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Research Article

Effect of Lactic Acid Bacteria Inoculation on Fermentation Characteristics of Whole Crop Barley Silage

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ABSTRACT

An experiment was carried out to determine the homofermentative activity of *Lactobacillus plantarum* KCC-10 and KCC-19 on the ensiling of whole crop barley (WCB). The crude protein in the silages was slightly higher in the KCC-10 and KCC-19 treatments compared to the control, but there was no significant difference between the two inoculant-treated silages. Nutrient parameters such as acid detergent fiber, neutral detergent fiber and *in vitro* dry matter digestibility in *L. plantarum* KCC-10 and KCC-19 treated silages did not differ from those in the control silage. The lactic acid content increased in KCC-10 and KCC-19 treated silage when compared with the control silage but the contents of acetic acid and butyric acid produced in KCC-10 and KCC-19 treated silages were similar with the control silage. Further, the number of lactic acid bacteria (LAB) in KCC-10 treated silage demonstrated a significant increase when compared to the control. Especially, KCC-19 treated silage showed greater lactic acid bacterial growth potential. Other microbes such as yeast and fungi were not detected in KCC-10 and KCC-19 treated WCB silages. Hence, this study suggests that the addition of *L. Plantarum* KCC-10 and KCC-19 to the WCB silage can improve fermentation quality for the production of high-quality silage.

(**Key words :** Whole crop barely, *Lactobacillus plantarum*, Silage, Lactic acid, Fermentation)

Ⅰ. INTRODUCTION

Ensiling forage crops is a traditional way to provide animal feed and this process gaining significance and is the alternate way for hay production and direct feeding green forage. This forage storage technology comprises its compression, followed by the airtight sealing. Then fermentation is initiated by the airtight sealing and stimulated by the lactic acid bacterial inoculants which subsequently convert the free sugars into lactic acid (Weinberg et al., 1993). Nowadays the use of starter culture like lactic acid bacterial strains is growing in order to produce high quality silages that ensure the immediate decrease of pH and prevent the growth of undesirable microorganisms such as fungi and yeasts. These unwanted microbes produce butyric acid and also increase the degradation of protein into ammonia. Inoculation of silages with homofermentative LAB improved the silage fermentation (Moon et al., 1980). The rate of production of lactic acid has been increased when the silages inoculated with LAB. This further decreases the rate of proteolysis as well as the production of volatile organic acid (Cooke, 1995; Honig, 1990). Silage inoculants has been applied as dry as well as liquid both inoculants can be effective, especially liquid applications have some more advantage over dry application when crops with low moisture (Weinberg and Muck, 1996; Holzer, 2001).

The aerobic deterioration causes a worse impact on silage quality and the stability of silages against aerobic deterioration can vary severely (Choi et al., 2011a). The mechanism that prevents aerobic spoilage is not well understood yet. But the aerobic spoilage had been disallowed when silages inoculated with homo fermentative LAB. Current studies stated that aerobic stability is not related to dry matter content, pH and addition of glucose at the time of ensiling.

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At the same time the production of organic acids such as lactic acid and propionic acid may contribute the role in aerobic stability (Bucher, 1970; Choi et al., 2011b). Other studies have described that acetic acid production by homo fermentive *Lactobacillus* strains could protect the silages from aerobic spoilage (Pirt, 1975; Moon, 1983).

Ensilling barley is the simple process because of its low buffering capacity and the availability of abundant fermentable carbohydrates (Hristov and McAllister, 2002). Despite its easiness of ensiling, LAB inoculation experiments showed improvement in barley silage fermentation, digestibility of WCB and average daily gain as well as nutrient intake (Hristov et al., 2000). Till now the silage experiments carried in our institute, we observed that inoculation of silages with LAB is very stable against aerobic spoilage which is evident from the quality of the silage (Ilavenil et al., 2014). Recently, Valan Arasu et al. (2014) and Valan Arasu et al. (2013) report that *L. plantarum* KCC-10 and KCC-19 isolated from forages interestingly showed best antifungal and probiotic effects. Therefore, a study was conducted to investigate the effect of *L. plantarum* KCC-10 and KCC-19 on nutritive value, quality and microbe characteristics of WCB silage.

Ⅱ. MATERIALS AND METHODS

1. Collection and preparation of silage

WCB 'Youngyang' was harvested in early heading stage at the experimental field of National Institute of Animal Science - RDA, Cheonan. Initially, two hundred grams of WCB, was packed in an air-diffusible bag which served as control and three set of two hundred grams of WCB weighed and was packed in three different air diffusible bags. Subsequently, silage was made with a fermentation additive containing *L. Plantarum* KCC-10 or KCC-19. Each *L. Plantarum* (1.5×10^{10}cfu/g) was dissolved by distilled water (0.004 g/10ml) and inoculated to 200 g of wilted forage. Silage was sealed to prevent air flow. Each of the samples with or without strains was prepared in triplicate. Silage was stored in underground and opened after 45 days. The nutritive values, microbial counts and fermentative metabolites were analyzed.

2. Mass cultivation of *Lactobacillus plantarum*-KCC-10 and KCC-19

Fresh culture of KCC-10 and KCC-19 were inoculated in mass cultivation medium containing Glucose 1%, soy peptone 0.25%, yeast extract 1%, $MgSO_4$ 0.01%, $MnSO_4$ 0.04%, NaCl 0.1%, $CaCO_3$ 0.2%, Na_2HPO_4 0.6% in a fermenter. After mass cultivation, the cultures were freeze dried. The powder form of KCC-10 and KCC-19 was used in silage preparation.

3. Nutrient composition and analysis

The content of crude protein was quantified by standard procedure (AOAC, 1990). Neutral detergent fiber (NDF) and Acid detergent fiber (ADF) were analyzed according to (Van Soest et al., 1993). Total digestible nutrient (TDN) was calculated as follow; 88.9-(ADF% × 0.79) (Holland et al., 1990; Seo et al., 2010). Samples were ground and pass through a 1 mm sieve prior to analysis. The *in-vitro* dry matter digestibility (IVDMD) was analyzed by the modified method of Moore (1970).

4. Microbial population enumeration

Ten grams of wet silage samples were transferred into sterile flasks containing 100 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 (Miller and Wolin, 1974). 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. Incubation under the microaerobic condition at 28±1℃ 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℃ for two 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℃ for 3 days.

5. Analyzes of fermentation metabolites

10 g of sample was weighed and blended with 90 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 of the supernatant was measured after centrifugation using a combination electrode. The filtrate was stored at −70℃ with and without stabilization with 5% meta-phosphoric acid (final concentration). Fermentation byproduct lactic acid content was analyzed by HPLC. The levels of acetic acid and butyric acid were analyzed by Gas Chromatography (GC-450, Varian Co., USA) (Kristensen et al., 2007).

6. Statistical analysis

All 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 treatment mean difference at the 5% probability level.

Ⅲ. RESULTS AND DISCUSSION

In the present study ensiling of WCB silage was analyzed with the homofermentative *L. Plantarum* KCC-10 and KCC-19. The nutritive profiles such as CP, ADF, NDF, TDN and IVDMD were accounted to study the WCB silage quality that presented in Table 1. The crude protein level increased slightly in LAB inoculated group as compared to the control WCB silage, but there was no significant changes noted between control and LAB inoculated WCB silage. Conversely, previous reports demonstrated that degradation of protein in the silage increased because of the production of volatile organic acids (Danner et al., 2003). But the silage inoculated with *Lactobacillus* strains showed barrier to volatile organic acid production and preventing aerobic spoilage (Avila et al., 2009). Other nutrient parameters such as ADF, NDF, TDN and IVDMD showed almost similar with control. They had similarity with LAB inoculated WCB silage and incredibly reduced the butyric acid, ethanol production, dry matter loss and also increasing the metabolizable energy (Acosta Aragon et al., 2012).

The count of microbial colonies in the silages has been presented in Table 2. Control silage group showed significantly lower growth of LAB (11.50×10^7CFU/g) as compared to *L. plantarum* KCC-10 (43.50×10^7CFU/g) and KCC-19 (74.50×10^7CFU/g) inoculated silages. Significant growth of LAB strains inoculated silages assure high production of lactic acid and thus improve the silage fermentation as well as quality with increased dry matter recovery (Oude Elferink et al., 2001). Additionally, the growth of yeast colonies significantly lowered in *L. plantarum* KCC-10 as well as *L. plantarum* KCC-19 inoculated WCB silages when compared to control silage. Increased growth of yeast colonies leads to aerobic deterioration of silages (Carvalho et al., 2014) which further decrease the silage quality as well as aerobic instability. But silages inoculated with *Lactobacillus* strain inhibited the yeast growth with increased production of lactic acid and may provide aerobic stability during silage fermentation process (Ilavenil et al., 2014). In the case of fungal growth, there was a significant reduction in fungal growth in *L. plantarum* KCC-19 inoculated group as compared to control WCB silage group and there was no fungal growth in *L.*

Table 1. Nutritive value of whole crop barley silage according to inoculation of lactic acid bacteria

Treatment	CP[2] (%)	ADF[3] (%)	NDF[4] (%)	TDN[5] (%)	IVDMD[6] (%)
Control	18.96	27.72	46.96	68.65	67.00
LAB[1] KCC-10	19.17	28.21	46.40	70.35	66.61
LAB KCC-19	19.37	27.48	46.39	70.29	67.19

[1] LAB: lactic acid bacteria, [2] CP: Crude protein, [3] ADF: Acid detergent fiber, [4] NDF: Neutral detergent fiber, [5] TDN: Total digestible nutrient, [6] IVDMD: *in vitro* dry matter digestibility.

Table 2. Changes of microbes on whole crop barley silage according to inoculation of lactic acid bacteria

Treatment	LAB ($\times10^7$CFU[2)]/gram)	Yeast ($\times10^4$CFU/gram)	Fungi ($\times10^4$CFU/gram)
Control	11.50[b]	10.50[a]	5.00[a]
LAB[1)] KCC-10	43.50[a]	1.00[b]	0.01[b]
LAB KCC-19	74.50[a]	6.50[b]	1.00[b]

[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. Effect of Lactic acid bacteria inoculation on pH and organic acids of whole crop barley silage

Treatment	pH	Lactic acid (DM[2)]%)	Acetic acid (DM%)	Butyric acid (DM%)
Control	3.99	6.04[b]	0.60	0.02
LAB[1)] KCC-10	3.71	12.33[a]	0.95	0.05
LAB KCC-19	3.72	10.72[a]	0.72	0.05

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

plantarum KCC-10 inoculated group. Low fungal growth in homofermentative *Lactobacillus* inoculated silages related to organic acid production in the silages (Borreani and Tabacco, 2010). The increased rate of production of lactic acid, acetic acid and butyric acid in LAB inoculated group leads to lower fungal growth in this study.

Interestingly, the pH of the LAB inoculated WCB silage was decreased when compared to the control silage that further strengthen the quality of the silage (Avila et al., 2012) and corroborate the enhanced combination of organic acid production. Interestingly, silages inoculated with *L. plantarum* promotes lactic acid production and can grow well in the low pH environment (Cai et al., 1999). Silages prepared using homofermentative *Lactobacillus* bacteria showed that decrease in pH, inhibit the growth of clostridia and aerobic bacteria as well as improve the silage quality (Cao et al., 2011). Similarly, significant production of lactic acid in LAB inoculated group has been noted as compared to control group, especially KCC-10 (12.33 DM%) inoculated group showed more lactic acid production than *L. plantarum* KCC-19 (10.72 DM%). Production of acetic acid was low in all experimental groups with no significance, and there was almost no butyric acid production in all experimental groups. Decreased production of butyric acid in LAB inoculated silage indicated improved fermentation and reduction of propionic acid, ammonia-N, indicating reduced protein degradation (Cao et al., 2010).

In this study, the ensiling property of *L. plantarum* KCC-10 and KCC-19 inoculated on WCB was analyzed. Both strains showed ensiling property such as propagation, high organic acid production and improved fermentation. There was no undesirable microbial growth that could be due to the large amount of organic acid production and decreased protein degradation that confirmed by the very less amount of butyric acid production. Further, both strains demonstrated some potential of boosting aerobic stability and preventing aerobic deterioration as indicated from limited growth of yeast colonies. Therefore, this study concluded that the *L. plantarum* KCC-10 and KCC-19 could be used as the effective inoculants in WCB ensiling with good economical value to feed live stocks of farmers in developing country.

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