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Research Article

Comparison of Treatment Effect of Domestically Distributed Major Silage Inoculant

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ABSTRACT

Silage inoculants, crucial in modern silage production, comprise beneficial microorganisms, primarily lactic acid bacteria (LAB), strategically applied to forage material during ensiling. This study aimed to compare the effectiveness of various inoculants produced by different companies. Five treatments were evaluated, including a control group: T1 (*Lactobacillus plantarum*), T2 (*Lactobacillus plantarum* + *Pediococcus pentosaceus*), T3 (*Lactobacillus plantarum* + *Pediococcus pentosaceus* + *Lactobacillus buchneri*), T4 (*Lactobacillus plantarum* + *Lactobacillus acidophilus* + *Lactobacillus bulgaricus*), and T5 (*Lactobacillus plantarum* + *Pediococcus pentosaceus* + *Enterococcus faecium*). Italian ryegrass was harvested at the heading stage and treated with these silage inoculants. Samples were collected over a 60-day ensiling period. Co-inoculation with *L. plantarum* and *P. pentosaceus* (T2) resulted in significantly higher CP compared to the control group co-inoculation exhibited with resulted in *Lactobacillus plantarum* and *Pediococcus pentosaceus* in the T2 treatment exhibited higher CP content of 106.35 g/kg dry matter (DM). The T3 treatment, which included heterofermentative bacterial strains such as *Lactobacillus buchneri*, exhibited an increase in acetic acid concentration (11.15 g/kg DM). In the T4 treatment group, which utilized a mixed culture of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*, the NH_3-N/TN content was observed to be the lowest (20.52 g/kg DM). The T5 containing *Enterococcus faecium* had the highest RFV (123) after 60 days. Expanding upon these findings, the study underscores not only the beneficial effects of particular inoculant treatments on silage quality but also underscores the potential of customized inoculation strategies in maximizing nutrient retention and overall silage preservation.

(Key words: Fermentation, Inoculant, Lactic acid bacteria, Quality, Silage)

Ⅰ. INTRODUCTION

Silage inoculants are microbial additives strategically applied to enhance ensiling fermentation. The principal role of silage inoculants is to accelerate and enhance the fermentation process by stimulating the growth of beneficial LAB strains (Bai et al., 2021). These bacteria convert water-soluble carbohydrates in forage crops into organic acids, predominantly lactic acid (Gonda et al., 2023). This acidification process reduces the pH level, creating an environment unfavorable for spoilage organisms and pathogens, thus preserving the forage (Kung, 2018). Additionally, the fermentation of water-soluble carbohydrates into lactic acid, a process known as homolactic fermentation, is preferred for its efficiency in pH reduction compared to alternative acids such as acetic acid. This efficiency helps mitigate dry matter losses associated with gas production during fermentation by heterofermentative bacteria (Li et al., 2022). In addition to acidification, silage inoculants aim to minimize nutrient losses during ensiling, preserve dry matter content, enhance aerobic stability, and ultimately improve the nutritional value of the silage fed to livestock (Muck et al., 2018).

Advances in microbial technology have facilitated the development of diverse inoculant formulations, including combinations of distinct bacterial species and strains (Lobo et al., 2019). These consortia target specific biochemical pathways within the ensiling process, aiming to optimize fermentation efficiency and cater to the varied requirements of silage producers. This ultimately results in the production of silage

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with superior quality characteristics. This study aims to assess various silage inoculant products from different companies, including those utilizing combination inoculants, to provide a comprehensive view of their compositions, efficacy, and potential variations in their impact on silage quality.

Ⅱ. MATERIALS AND METHODS

1. Experimental design

This experiment was conducted at the experimental field of Pyeongchang Campus, Seoul National University, Gangwon-do, Republic of Korea (Latitude: 37° 32' 46.10" N, Longitude: 128° 26' 17.90" E). The Italian ryegrass (IRG) was seeded In the "Kowinearly" variety, developed by the National Institute of Animal Science (Choi et al., 2011) on October 6, 2022, and harvested from the heading stage on May 18, 2023. The chemical composition of pre-ensiled Italian ryegrass is in Table 1. After harvest with manual mixing, the IRG was chopped into 2-3 cm by a cutter (SC-7000, Agricultural Machinery, Inc., Daegu, South Korea), and then treated with silage inoculant. The silage samples were individually packed into 20-liter mini-buckets at a weight of 10 kg per bucket, with three repetitions performed. These buckets were then stored at room temperature for the duration of the study. The composition of the treatment product is as follows: (Control): 100 mL distilled water /50 kg of fresh matter, (T1): Lactobacillus plantarum at the rate of 3×105 cfu/50 kg, (T2): Lactobacillus plantarum + Pediococcus pentosaceus at the rate of 2×104 cfu/50 kg, (T3): Lactobacillus plantarum + Pediococcus pentosaceus + *Lactobacillus buchneri* at the rate of 2.5×105 cfu/50 kg, (T4): *Lactobacillus plantarum* + *Lactobacillus acidophilus* + *Lactobacillus bulgaricus* at the rate of 4.25×10^3 cfu/50 kg, (T5): *Lactobacillus plantarum* + *Pediococcus pentosaceus* + *Enterococcus faecium* at the rate of 5×10^5 cfu/50 kg. All additives were produced by different companies. The fermentation period for silage was 60 days.

2. Laboratory analyses

The acidity analysis was 10 g sample of fresh silage from each treatment was placed in triplicate into 250 mL conical flasks containing 90 mL of distilled water, sealed tightly to prevent air entry. The flasks were placed on a shaker (Green Seriker, Vision Scientific, Korea) and shaken for approximately one hour. Subsequently, the sealed flasks were stored in a refrigerator for 24 hours. During this period, the conical flasks were manually shaken every two hours. After the 24-hour stored in refrigerator, the mixture was filtered through Whatman No. 6 filter paper (AVANTEC) into a 50-mL centrifuge tube. The filtrate was constantly shaken during filtration. The pH of the extract was promptly measured with a pH meter (AB 150, Fisher Scientific International, Inc., Pittsburgh, US). The silage extraction was preserved at -20℃ in the refrigerator until further analysis for volatile fatty acids, lactic acid, and ammonia nitrogen.

The organic acid analysis proceeded as follows: The thawed extract underwent thorough shaking, and 1.5 mL of the sample extracts were centrifuged at 3000 revolutions per minute (rpm), at 4 degrees Celsius for 15 minutes using a Centrifuge (Smart 15, Hanil Science Industrial, South Korea). Subsequently, the resulting supernatant was withdrawn with a syringe (KOVAX-SYRINGE 1 mL) and injected into a vial through a filter (13 mm Syringe Filter, w/0.45 μm PVDF Membrane). Following this preparation, the supernatants were analyzed for organic acid utilizing an Agilent HPLC 1260 (HPLC, Agilent Technologies, Santa Clara, California, US) equipped with a refractive index detector. The column employed was the Agilent Hi-Plex H (7.7 x 300 mm, 8 μm, part number PL1170-6830); Mobile phase: 0.005 M H_2SO_4; Flow rate: 0.7 mL/min). The column temperature was maintained at 60°C, and the pressure was set at 4.6 MPa (46 bar, 670 psi). Detection of the relative volatility (RV) occurred at 55°C.

Ammonia-N concentration was determined using the modified

Table 1. Chemical composition of pre-ensiled Italian ryegrass

pH	DM	WSC	CP	NDF	ADF	IVDMD	TDN	RFV
	(g/kg DM)						(% DM)	
6.28	236.50	129.30	98.83	575.62	344.38	71.33	61.70	100

DM=dry matter; WSC=water soluble carbohydrate; CP=crude protein; NDF=neutral detergent fiber; ADF=acid detergent fiber; IVDMD=*in vitro* dry matter digestibility; TDN=total digestible nutrient; RFV=relative feed.

phenol-hypochlorite procedure as outlined by Broderick and Kang (1980). The thawed extract was mixed thoroughly under natural environmental conditions. Subsequently, 12 mL of the extract were transferred into a 15 mL centrifuge tube using a pipette and then centrifuged at 3000 rpm, maintaining a temperature of 4°C for 15 minutes. A 0.02 mL aliquot of the supernatant was injected into a 25 mL test tube, followed by the sequential addition of 1 mL of phenol reagent and 1 mL of alkali-hypochlorite reagent. The test tube was immediately covered and shaken vigorously to ensure thorough mixing of the reagents and supernatant. The test tube was then placed in a 37°C water bath for 15 minutes to allow for color reaction. Following that, 8 mL of distilled water were added, and the mixture and combination was vortexed thoroughly to ensure proper mixing. The spectrophotometric analysis was carried out at 630 nm wavelength using a Libra S70 Double Beam Spectrophotometer (Biochrom, Korea). Calibration at 630 nm was performed using a blank, and the ammonia nitrogen concentration of both standard and sample extractions was measured.

Fresh Italian ryegrass samples taken before ensiling and silage samples obtained upon opening were sub-sampled for the analysis of dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), water-soluble carbohydrates (WSC), and *in vitro* dry matter digestibility (IVDMD). All chemical analyses were conducted and reported on a dry matter basis. Upon opening the polyethylene bags containing silage, the samples were meticulously mixed, and approximately 300 g of each sample was extracted.

The dry matter analysis proceeded as follows: The samples were first placed in paper bags, weighed, and then subjected to air-forced drying in an oven at 65°C for 72 hours. After the drying process, all samples were ground to pass through a 1 mm screen using the "Wiley Mill 4 1/2 Horse Power Unit Motor" instrument (Thomas Scientific, Inc., New Jersey, USA). Following grinding, the ground samples were transferred into plastic bottles and stored in a dried storage room at 4°C until further analysis.

The crude protein (CP) analysis was conducted using the Dumas method, as originally described by Dumas (1831). Before sampling, the contents of the plastic bottle were meticulously mixed to minimize errors. Dried sample powder ranging from 9 mg to 11 mg was accurately weighed and enclosed in tin foil, where it was compressed into small squares for precise protein content analysis. The CP analysis was performed using the "Automatic Vector Analyzer Euro Vector EA3000" instrument (EVISA Co., Ltd, Milan, Italy), adhering to established protocols and methodologies.

For neutral detergent fiber (NDF) and acid detergent fiber (ADF) analysis, 0.5 ~ 0.6 g of dried sample powder was precisely weighed and enclosed in Nylon filter bags (50 mm × 55 mm, ANKOM F57, ANKOM Tech., Fairport, NY). In the NDF procedure, 20 g of sodium sulfate and 12 mL of alpha-amylase were added. However, these additions were not necessary for ADF analysis. The NDF and ADF analyses were conducted following the methods outlined by Van Soest et al. (1991) utilizing the ANKOM A2000 Automated Fiber Analyzer (Ankom Technologies, Inc., Fairport, NY, USA).

The determination of water-soluble carbohydrates (WSC) in oven-dried materials was conducted using a modified Anthrone-Sulfuric acid colorimetry method as described by Yemm and Willis (1954). Ground samples weighing 0.2 g from each treatment were taken in triplicate and placed in labeled 250 mL conical flasks with 200 mL distilled water, securely sealed, and shaken using a shaker (Green Sseriker, Vision Scientific, Korea) for one hour. The mixture was subsequently filtered through (Whatman No. 1, AVANTEC).

Next, 2 mL of the filtrate was pipetted into labeled test tubes, and 10 mL of anthrone reagent was rapidly added. The test tubes were capped and shaken thoroughly to ensure proper mixing of the reagent and filtrate. The capped tubes were then placed in a boiling water bath (approximately 100°C) for 20 minutes to initiate the color reaction. Afterward, they were cooled in running tap water for 10 minutes and vortexed to mix. The WSC content was analyzed using the Libra S70 Double Beam Spectrophotometer (Biochrom, Korea) at 620 nm wavelength. The spectrophotometer was adjusted to “0” using a blank, and then the WSC of standard and sample extractions were measured at 620 nm wavelength. The formula utilized to determine the Water-Soluble Carbohydrate (WSC) content is as follows: The percentage of WSC is calculated by multiplying the value of G (milligrams of glucose read from the graph) by the dilution factor (D), the extract volume (E, set at 200 mL), and a constant (0.1). This product is then multiplied by 100 and divided by the product of the sample weight (W, measured in milligrams) and the sample's laboratory dry matter percentage.

The *in vitro* dry matter digestibility (IVDMD) was analyzed using the two-stage technique method detailed by Tilley and Terry (1963). Nylon filter bags were first soaked in acetone for 5 minutes and then dried in an air-forced drying oven at 105°C for over 4 hours. Ground samples weighing between 0.5 to 0.6 g were placed into the labeled nylon filter bags, which were then sealed tightly using a heat sealer. These sealed sample bags, along with 2 blank bags, were placed into incubation bottles (Ankom Technologies, Inc., Fairport, NY, USA) containing 1330 mL of buffer solution A and 266 mL of buffer solution B (in a 5:1 ratio, v/v). The incubation bottles were then placed in an incubator set at 39°C.

Rumen fluid was collected from cows with cannulas. Holstein heifers before their morning feeding. The collected rumen fluid was pooled and filtered through four layers of cheesecloth into preheated thermos bottles. To each prepared incubation bottle, 400 mL of filtered rumen fluid was added. The bottles were then continuously filled with CO_2 gas for 30 seconds and tightly closed to achieve anaerobic conditions. The preparation of In vitro culture solution by combining rumen fluid and buffer solution at a ratio of 1:4. The incubation bottles were placed back into the incubator at 39°C and continuously rotated for 48 hours. After 48 hours, the incubation bottles were removed, and the sample bags were washed until the water ran clear. The neutral detergent fiber (NDF) procedure was then performed. The buffer solution A contains the following components per liter: 10.0 grams of KH_2PO_4, 0.5 grams of $MgSO_4 \cdot 7\ H_2O$, 0.5 grams of NaCl, 0.1 grams of $CaCl_2 \cdot 2H_2O$, and 0.5 grams of reagent-grade urea. On the other hand, buffer solution B consists of 15.0 grams of Na_2CO_3 and 1.0 gram of $Na_2S \cdot 9H_2O$ per liter.

The calculation of total digestible nutrients (TDN), as outlined by Rohweder et al. (1978), follows a specific formula: Legumes and grasses: TDN% = 88.9 - (0.79 × ADF%). Relative feed value (RFV) serves as a predictive measure of feeding value, amalgamating estimated intake and digestibility into a unified index. The calculation of RFV, as proposed by Rohweder et al. (1978), is derived from the following formula: Digestible dry matter (DDM) = 88.9 − (0.779 × ADF%), Dry matter intake (DMI) = 120 / NDF%, Relative feed value (RFV) = (DMI × DDM) / 1.29.

3. Statistical analysis

All data were analyzed for variance by the General linear modal (GLM) procedure of SPSS statistical software (IBM SPSS Statistics 26 program SPSS Inc., Chicago, Illinois, USA). Mean treatment differences were obtained by Duncan's multiple range tests. The significant differences were significant of 5%.

Ⅲ. RESULTS AND DISCUSSION

1. Effect of inoculant on silage quality

The chemical composition of Italian ryegrass is shown in

Table 2. Effect of chemical composition of Italian ryegrass silage with inoculant

Additives	DM	WSC	CP	NDF	ADF	IVDMD	TDN	RFV
	(g/kg DM)						(% DM)	
Control	217.39^{d}	12.63^{c}	89.10^{c}	538.93^{a}	340.43^{a}	75.43^{ab}	62.00^{b}	108^{b}
T1	222.97^{c}	42.81^{a}	93.44^{bc}	527.24^{a}	333.86^{a}	75.79^{ab}	62.52^{b}	111^{b}
T2	216.42^{d}	34.39^{b}	106.35^{a}	537.82^{a}	335.54^{a}	75.91^{ab}	62.63^{b}	109^{b}
T3	245.00^{a}	34.74^{b}	97.32^{abc}	535.67^{a}	334.16^{a}	76.66^{a}	62.50^{b}	109^{b}
T4	225.58^{bc}	30.88^{b}	92.53^{bc}	538.36^{a}	337.22^{a}	73.80^{b}	62.26^{b}	108^{b}
T5	228.18^{b}	34.38^{b}	101.89^{ab}	487.28^{b}	316.88^{b}	76.58^{a}	63.86^{a}	123^{a}
Mean	225.92	31.64	96.77	527.55	332.52	75.69	62.63	111
SEM	2.35	2.29	1.81	5.77	2.48	3.57	0.20	1.54

DM=dry matter; CP=crude protein; WSC=water soluble carbohydrate; NDF=neutral detergent fiber; ADF=acid detergent fiber; IVDMD=*in vitro* dry matter digestibility; TDN=total digestible nutrient; RFV=relative feed value. $^{a-d}$ Mean within rows with unlike superscripts differ ($p<0.05$); SEM=standard error of mean; T1=*Lactobacillus plantarum* at the rate of 3×10^5 cfu/50kg; T2=*Lactobacillus plantarum* + *Pediococcus pentosaceus* at the rate of 2×10^4 cfu/50kg; T3=*Lactobacillus plantarum* + *Pediococcus pentosaceus* + *Lactobacillus buchneri* at the rate of 2.5×10^5 cfu/50kg; T4=*Lactobacillus plantarum* + *Lactobacillus acidophilus* + *Lactobacillus bulgaricus* at the rate of 4.25×10^3 cfu/50kg; T5=*Lactobacillus plantarum* + *Pediococcus pentosaceus* + *Enterococcus faecium* at the rate of 5×10^5 cfu/50kg.

Table 2 and 3. The DM content of T3 treated silage was higher at 245.00 g/kg compared to other groups. The decline in WSC was consumed by LAB or other bacteria during ensiling. Italian ryegrass silages treated with Control showed the lowest WSC content at 12.63 g/kg DM. These WSC were consumed by both undesirable bacteria and LAB (Shao et al., 2007). Microbial inoculants ensure the presence of sufficient lactic acid bacteria, which efficiently ferment WSC to produce a substantial amount of LA and reduce the pH (Filya et al., 2000). During this experiment, a decrease in CP content after 60 days was noted. This decrease reflects the inevitable degradation of plant protein during ensiling, leading to alterations in the nitrogen content of the silage. Lower pH levels inhibit protein degradation, while some microbial activities contribute to amino acid synthesis (Neis et al., 2015). Among Italian ryegrass silages, T2 displayed the highest CP content at 106.35 g/kg DM, followed by T5 at 101.89 g/kg DM. This concurs with the observations of Ran et al. (2022), who noted elevated CP content in inoculant-treated silages compared to the Control. These findings imply that the combination of bacterial strains in T2 and T5 treatments positively affected CP preservation during ensiling. The synergistic metabolic activities of these bacteria likely facilitated more efficient fermentation processes, resulting in heightened CP retention and enhanced preservation of proteinaceous compounds within the silage.

After 60 days of ensiling, reductions in ADF and NDF levels were observed, indicating improved nutrient preservation and enhanced digestibility and intake for livestock. NDF, comprising cellulose, hemicellulose, and lignin, contributes to the rigidity of plant cell walls. A decline in NDF levels signifies a breakdown of these structural components, potentially improving palatability and digestibility for animals. Similarly, ADF, consisting of cellulose and lignin but excluding hemicellulose, represents the less digestible fraction of plant material. Reduced ADF levels suggest a decrease in lignin and cellulose content, further enhancing digestibility (Li, 2021).

The significant decrease in both NDF and ADF content, particularly notable in the T5 treatment, underscores the effectiveness of this inoculant in facilitating the breakdown of plant cell walls. *Enterococcus faecium* likely contributes to enzymatic activities that degrade structural carbohydrates, leading to reduced NDF and ADF content. This enhanced breakdown implies improved nutrient accessibility and utilization by animals, highlighting the potential of T5 for nutrient preservation in ensiled forage (Zhao et al., 2021).

The increase in IVDMD might be attributed to the intricate nature of the rumen, influenced by various complex factors affecting digestibility (Merchen and Bourquin, 1994). The study of Weinberg et al. (2003) demonstrated that lactic acid bacteria (LAB) strains could migrate from silage to the rumen fluid and persist there for a minimum of 96 hours. These LAB strains might engage with rumen microorganisms, potentially

Table 3. Effect of pH, NH_3-N/TN and organic acid content of Italian ryegrass silage with inoculant

Additives	pH	NH_3-N/TN	LA	AA	PA	BA	LA / AA
				(g/kg DM)			
Control	4.70^{a}	86.67^{a}	28.23^{b}	ND	51.69^{a}	33.15^{a}	0.00
T1	3.79^{b}	27.86^{cd}	107.71^{a}	5.76^{bc}	11.00^{b}	ND	25.11^{a}
T2	3.80^{b}	39.79^{b}	107.70^{a}	5.42^{c}	ND	ND	26.68^{a}
T3	3.76^{b}	33.61^{bc}	101.30^{a}	11.15^{a}	ND	ND	12.35^{c}
T4	3.80^{b}	20.52^{d}	105.15^{a}	5.56^{bc}	5.74^{c}	ND	25.39^{a}
T5	3.79^{b}	39.55^{b}	107.96^{a}	6.36^{b}	ND	ND	22.90^{b}
Mean	3.94	41.33	93.01	5.07	16.24	3.06	13.96
SEM	0.08	5.27	7.09	-	-	-	1.75

DM=dry matter; NH_3-N=ammonia nitrogen; TN=total nitrogen; LA=lactic acid; AA=acetic acid; PA=propionic acid; BA=butyric acid. $^{a-d}$Mean within rows with unlike superscripts differ ($p<0.05$); SEM=standard error of mean;T1=*Lactobacillus plantarum* at the rate of 3×10^5 cfu/50kg; T2=*Lactobacillus plantarum* + *Pediococcus pentosaceus* at the rate of 2×10^4 cfu/50kg; T3=*Lactobacillus plantarum* + *Pediococcus pentosaceus* + *Lactobacillus buchneri* at the rate of 2.5×10^5 cfu/50kg; T4=*Lactobacillus plantarum* + *Lactobacillus acidophilus* + *Lactobacillus bulgaricus* at the rate of 4.25×10^3 cfu/50kg; T5=*Lactobacillus plantarum* + *Pediococcus pentosaceus* + *Enterococcus faecium* at the rate of 5×10^5 cfu/50kg.

improving rumen functionality and subsequently enhancing animal performance. Hence, the precise cause remains unclear, necessitating further research in this area. RFV and TDN typically correlate with NDF and ADF. In this study, the reduction in NDF and ADF across all silage samples increased TDN and RFV overall.

2. Effects of inoculant on fermentation quality

The rate at which the pH declines during silage fermentation is as crucial as the final pH value achieved (Makoni et al., 1997). This decline in pH determines how rapidly undesirable microorganisms are either eliminated or inhibited during the initial stages of silage fermentation. In this experiment, the Control silage exhibited a higher pH value than the other groups, registering at 4.70.

The Italian ryegrass silage treated with the Control had a lower LA content compared to the other silages treated with inoculants. The inoculant-treated silages could effectively accelerate fermentation, resulting in higher amounts of lactic acid, which plays a key role in inhibiting the growth of undesirable bacteria (Özogul and Hamed, 2018).

The AA primarily originates from acetic acid bacteria and heterofermentative lactic acid bacteria (McDonald et al., 1991). In Italian ryegrass silage treated with T3, the AA content was notably higher at 19.07 g/kg DM, indicating a tendency toward heterogeneous fermentation. Numerous studies have highlighted *Lactobacillus buchneri* inclination toward this fermentation type, leading to increased acetic acid production (McDonald et al., 1991; Kung Jr. et al., 2003). This experiment observed the highest AA concentrations in T3, attributable to the presence of *Lactobacillus buchneri* within the T3 inoculant. Acetic acid production during ensiling contributes significantly to improving fermentation, preserving silage quality, and bolstering its stability, thus ensuring high-quality livestock feed.

In this experiment, the PA content in Italian ryegrass silage treated with the Control group was the highest at 51.68 g/kg DM. Typically, biological additives intended to increase propionic acid in silage contain bacteria from the *Propionibacteria* family (Piwowarek et al., 2018). However, research indicates that these organisms often struggle to compete effectively in regular silage conditions, rendering their impact typically ineffective.

The BA was only detected in the Control group at 33.15 g/kg DM. Butyric acid, a natural byproduct of fermentation, is generally deemed undesirable in silage production due to its adverse effects (Dunière et al., 2013). Elevated butyric acid levels indicate clostridial fermentation, considered one of the least favorable fermentation types. Silages with high butyric acid content tend to have lower nutritional value and higher NDF and ADF levels, as many soluble nutrients are degraded. These silages may also contain elevated levels of soluble proteins and small protein compounds known as amines, which have occasionally been shown to impact animal performance (Bolsen et al., 1996a). Butyric acid contributes to reduced silage intake owing to its pungent, rancid-butter odor. In silage, its content decreased from 1.16 to 0.84% DM, while in rice straw silage, it dropped from 0.01% DM to undetectable levels due to *Lactobacillus plantarum*. This reduction might be attributed to decreased pH resulting from *Lactobacillus plantarum* addition, inhibiting clostridium activity. Protein degradation during ensiling leads to the buildup of nonprotein-N and ammonia-N (Zhang et al., 2021).

High ammonia concentrations can stem from excessive protein breakdown in silage due to a slow decline in pH or the activity of clostridia. Generally, wetter silages tend to have higher ammonia levels (Kung Jr. et al., 2018). Silages with very high moisture content (< 30% DM) have even greater ammonia concentrations due to the potential for clostridial fermentation (Kung and Shaver, 2001). Additionally, poorly packed or slowly filled silages can also exhibit elevated ammonia concentrations. In this experiment, the NH_3-N/TN concentration of the Control group was the highest at 86.67 g/kg DM, albeit still below 100 g/kg DM, indicating that the protein content in all silages was well preserved (Nkosi et al., 2010). Notably, the T4 treatment group, which utilized a mixed culture of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*, exhibited the lowest NH_3-N/TN content.

Distinct variations in organic acid production were observed across varying moisture levels. Elevated moisture content exhibited a predominant presence and higher concentrations of ammonia and butyric acids. Conversely, lower moisture levels favored the maximization of lactic and acetic acids in most experiments. The heightened levels of ammonia and butyric acid suggest the proliferation of *Clostridium* (Pahlow et al., 2003).

Ⅳ. CONCLUSIONS

During the 60-day ensiling period, the inoculant-treated Italian ryegrass silages exhibited significant alterations in their chemical compositions compared to the Control group. The inoculants, particularly those containing *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Lactobacillus buchneri*, and *Enterococcus faecium*, played distinct roles in enhancing fermentation efficiency, preserving nutrients, and preventing undesirable changes during ensiling.

Notably, the inclusion of *Enterococcus faecium* in T5 resulted in the most effective reduction in NDF and ADF content, highlighting its potential for nutrient preservation. Additionally, the synergy between *Lactobacillus plantarum* and *Pediococcus pentosaceus* facilitated lactic acid fermentation, ensuring superior silage quality. The introduction of *Lactobacillus buchneri* in T3 contributed to increased acetic acid production, a key factor in preventing aerobic deterioration in silages.

Building on these insights, the study not only emphasizes the positive impact of specific inoculant treatments on silage quality but also suggests the potential of tailored inoculation strategies for optimizing nutrient retention and overall silage preservation.

Ⅴ. ACKNOWLEDGEMENTS

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