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Research Article

Beneficial Effects of Lactic Acid Bacteria Inoculation on Oat Based Silage in South Korea

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ABSTRACT

The objective of the study was to measure the beneficial effects of lactic acid bacteria (LAB) inoculation on the nutritive value of oat silage collected from thirteen regions in the Republic of Korea. The contents of crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude ash (CA) were slightly lower in LAB inoculated silage when compared with the control silage, whereas inoculation of LAB resulted in increased total digestible nutrient (TDN). Higher number of LAB, but lower count of yeast and fungi indicated the effectiveness of the LAB inoculation on oat silage fermentation. LAB inoculation resulted in low pH silage, which may prevent undesirable microbial growth. The LAB inoculation promoted lactic acid dominant fermentation with marginal levels of acetic acid and butyric acid in oat silage. These data suggest that the LAB inoculation may preserve oat silage at better quality for ruminant animal production.

(**Key words :** Oat, lactic acid bacteria, Silage, Lactic acid, Quality)

Ⅰ. INTRODUCTION

Corn, sorghum, Italian ryegrass, rye, oats and rape are the most important forage crops in Republic of Korea. Corn, sudangrass, and sorghum are growing during the summer season. Italian ryegrass, rye, oats, and rape were growing during winter season. Forage crops were planted (approximately 259,000 ha area) in South Korea for beef cattle feeding in 2014. Approximately 74% of annual type forage crop was produced from paddy land as winter forage crops and 12% was from higher lands during summer season. Especially, oats have been an important crop as forage and food in whole country. Song et al. (2010) reported that oat was cultivated as a silage crop in Korea since long time ago. Silage is most important and common preserved ruminant feed. The epiphytic lactic acid bacteria (LAB) are naturally found in the forage crops which are responsible for silage fermentation and silage quality (Cai, 1999). The *Lactobacillus* species are major components of the microbial flora in various forage crops and silage. It plays major role in lactic acid production and pH reduction during silage fermentation (Cai et al., 1998). Natural populations of LAB on plant materials are major responsible for conservation of crops as silage by converting the water soluble carbohydrates (WSC) into organic acids especially lactic acid that reduce the pH of the silage (Chen and Weinberg, 2009.). However, numbers of the naturally occurring LAB are very less. Therefore, addition of LAB to the silage is much essential for silage fermentation. Application of LAB inoculation becomes popular to prevent the aerobic deterioration and to improve the nutritive profiles in silage (Choi et al., 2014; Ilavenil et al., 2014; Vijayakumar et al., 2014). Therefore, a study was conducted to investigate nutritive profiles, fermentation quality, and microbial populations in oat silage manufactured in the field condition of livestock farmers.

Ⅱ. MATERIALS AND METHODS

1. Collection and process of silage preparation

The samples of oat silage used in this study were

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randomly collected from 13 different regions of Korea since 2013 to 2014. In the field condition of livestock farmers, oat was harvested at heading stage. After wilting for 12 hr., oat was packed with *L. plantarum* (Chung-Mi Bio Co., Korea) in round bale wrapper using silage wrapping machine. The known amount of *L. plantarum* was dissolved in sterile water by the method of Chung-Mi Bio Co., Korea. Then, this dissolved bacteria were loaded into machine. During silage preparation, this machine automatically spraying the microbes on silage during every scrolling of silage when the ensiling time. After three months incubation under the field conditions, the nutritive values, microbial counts and quality of silage were evaluated by directly collecting the samples.

2. Nutritive profile analysis

Then samples were powdered and passed through a1 mm sieve prior to analysis. The nutritive profiles such as acid detergent fiber (ADF), neutral detergent fiber (NDF), were investigated according to Van Soest method (Goering and Van Soest, 1970). The total digestible nutrient (TDN) was calculated from 88.9-(ADF%×0.79) (Seo et al., 2010; Holland et al., 1990). According to AOAC (1990), the crude protein (CP) and crude ash (CA) content in silage was quantified.

3. Microbial contents

Ten grams of wet silage samples were transferred into sterile flasks containing 90 ml of sterile water. Then it was kept in an orbital incubator shaker at 150 rpm for 30 min and then made a tenfold serial dilutions with water. 0.1 ml of the sample was spread on selective media (Rogosa, and Sharpe agar (Diffco) and Bromocresol purple blue agar medium) for enumeration of LAB colony. The plates were incubated at 28±1°C for two days. Yeasts and molds were enumerated on 3 M petrifilm (3 M Microbiology Products, St.Paul, USA), and following aerobic incubation at 28±1°C for 4 days. Fungi were enumerated on Potato Dextrose agar (PDA) [4 g/L of potato starch (Diffco), 20 g/L of starch (Diffco), and 20 g/L of agar (Diffco)] following aerobic incubation at 28±1°C for 4 days.

4. Fermentation metabolites profiles

10 g of sample was weighed and blended with 100 ml of deionized water for 24 hr in a refrigerator at 4℃. Firstly, samples were filtered through the filter paper (Whatman No. 6) and the filtrate was re-filtered using 0.22 ㎛ syringe filter before injection of samples in high-performance liquid chromatography (HP1100. Agilent Co. USA). The pH measured after centrifugation using a combination electrode. Fermentation byproduct lactic acid content was analyzed by HPLC (HP1100 Agilent Co. USA). The levels of acetic acid and butyric acid were analyzed by Gas Chromatography (GC-450, Varian Co., and USA) (Kristensen et al., 2007).

5. Statistical analysis

Samples were analyzed in three replicates and analysis of variance were performed on all the variables measured using SPSS/PC (Statistical Package for the Science, ver 12.0. USA). The Duncan's multiple range tests were used to determine the effect of inoculants on the quality of oat silage. Tests were run at the 5% probability level.

Ⅲ. RESULT AND DISCUSSION

The present study, we analyzed changes in content of CP, ADF, NDF, CA and TDN in control and experimental silages (Table 1). In LAB inoculated silage exhibited slight decrease in the contents of CP, ADF, NDF and CA as

Table 1. The contents of CP, ADF, NDF CA and TDN in experimental silages

Experimental group	CP[2] (%)	ADF[3] (%)	NDF[4] (%)	CA[5] (%)	TDN[6] (%)
Non-inoculation	10.16±0.54	38.67±1.14	59.16±2.13	9.15±0.50	58.35±0.90
[1]LAB- Inoculation	9.16±0.67	35.68±1.44	55.06±1.82	7.71±0.57	60.72±1.14

[1] LAB: lactic acid bacteria, [2] CP: crude protein, [3] ADF: Acid detergent fiber, [4] NDF: Neutral detergent fiber, [5] CA: Crude ash, [6] TDN: Total digestible nutrient.

compared to control silage. The content of TDN was comparatively higher in LAB inoculated silage (60.72±1.14%) than the control silage (58.35±0.90). But there is no significant changes were observed in nutritive profiles between control and LAB inoculated silage except the content of TDN.

Table 2 shows the microbial population in experimental silages. The results revealed that the higher number of LAB was noted in LAB inoculated silage as compared with control ($p<0.05$). But less no of the yeast (0.10±0.02) and fungi (0.01±0.01) colonies were noted in LAB inoculated silage as compared to control silage. Natural populations of LAB in the plant material are very low (Eitan et al., 2006; Cai et al., 1999). It is not enough to dominate undesirable microbes. Therefore, addition of fast growing homo fermentative LAB as an additive to the silage enhances the fermentation process (Choi et al., 2014; Valan Arasu et al., 2015). Some homofermentative bacteria such as *Lactobacillus plantarum, L. rhamnosus, L. acidophilus, Pediococcus acidilactici* and *Enterococcus faecium* are used as inoculants for silage preparations and some hetrofermentative bacteria such as *L. brevis, L. fermentum* and *L. reuterin,* were also used as additives in silage (Avila et al., 2010). *L. casei* and *L. plantarum* are generally found in the forage crops and silages (Lin et al., 1992; Lin et al., 1991). Numbers of the researchers have been reported that the lactobacilli are the dominant microbial population in the forage crops and it contributes to fermentation of silage. Commercially, available inoculants contain one or more of these bacteria which have selected for their ability to promote the fermentation process (Eitan et al., 2006). Here we noted higher number of LAB with lowest population of yeast and fungi in LAB inoculated silage as compared to control. These increases indicated that the LAB had ability to utilize the carbohydrates found in the oat based silage and ferment them to produce fermentative metabolites which inhibit undesirable microbial growth.

The fermentative metabolites such as lactic acid, acetic acid and butyric acid concentration in experimental silage were given in Table 3. LAB inoculated silage exhibited higher concentration of lactic acid (5.39±0.38%) as compared to control (3.14±0.21%) ($p<0.05$), while acetic acid (0.45±0.09%) and butyric acid (0.09±0.03%) concentration was reduced in LAB inoculated silage than the control silage. The flieg score was higher in LAB inoculated silage as compared with control. LAB inoculation reduces the pH of the silage (4.40±0.10) as compared to control silage (4.78±0.08). Reducing the pH in silage is an important criterion for silage preparation, because the decline of pH inhibits spoilage microorganism growths, which preserve the silage for the long time. LAB plays an essential role in the fermentation process and effectively stimulates lactic acid

Table 2. Microbial population in oat based silage

Experimental group	LAB ($\times10^7$CFU[2)]/gram)	Yeast ($\times10^2$CFU/gram)	Fungi ($\times10^1$CFU/gram)
Non-inoculation	1.57±0.18[b]	0.52±0.12	0.04±0.02
[1)]LAB-Inoculation	3.58±0.20[a]	0.10±0.02	0.01±0.01

[1)] LAB: lactic acid bacteria, [2)] CFU: Colony forming unit.
[a and b]: Means with different letters within a column are significantly different at the 5% level.

Table 3. Fermentative metabolites in oat based silages

Experimental group	pH	Lactic acid (DM %)	Acetic acid (DM %)	Butyric acid (DM %)	Flieg's score
Non-inoculation	4.78±0.08	3.14±0.21[b]	0.62±0.19	0.27±0.18	87.40±2.67
[1)]LAB-Inoculation	4.40±0.10	5.39±0.38[a]	0.45±0.09	0.09±0.03	94.88±1.09

[1)] LAB: lactic acid bacteria, [2)] DM: Dry matter.
[a and b]: Means with different letters within a column are significantly different at the 5% level.

fermentation for the longer time (Cai and Kumai, 1994).

The addition of LAB to the silage at ensiling process is intended to confirm the rapid and vigorous fermentation that results showed higher lactic acid production with lower pH values. It improves the forage conservation and reduces the wasteful byproduct like-N and volatile fatty acids. As a result in poor conversion efficiency and higher in-silo dry matter losses (Cai et al., 1997; Valan Arasu et al., 2014; Jatkauskas and Vrotniakiene, 2004; Weinberg and Muck, 1996; McDonald et al., 1991). Some author reports that the acetic plays an important role in inhibition of spoilage microorganisms. Production of lactic acid and acetic acid combined with low residual butyric acid is an important factor to improve the aerobic stability of silage (Rooke, 1991; Cooke, 1995; Danner et al., 2003). From these data, we concluded that the addition of LAB inoculants to oat silage increased LAB population and produced the greater amount of lactic acid with the marginal amount of acetic acid and butyric acid. It leads inhibit the undesirable microbes through reducing the pH of silage. LAB inoculated silage exhibited higher flieg score. It indicated that the quality silage. Therefore, the addition of LAB would be useful in high-quality silage preparation for ruminant animals.

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