives showed consistent improvement, regardless of the moisture content (Franco, Tapio, et al., 2022). In addition, inoculation of high and low DM alfalfa forages with *L. plantarum* 24–7 g for 60 days showed ameliorated silage quality compared to the control, which was more pronounced at high DM than low DM (Zhang et al., 2021).

As described earlier, proteolysis in silage may be related to plant and/or microbial enzymes (Pauly & Wyss, 2019). In this study, all treatments successfully reduced the NH_4-N%TN. This parameter was positively correlated with the acetic acid content in Experiment 1 and 2. All treated forages were negatively correlated with the ammonia-N fraction, especially with the high DM once, in both experiments (Figures 1 and 2). When considering microbial treatments alone, the acetic acid concentration was higher but not statistically different from that of the control when *L. plantarum* (LP2, or LP3) was used as an additive in the Experiment 1, whereas LP1 addition significantly reduced the content. Strictly homofermentative bacteria are unable to produce acetic acid because of their lack of phosphoketolase, which enables pentose utilization (Gänzle, 2015). *L. plantarum* strains exhibit facultative heterofermentative metabolism and, therefore possess inducible phosphoketolase when pentoses are present, which allows acetic acid production while maintaining high acidification; the latter is related to high lactic acid production as acetic acid has a weak acidification ability (Honig, 1990; McDonald et al., 1991; McDonald & Henderson, 1962). In the PCA (Figure 1), the aforementioned treatment-forage type combinations (*L. plantarum* inoculated forages) were also separated from the other forage types because of their high aerobic stability and lactic acid content.

A recent meta-analysis exploring the different effects of inoculation with homofermentative and facultative heterofermentative LAB, including their effect on aerobic stability improvement, concluded that these microbial inoculants had no significant impact on the aerobic stability (Oliveira et al., 2017). Here, in Experiment 1, LP2 significantly increased ASTA, whereas in Experiment 2, no treatment effects were observed.

Both *Pediococcus* inoculants, PP1 and PP2, showed different fermentation patterns in both experiments. The numerically lower lactic acid production and high residual WSC in forages treated with PP1 suggest that although the fermentation led by this inoculants was not as fast as that of the other inoculants, the lower ethanol, ammonia-N, acetic acid, and butyric acid contents (compared with the control) confirmed that it did not give rise to uncontrolled fermentation or signs of pathogen growth. Conversely, the PP2 showed significantly higher lactic acid production and no WSC difference than control, while maintaining the same fermentation profile for the rest of the parameters as PP1. *Pediococcus* species replicate quickly in the first 24 h of fermentation, whereas *Lactobacillus* species are more active in the later stages (McDonald et al., 1991). This may suggest that both *Pediococcus* inoculants established successful initial fermentation, and it could be speculated that inhibitory substances (i.e., bacteriocins) might have impacted the epiphytic LAB or forage contaminants (Jiang et al., 2019).

These observations confirm previous findings concerning the efficacy of the six microbial strains as silage additives (EFSA FEEDAP Panel et al., 2021a, 2021b, 2021c, 2021d; Franco, Nikodinoska, et al., 2022; Gonda et al., 2022; Nikodinoska, Gonda, & Moran, 2022a, 2022b; Apajalahti et al., 2022; Ferrero et al., 2022; Wambecq et al., 2022).

5 | CONCLUSIONS

All six microbial inoculants significantly improved the fermentation quality of the silages compared to the control (measured based on low pH, acetic acid, propionic acid, ethanol, and high lactic acid production). In addition, significant inhibition of DM loss and proteolysis was observed in all treated forages compared to spontaneously fermented silages. Among different treatments, the *L. planatrum* (LP1, LP2, and LP3) and *L. rhamnosus* (LR) strains were more performant compared with the *P. pentosaceus* (PP1 and PP2) strains, primarily due to their higher lactic acid content and higher aerobic stability. Future studies will focus on looking at synergistic fermentation quality effects from combining different *Pediococcus pentosaceus* and *Lactoplantibacillus plantarum* strains. Moreover, alternative strategies to improve ASTA beyond microbial inoculants need to be investigated.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Apajalahti, J., Nikodinoska, I., & Moran, C. A. (2022). Effect of lactic acid bacteria on the silage quality prepared with two different forage crops. Abstracts of the 2022 American dairy science association® annual meeting. Abstract number 86753. *Journal of Dairy Science*, *105*(1), 418.

Borreani, G., Tabacco, E., Schmidt, R. J., Holmes, B. J., & Muck, R. E. (2018). Silage review: Factors affecting dry matter and quality losses in silages. *Journal of Dairy Science*, *101*, 3952–3979.

Carvalho, B. F., Sales, G. F. C., Schwan, R. F., & Ávila, C. S. (2021). Criteria for lactic acid bacteria screening to enhance silage quality. *Journal of Applied Microbiology*, *130*, 341–355.

Downing, T. W., Buyserie, A., Gamroth, M., & French, P. (2008). Effect of water soluble carbohydrates on fermentation characteristics of ensiled perennial ryegrass. *The Professional Animal Scientist*, *24*, 35–39. https://doi.org/10.15232/s1080-7446(15)30807-x

EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., Lopez-Alonso, M., Lopez Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Martino, L. (2018). Guidance on the assessment of the efficacy of feed additives. *EFSA Journal*, *16*(5), 5274.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances usedin Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Dusemund, B., Fasmon Durjava, M., Kouba, M., Lopez-Alonso, M., Lopez Puente, S., Marcon, F., Mayo, B., Pechova, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Cocconcelli, P. S., … Revez, J. (2021a). Scientific opinion on the safety and efficacy of a feed additive consisting of *Lacticaseibacillus rhamnosus* (formerly lactobacillus rhamnosus) IMI 507023 for all animal species (all-technology (Ireland) limited [Alltech Ireland]). *EFSA Journal*, *19*(7), 6700.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances usedin Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Dusemund, B., Fasmon Durjava, M., Kouba, M., Lopez-Alonso, M., Lopez Puente, S., Marcon, F., Mayo, B., Pechova, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Cocconcelli, P. S., … Revez, J. (2021b). Scientific opinion on the safety and efficacy of a feed additive consisting of *Lactiplantibacillus plantarum* (formerly lactobacillus plantarum) IMI 507026 for all animal species (all-technology (Ireland) limited [Alltech Ireland]). *EFSA Journal*, *19*(7), 6703.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances usedin Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Dusemund, B., Fasmon Durjava, M., Kouba, M., Lopez-Alonso, M., Lopez Puente, S., Marcon, F., Mayo, B., Pechova, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Cocconcelli, P. S., … Revez, J. (2021c). Scientific opinion on the safety and efficacy of a feed additive consisting of *Lactiplantibacillus plantarum* (formerly lactobacillus plantarum) IMI 507027 for all animal species (all-technology (Ireland) limited [Alltech Ireland]). *EFSA Journal*, *19*(7), 6704.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances usedin Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Dusemund, B., Fasmon Durjava, M., Kouba, M., Lopez-Alonso, M., Lopez Puente, S., Marcon, F., Mayo, B., Pechova, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Cocconcelli, P. S., … Revez, J. (2021d). Scientific opinion on the safety and efficacy of a feed additive consisting of *Lactiplantibacillus plantarum* (formerly lactobacillus plantarum) IMI 507028 for all animal species (all-technology (Ireland) limited [Alltech Ireland]). *EFSA Journal*, *19*(7), 6705.

Ericson, B., & André, J. (2010). HPLC - applications for agricultural and animal science. Proc. of the 1st Nordic Feed Science Conference (June 22–23, 2010), Uppsala, Sweden. Swedish University of Agric. Sciences (SLU), Department of Animal Nutrition & Management, Uppsala, Sweden. SLU Report #274, p.23–26. https://www.slu.se/globalassets/ew/org/inst/huv/konferenser/nfsc/proceedings/nfsc-2010-proceedings_100617.pdf

Fabiszewska, A. U., Zielińska, K. J., & Wróbel, B. (2019). Trends in designing microbial silage quality by biotechnological methods using lactic acid bacteria inoculants: A minireview. *World Journal of Microbiology and Biotechnology*, *35*, 76. https://doi.org/10.1007/s11274-019-2649-2

Ferrero, F., Nikodinoska, I., Moran, C. A., Tabacco, E., & Borreani, G. (2022). Effectiveness of six lactic acid bacteria as silage inoculants for aerobic stability improvement. Abstracts of the 2022 American dairy science association® annual meeting. Abstract number 86365. *Journal of Dairy Science*, *105*(1), 418–419.

Franco, M., Nikodinoska, I., Moran, C. A., & Rinne, M. (2022). Effects of innovative inoculants on fermentation quality of grass silage. 11th Nordic Feed Science Conference, Uppsala, Sweden, August 22–23, 2022. https://www.slu.se/globalassets/ew/org/inst/huv/konferenser/nfsc/nfsc2022/nfsc-2022-proceedings.pdf

Franco, M., Tapio, I., & Rinne, M. (2022). Preservation characteristics and bacterial communities of crimped ensiled barley grains modulated by moisture content and additive application. *Frontiers in Microbiology*, *13*, 1092062. https://doi.org/10.3389/fmicb.2022.1092062

Gänzle, M. G. (2015). Lactic metabolism revisited: Metabolism of lactic acid bacteria in food fermentations and food spoilage. *Current Opinion in Food Science*, *2*, 106–117.

Gonda, H., Nikodinoska, I., & Moran, C. A. (2022). Innovative microbial inoculants as silage additives in difficult to ensile forage. Abstracts of the 2022 American dairy science association® annual meeting. Abstract number 86140417. *Journal of Dairy Science*, *105*(1), 417.

Guo, X., Xu, D., Li, F., Bai, J., & Su, R. (2023). Current approaches on the roles of lactic acid bacteria in crop silage. *Microbial Biotechnology*, *16*(1), 67–87.

Honig, H. (1990). Evaluation of aerobic stability. Proceedings of the EUROBAC conference Aug. 1986, Uppsala, Sweden. Swedish University of Agric. Sciences. *Grass and Forage Reports*, *3*, 76–82.

IPCC. (2022). IPCC Sixth Assessment Report. Climate change 2022: Mitigation of climate change. CHAPTER 7: Agriculture, forestry, and other land uses (AFOLU). https://www.ipcc.ch/report/ar6/wg3/

Jiang, D., Li, B., Zheng, M., Niu, D., Zuo, S., & Xu, C. (2019). Effects of Pediococcus pentosaceus on fermentation, aerobic stability and microbial communities during ensiling and aerobic spoilage of total mixed ration silage containing alfalfa (*Medicago sativa* L.). *Grassland Science*, *1*, 1–10.

Kagan, I. A., Anderson, M. L., Kramer, K. J., Seman, D. H., Lawrence, L. M., & Smith, S. R. (2020). Seasonal and diurnal variation in water-soluble carbohydrate concentrations of repeatedly defoliated red and white clovers in Central Kentucky. *Journal of Equine Veterinary Science*, *84*, 102858.

Kung, L., Jr., Shaver, R. D., Grant, R. J., & Schmidt, R. J. (2018). Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *Journal of Dairy Science*, *101*, 4020–4033.

Larsson, K., & Bengtsson, S. (1983). Determination of non-structural carbohydrates in plant material. Method description no. 22. National Laboratory for Agricultural Chemistry.

McDonald, P., Henderson, A., & Heron, S. (1991). *The biochemistry of silage*. Chalcombe Publications.

McDonald, P., & Henderson, A. R. (1962). Buffering capacity of herbage samples as a factor in ensilage. *Journal of the Science of Food & Agriculture*, *13*, 395–400.

Muck, R. E., Nadeau, E. M. G., McAllister, T. A., Contreras-Govea, F. E., Santos, M. C., & Kung, L. J. (2018). Silage review: Recent advances and future uses of silage additives. *Journal of Dairy Science*, *101*, 3980–4000. https://doi.org/10.3168/jds.2017-13839

Nikodinoska, I., Gonda, H., & Moran, C. A. (2022a). Effect of lactic acid bacteria on high dry matter forage preservation (abstract number 39798). In European Federation of Animal Science (Ed.), *Book of abstracts of the 73rd annual meeting of the European federation of animal science* (p. 445). Online Academic Submission and Evaluation System https://www.eaap2022.org/docs/AbstractBookEAAP.pdf

Nikodinoska, I., Gonda, H., & Moran, C. A. (2022b). Effect of lactic acid bacteria on low dry matter forage preservation(abstract number 39987). In European Federation of Animal Science (Ed.), *Book of abstracts of the 73rd annual meeting of the European federation of animal science* (p. 446). Online Academic Submission and Evaluation System https://www.eaap2022.org/docs/AbstractBookEAAP.pdf

Nikodinoska, I., Makkonen, J., Blande, D., & Moran, C. (2022c). Draft genome sequence of *Lacticaseibacillus rhamnosus* IMI 507023. *Microbiology Resource Announcements*, *11*(5), e01217–e01221.

Nikodinoska, I., Makkonen, J., Blande, D., & Moran, C. (2022d). Draft genome sequence of *Pediococcus pentosaceus* IMI 507024. *Microbiology Resource Announcements*, *11*(4), e01216–e01221.

Nikodinoska, I., Makkonen, J., Blande, D., & Moran, C. (2022e). Draft genome sequence of *Lactiplantibacillus plantarum* IMI 507026. *Microbiology Resource Announcements*, *11*(6), e00305–e00322.

Nikodinoska, I., Makkonen, J., Blande, D., & Moran, C. (2022f). Genome sequence data of *Lactiplantibacillus plantarum* IMI 507028. *Data in Brief*, *42*, 108190. https://doi.org/10.1016/j.dib.2022.108190

Nikodinoska, I., Makkonen, J., Blande, D., & Moran, C. (2022g). *Pediococcus pentosaceus* IMI 507025 genome sequencing data. *Data in Brief*, *43*, 108446. https://doi.org/10.1016/j.dib.2022.108446

Nikodinoska, I., Makkonen, J., Blande, D., & Moran, C. (2022h). Whole genome sequence data of *Lactiplantibacillus plantarum* IMI 507027. *Data in Brief*, *42*, 108025. https://doi.org/10.1016/j.dib.2022.108025

Nordic Committee in Food Analysis. (1976). Nitrogen. Determination in foods and feeds according to Kjeldahl. Method description No 6. Esbo: Statens Teknologisk Forskning central.

Oliveira, A. S., Weinberg, Z. G., Ogunade, I. M., Cervantes, A. A. P., Arriola, K. G., Jiang, Y., Kim, D., Li, X., Gonçalves, M. C. M., Vyas, D., & Adesogan, A. T. (2017). Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. *Journal of Dairy Science*, *100*(6), 4587–4603.

Pauly, T., & Wyss, U. (2019). Efficacy testing of silage additives—methodology and existing schemes. *Grass and Forage Science*, *74*, 201–210.

R Core Team. (2021). *R: A language and environment for statistical computing* (Version 4.0.3). R Foundation for Statistical Computing. https://www.R-project.org/

Tabacco, E., Comino, L., & Borreani, G. (2018). Production efficiency, costs and environmental impacts of conventional and dynamic forage systems for dairy farms in Italy. *European Journal of Agronomy*, *99*, 1–12.

van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fibre, neutral detergent fibre, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*, 3583–3597.

Wambecq, E., Nikodinoska, I., Haesaert, G., & Moran, C. A. (2022). Efficacy of innovative inoculants on the silage aerobic stability – A short screening study. Abstracts of the 2022 American dairy science association® annual meeting. Abstract number 86265. *Journal of Dairy Science*, *105*(1), 417–418.

Wang, M., Franco, M., Cai, Y., & Yu, Z. (2020). Dynamics of fermentation profile and bacterial community of silage prepared with alfalfa, whole-plant corn and their mixture. *Animal Feed Science and Technology*, *270*, 114702.

Wang, S., Li, J., Dong, Z., Chen, L., Yuan, X., & Shao, T. (2018). The effects of lactic acid bacteria strains isolated from various substrates on the fermentation quality of common vetch (*Vicia sativa* L.) in Tibet. *Grass Forage Science*, *73*, 637–647.

Wilkinson, J. M., & Muck, R. E. (2019). Ensiling in 2050: Some challenges and opportunities. *Grass and Forage Science*, *74*, 178–187.

Windle, M. C., Walker, N., & Kung, L., Jr. (2014). Effects of exogenous protease on the fermentation and nutritive value of corn silage harvested at different dry matter contents and ensiled for various lengths of times. *Journal of Dairy Science*, *97*, 3053–3060.

Zhang, Y. X., Ke, W. C., Vyas, D., Adesogan, A. T., Franco, M., Li, F. H., Bai, J., & Guo, X. S. (2021). 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. *Animal Feed Science and Technology*, *272*, 114751.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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