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Research Article

Improvement of Orchardgrass (*Dactylis glomerata* L.) Silage Quality by Lactic Acid Bacteria

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ABSTRACT

In the current study, lactic lactic acid bacteria (LAB) *Lactobacillus plantarum and Pediococcus pentosaceus* were used as a mixed additive for the production of Orchardgrass silage by ensiled method and nutritional change fermentation ability and microbial content of experimental silages. The addition of LAB to Orchardgrass during ensiling process rapidly reduced the pH of the silages than the non-inoculated silages. In addition, the lactic and acetic acid content of silage was increased by LAB strains than the non-inoculated silages whereas butyric acid content was reduced in silage treated with LAB. A microbiological study revealed that higher LAB but lower yeast counts were observed in inoculated silages compared to non-inoculated silage. Overall data suggested that the addition of LAB stains could have ability to induce the fermentation process and improve the silage quality via increasing lactic acid and decreasing undesirable microbes.

(**Key words**: Lactic acid bacteria, Orchardgrass, Silage, Organic acid)

Ⅰ. INTRODUCTION

The ensiling is commonly used for the preservation of forages for a long time; it is considered effective storage of harvested forages due to its easy operation, economic and prevents the loss of nutrition (Wang et al., 2019). The natural fermentation process largely determined the quality of silage. LAB ferments water-soluble carbohydrates immediately after the forage enters the anaerobic status and then converts into organic acids and other valuable products, which leads to induce rapid acidification and inhibits spoilage microorganisms including undesirable bacteria, yeast and mold (Burns et al., 2018; McDonald et al., 1991). In addition, LAB has produced acetic acid, ethanol, CO_2, 1,2-propanediol and other products via various metabolic pathways of carbohydrate use (Lahtinen et al., 2011), which also possess significant biological activities. Particularly, lactic acid produced by LAB is primarily responsible for silage conservation, so it could be considered as the most prominent group of bacteria which used as additives (Burns et al., 2018; Guo et al., 2021; Muck et al., 2018). LAB improved silage quality, aerobic stability, and reduced aflatoxin B1 level. The use of inoculants as additives for silage production is recommended (Muck et al., 2018). These inoculants altered many microbiological and nutritional qualities of silages (Burns et al., 2018) . The positive effects on fermentation process of silage are based on the strain characteristics. One of the common challenges in the livestock's industries is the extent of variability in the effects of inoculant bacteria on the fermentation of silages and their preservation, nutritional quality, and animal performance (Kristensen et al., 2007; Muck, 1997). Lactic acid bacteria (LAB), *Lactiplantibacillus plantarum* subsp. *Pediococcus pentosaceus* and *Enterococcus faecium* are the homofermentative LAB which is most extensively used as a microbial additive for silage production (Muthusamy et al., 2020; Ogunade et al., 2018; Oliveira et al., 2017), Among these, *Lactiplantibacillus plantarum* sub is the most commonly used silage inoculant. In addition, some other LAB species are also considered silage inoculants due to their rapid growth at higher pH compared to *L. plantarum* sub (Oliveira et al., 2017). Some researchers recommended synergistic mixture of LAB can be used for silage production during a different phase of fermentation. For example, *Pedioccous*

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strains are more tolerant to high dry matter conditions than *Lactobacillus* spp and show a broad range of optimal temperatures and pH values for their growths. Silage treated with one or more bacteria as dual inoculants often have a lower pH, acetic, butyric acid, ammonia nitrogen contents and higher lactic acid level with better DM recovery compared to untreated silages(Muck, 1997).

Orchardgrass is a perennial, tall-growing under cool season grasses and fairly drought resistant. It considers as valuable forages that can be used for hay, pasture and silage. It provides nutritious feed to livestocks including cattle, sheep, goats and horses. Orachardgrass had higher nutritive contents than the other forages such as bromegrass, tall fescue, and reed canarygrass (Butkutė et al., 2014; Turner et al., 2007). The inclusion of Orchardgrass hay and silage in steers and sheep must around 60-75% to avoid negative impacts on rumen fermentation (Bourquin et al., 1994; Niderkorn et al., 2015). Silage produced from a mixed ration of orchardgrass and alfalfa at the ratio of 50:50 favors the growth of rumen microorganisms without altering nutrient digestion and rumen fermentation (Xue et al., 2019). In the present study, Orchardgrass silage was produced using Top silage bacteria (Jungnongbio, Co. South Korea) by ensiling method and analyzed their organic acid content, microbial and nutrients profiles of experimental silages.

Ⅱ. MATERIAL AND METHODS

1. Place and collection of Orchardgrass

The *Orchardgrass* (Onnuri) was cultivated at Grassland and Forage field, National Institute of Animal Science, Seonghwan-eup, Cheonan, Korea by standard grassland cultivation guidelines given by Rural Development Administration recommendations. Orchardgrass was sown in narrow strips in plots 2m by 3m in a randomized block in the late middle of September. It was harvested at the heading stage (30%) in the middle of May. The total soluble carbohydrate was 10.2 ± 0.25%, determined by anthrone method (Murphy, 2010) and used for silage production. Top Silage bacteria (*L. plantarum* KCC-10, KCC-19, K46 and *Pediococcus pentosaceus* KCC-23, 100g/50tone, 10^7 CFU/g) were obtained from Jungnong Company Pvt. Ltd. and used as additives for silage production by the ensiling method.

2. Silage production from Orchardgrass plant

The Orchardgrass first cut was harvested and dried under field conditions for 36h and then the moisture content of samples was analyzed frequently by microwave Oven method. After reaching the expected moisture content (50-55%), 200g for Orchardgrass was weighed and chopped to a theoretical cut of 1.5-2.5 cm with a manual cutter(Muthusamy et al., 2020). The samples were packed in a silage bag (28×36 cm, Aostar Co., Ltd., Seoul, Korea) with/without LAB 100g/ 50 tone of forage. Top silage bacteria were used as an additive for silage production. The air was evacuated from all bags by a vacuum sealer (Food saver V48802, MK Corporation, Seoul, Korea). All vacuum sealed bags were kept at room temperature for 45 days. After opening at day 45, the pH and nutrient profiles such as CP, ADF(AOAC, 2000), NDF(Van Soest et al., 1991), and TDN (TDN = 89.9 － (ADF * 0.79) contents of silage were determined (Guo Qiang Zhao et al., 2020)

3. Quantification of organic acids and microbial population enumeration in ensiled silages

Ten grams of silage samples were taken and mixed with 90 mL sterile water and shake vigorously in an orbital shaker for 60 minutes. The extract was filtered via double layers of sterilized cheesecloth and divided into three portions. A portion was used to analyze the pH of silage samples (Lab pH meter, Thomas Scientific, Swedesboro, NJ, USA). Other portions were used to determine the content of the organic acid by the HPLC method (Arasu et al., 2014) and enumerated LAB, yeast and mould by MRS agar and 3M petriflim (3M Microbiology Products, USA) (Soundharrajan et al., 2020)

4. Statistical Analysis

The obtained data were subjected into statistical analysis using a statistical Package for the Social Science-16 (SPSS-16, Chicago; SPSS Inc). Means and standard errors were calculated for all the amino acid content using the means procedure of the SPSS. The significant between amino acids was performed by the general linear model containing multivariate analysis with Duncan’s multiple range tests. Significance was defined at $p<0.05$.

Ⅲ. RESULTS AND DISCUSSION

Table 1 shows moisture content and nutrient composition of Orchardgrass silages after LAB treatments. The moisture contents of the control and LAB inoculated silages were 52.41% and 53.3%, respectively. The nutrient contents of silages such as crude protein (CP), Acid detergent fiber (ADF), Neutral detergent fiber (NDF), Total digestible nutrients (TDN) were not altered significantly between control and LAB inoculated silages.

Table 2 shows the acidification and microbial composition of experimental silages. The pH of the non-inoculated silage was 5.81 and inoculated silage was 4.73 pH value. The non-inoculated had higher pH indicates a failure to induce fermentation process due to insufficient LAB population. By contrast, LAB treatments reduced the pH of the silages due to the microbial changes compared to the control group. Reduction in pH of silages is majorly dependent on microbial changes in silages particularly higher LAB population with lower *enterobacteria*, *clostridium*, yeast, and mold counts have been considered essential criteria for silage production by ensiling method. The previous finding suggested that Orchardgrass treated with different LAB inoculants sharply reduced the pH of silages (4.35 - 4.49 pH values) and increased lactic acid content (Jalc et al., 2009). The present study also reduced the pH of silage in response to LAB inoculants but the degree of pH reduction; it may have several reasons in particular moisture content of forages, cultivation places and methods etc. The pH values of the present findings were consistent with microbial changes in silages of control and LAB treatment. It shows silage without inoculum treatment had lower numbers of LAB (LAB: 8.0 × 10^7 CFU/g) but higher yeast counts. By contrast, higher LAB (31.5 × 10^7 CFU/g) and lower yeast counts (1.73 × 10^3CFU/g) were observed in silage treated with LAB than in the control group. According to the previous finding epiphytic LAB population widely varies in composition and the numbers in plant materials are based on various environmental factors (Pahlow et al., 2003). However, under suitable conditions such as an anaerobiosis, water activity, and temperature, the LAB can dominate other microbial growth and induce spontaneous lactic acid fermentation, and convert water-soluble carbohydrates into organic acids (Di Cagno et al., 2013).

The key acids identified in the silages are lactic, acetic, and butyric acids, these acids are highest concentration present in silages (Kung, 2001), particularly lactic acid was found at the highest level in silages during the ensiling process, and its key reason to reduce pH of silage during fermentation because it is approximately 10-12 times higher than other major acids (Kung et al., 2018b). The present study showed that silage produced without LAB inoculants had less concentration of lactate (0.01% DM) but silages inoculated with LAB significantly (*p<0.05*) increased lactate content (>150 fold). Acetic acid content for non-inoculated was 0.59% and inoculated was 1.02%. The level of butyric acid for non-inoculated and inoculated silages was 0.27% and 0.03% (Table 3). Control silages showed a very lower concentration of lactate than in the LAB treated silages thus indicating unable to induce lactate fermentation due to insufficient microbial populations found in the plants

Table 1. Nutrient profiles changes in Orchardgrass after LAB treatment

Groups	Moisture (%)	CP (%)	ADF (%)	NDF (%)	TDN (%)
Control	52.41 ± 0.01	17.58 ± 0.99	55.02 ± 1.41	35.12 ± 1.36	61.16 ±1.07
Inoculants	53.31 ± 0.71	16.67 ± 0.30	55.51 ± 0.56	35.32 ± 0.32	61.00 ± 0.25

Inoculants from Top silage; CP: Crude protein; ADF: Acid detergent fiber; NDF: Neutral detergent fiber; TDN: Total digestible nutrients. The results are presented as mean ± S.E.M of three replicates

Table 2. pH and Microbial population of experimental silages

Groups	pH	LAB (×10^7CFU/g)	Yeast(×10^3CFU/g)	Mould (×10^3CFU/g)
Control	5.81 ± 0.07^a	8.00 ± 0.94^b	7.0 ± 0.11^a	ND
Inoculants	4.73 ± 0.05^b	31.5 ± 2.54^a	1.7 ± 0.38^b	ND

Inoculants from Top silage; LAB: lactic acid bacteria; CFU: colony-forming Unit. ND: Not detected at 10^{-3}dilutions. The results are presented as mean ± S.E.M of three replicates. ^{ab}p<0.05 alphabets within columns indicate significant differences between experimental silages.

Table 3. Organic acids contents of Orchardgrass silages after LAB treatments

Groups	Lactate (DM %)	Acetate (%/DM)	Butyrate (%/DM)
Control	0.01 ± 0.05 [b]	0.58 ± 0.01[b]	0.27 ± 0.01[a]
Inoculants	1.53 ± 0.21 [a]	1.01 ± 0.11[a]	0.03 ± 0.02[b]

DM: Dry matter content; Inoculants from Top silage. The results are presented as mean ± S.E.M of three replicates. [ab]$p<0.05$ alphabets within columns indicate significant differences between experimental silages

especially LAB populations (Davies et ., 2005; Nascimento Agarussi et al., 2019). By contrast, adding inoculums to Orchardgrass during ensiling significantly increased lactate content confirmed the fermentation process was accelerated in the presence of inoculums. In addition, increased acetic acid (non-inoculated: 0.59 vs inoculated: 1.02 %DM) and decreased butyric acid level (non-inoculated: 0.27 vs inoculated: 0.03 %DM) was noted in silages treated with LAB than non-inoculated silages. Silages having acetic acid and butyric acid indicate poor quality. It reduces dry matter content and its energy during fermentation(Nascimento Agarussi et al., 2019). But, the significant level of acetic acid production has been acceptable because many reports exhibited that a moderate amount of acetic acid could act as antimicrobial agents (Danner et al., 2003; Kung et al., 2018a; Muck, 2010). The organic acids production was closely associated with a microbial population of experimental silages.

Ⅳ. CONCLUSION

In the present study, Orchardgrass silage was produced using mixed LAB by an ensiled method. The results exhibited that the addition of LAB strains to Orchardgrass during ensiling process significantly reduced the pH of the silages. The organic acids content particularly lactic acid was the dominant acid found in the silage treated with LAB confirms successful fermentation and also reduced butyric acid level of silage compared to non-inoculated silages. The microbiological study revealed that a higher LAB and lower yeast population was noted in silage treated with LAB. The microbial counts were closely associated with organic acids content in silages. It suggested that the addition of LAB significantly improved silage quality by increasing lactic acid content and decreasing undesirable microbial growths.

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