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Research Article

Effects of Ensiling Period and Bacterial Inoculants on Chemical Compositions and Fermentation Characteristics of Rye Silage

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ABSTRACT

The present study was aimed to estimate the effect of ensiling period and bacterial inoculants on chemical compositions and fermentation characteristics on rye silage harvested at delayed stage. Rye (*Secale cereale* L.) was harvested after 20 days of heading stage (29.4% dry matter, DM). The harvested rye forage was applied with different inoculants following: applications of distilled water (CON), *Lactobacillus brevis* (LBB), *Leuconostoc holzapfelii* (LCH), or mixture of LBB and LCH at 1:1 ratio (MIX). Each forage was ensiled into 20 L mini bucket silo (5 kg) for 50 (E50D) and 100 (E100D) days in triplicates. The E50D silages had higher *in vitro* digestibilities of DM (IVDMD, $p<0.001$) and neutral detergent fiber (IVNDFD, $p=0.013$), and lactate ($p=0.009$), and acetate ($p=0.011$) than those of E100D, but lower pH, lactic acid bacteria (LAB), and yeast. By inoculant application, LCH had highest IVDMD and IVNDFD ($p<0.05$), while MIX had highest lactate and lowest pH ($p<0.05$). The CON and LCH in E50D had highest LAB and yeast ($p<0.05$), whereas LBB in E100D had lowest ($p<0.05$). Therefore, this study concluded that LCH application improved the nutrient digesbility (IVDMD and IVNDFD) of lignified rye silage, and longer ensiling period for 100 days enhanced the fermentation characteristics of silage compared to ensiling for 50 days.

(Key words: Bacterial inoculant, Digestibility, Ensiling period, Fermentation characteristic, Rye silage)

Ⅰ. INTRODUCTION

Rye (*Secale cereale* L.) is known as one of the main winter crops in South Korea. It can be grown by double cropping with rice, and also has higher cold tolerance with a high growth rate at low temperature compared to other wither forages such as wheat, triticale, and oat (Paradhipta et al., 2020; Li et al., 2021). The optimal harvest stage of rye for making high-quality silage is known as the heading stage (Lee et al., 2020). The dry matter (DM) yield of rye can be increased dramatically by delaying the harvest stage. However, the delay of harvest date in rye decreases the feed values due to the increase of structural carbohydrate contents, and leads to a decrease of the rye silage quality (Zhao, 2019). In South Korea, the harvest of rye forage is difficult in the proper harvest stage due to frequent rain. For this reason, rye could be lignified and lead to low fermentation quality in rye silage.

Lactic acid bacteria (LAB) as silage inoculant are used to improve the fermentation quality of forage and animal performance. LAB are classified as homo or hetero type depending on fermentation type (McDonald et al., 1991). *Lactobacillus* sp. is known as homofermentative LAB and has been used in food preservation such as dairy products or fermented vegetables, and its optimal growth condition is known at 15 to 45℃ with pH 3.7 to 4.2. (Giraffa et al., 2010). *Lactobacillus brevis* has acidification ability and, Kim et al. (2018) reported that certain *L. brevis* has an antifungal gene as known as *lanC* gene. And, they also reported that *lanC* gene has a role to produce antifungal substances that inhibit mycotoxin-producing fungi. *Leuconostoc* sp., heterofermentative LAB, is used in the production of fermented foods such as dairy and meat products (Paradhipta et al., 2020). The optimal growth temperature and pH of *Leuconostoc*

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sp. are 10 to 37℃ and 3.9, respectively (De Bruyne et al., 2007). Kim et al. (2017) reported that certain *Leu. holzapfelii* can produce fibrinolytic enzymes such as cellulase, xylanase, and esterase. Paradhipta et al. (2020) demonstrated the enzyme function of the *Leu. holzapfelii* in rye silage. However, these LAB isolated by Kim et al. (2017) and Kim et al. (2018) were not conducted to study the optimal ensiling period in rye silage. Tran et al. (2018) reported that the fermentation patterns and effects of LAB inoculants could be differed by ensiling period. For this reason, Mohd-Setapar et al. (2012) reported that ensiling period should be considered to optimize the silage quality with inoculant effects.

Therefore, the present study was conducted to study of effects of ensiling period and isolated bacteria producing antifungal and fibrinolytic substances on lignified rye silage.

Ⅱ. MATERIALS AND METHODS

1. Silage production

On October 14th, rye forage (*Secale cereale* L., Gogu) was seeded at 50 kg/ha in the animal research unit, Gyeongsang National University, Jinju, South Korea and harvested after 20 days from the heading stage (29.4% DM). The harvested rye forage was chopped into approximately 4 cm lengths using a conventional harvester (BHC-90, BUHEUNG Machinery Ltd., Jinju, Korea) and applied with different inoculants following: 1) application with distilled water at 2 mL/kg of fresh forage **(CON)**; 2) *Lactobacillus brevis* (Accession Number of the Korean Culture Center of Microorganisms, KCCM11787P) with application rate at 1.2×10^5 colony-forming unit (cfu)/g of fresh forage **(LBB)**; 3) *Leuconostoc holzapfelii* (Accession Number of the Korean Culture Center of Microorganisms, KCCM11788P) with application rate at 1.0×10^5 cfu/g of fresh forage **(LCH)**; and 4) mixture of LBB and LCH inoculants at 1:1 ratio **(MIX)**. All inoculant diluted into the distilled water, and applied at each treatments with same volume as CON. Each forage was ensiled into the mini bucket silo of 20 L (5 kg) for 50 **(E50D)** and 100 **(E100D)** days in triplicates. Thus, a total of 24 silos was produced in the present study. The fresh forage and silage were sub-sampled at approximately 500 g to analyses chemical composition and *in vitro* digestibility. In addition, 20 g of rye silage was sub-sampled and blended with 200 mL of sterile ultrapure water for 30 s, and then filtered by two layers of cheesecloth to make silage extract. The silage extract was used to analyze pH, ammonia-N, lactate, and volatile fatty acids (VFA).

2. Chemical compositions and *in vitro* digestibility

The sub-sampled fresh forage and silage (10 g) were dried at 105°C for 24 h to measure the concentration of DM. Approximately 200 g of each silage sub-sample was dried at 60°C for 48 h and ground using a cutting mill (SHINMYUNG ELECTRIC Co., Ltd, Ansan, South Korea) to pass through a 1 mm screen. The concentration of crude ash (CA) was determined using a muffle furnace at 550°C for 5 h. The concentrations of crude protein (CP) and ether extract (EE) were analyzed by the Kjeldahl method (method 984.13) and the Soxhlet method (method 920.39), respectively. The concentrations of neutral detergent fiber (aNDF; method 2002.04) treated alpha amylase contains ash and acid detergent fiber (ADF; method 973.18) were determined using an Ankom^{200} fiber analyzer (Ankom Technology, Macedon, NY, USA). All protocols for the CP, EE, aNDF, and ADF analyses were described by AOAC (2005). Hemicellulose (HEMI) was determined by calculating the difference between the aNDF and ADF. The *in vitro* digestibility of DM (IVDMD) and NDF (IVNDFD) were determined following the method of Tilley and Terry (1963) using Ankom^{II} Daisy Incubators (Ankom Tech., Macedon, NY, USA). The rumen fluid was collected from two non-pregnant cannulated Hanwoo cows before morning feeding. Their diets consisted of rice straw and commercial concentrate mix at a ratio of 8:2. The collected rumen fluid was composited and filtered through 2 layers of cheesecloth. A rumen buffer was prepared by mixing rumen fluid with anaerobic culture medium at a 1:4 ratio, as described by Goering and Van soest (1970). Dried samples of rye silage (0.5 g) were weighed into an incubation jar with 2,000 mL of rumen buffer. Then, the incubation jar was gassed with CO_2 and closed tightly to reach anaerobic conditions. Samples were incubated in four replications for 48 h with 3 blanks.

3. Fermentation characteristics

The pH and the concentration of ammonia-N were measured

using pH meter (SevenEasy, Mettler Toledo, OH, USA) and colorimetry assay described by Chaney and Marbach (1962), respectively. The silage extract was centrifuged at 5645 ×g for 15 min and then, the supernatant was used to measure the concentrations of lactate and VFA using HPLC (L-2200; Hitachi, Tokyo, Japan) fitted with a UV detector (L-2400; Hitachi, Tokyo, Japan) and a column (Metacarb 87H; Varian, CA, USA) described by Adesogan et al. (2004).

4. Microbial enumerations

Approximately 20 g of silage sub-sample from each treatment were diluted with 180 mL of sterile ultrapure water and macerated in a blender to obtain the silage extract for the enumeration of LAB, yeast, and mold. Considering the silage extract as the first dilution, serial dilutions were prepared and 100 µl aliquots of three consecutive dilutions (10^{-4} to 10^{-6}) were plated in triplicates onto a selective agar medium. De Man, Rogosa and Sharpe agar media (MRS; Difco, Detroit, MI, USA) was used to culture LAB, and potato dextrose agar (PDA; Difco, Detroit, MI, USA) was used for yeast and mold. The MRS agar plates were incubated in a CO_2 incubator (Thermo Scientific, USA) at 30°C for 72 h as described by Pertruzzi et al. (2020), while the PDA plates were incubated at 30°C for 72 h in an incubator (Johnsam Corporation, Korea) as described by Kasaei et al. (2017). Visible colonies were counted from the plates, and the number cfu was expressed per gram of silage. The microbial count was transformed to log10.

5. Statistical analysis

The present study had a completely randomized design with a 2 (ensiling period; E50D vs. E100D) × 4 (inoculant; CON vs. LBB vs. LCH vs. MIX) factorial arrangement of the treatments. All data on the chemical composition, fermentation characteristics, and microbe counts of the silages were analyzed using PROC MIXED of SAS (2002) and a model containing the ensiling period, inoculant, and interactions of these terms. The model was $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$, where Y_{ijk} = response variable, μ = overall mean, α_i = effect of ensiling period, β_j = effect of inoculant, $(\alpha\beta)_{ij}$ = the interaction effect of ensiling and inoculant, and e_{ijk} = error term. Mean separation was performed using a Tukey's test. Significant differences were declared at $p<0.05$.

Ⅲ. RESULTS

1. Chemical compositions and *in vitro* digestibility

The concentrations of DM, aNDF, IVDMD, and IVNDFD in rye forage were 29.4, 67.4, 59.1, and 48.2% on DM basis, respectively (Table 1). The interaction effects between ensiling period and inoculant were shown in the concentration of EE ($p=0.019$) but no significant difference among all treatments ($p>0.05$) (Table 2). The E50D silages had a higher concentration of CA ($p=0.007$; 5.34% vs. 5.09%) and a lower concentration of HEMI ($p<0.001$; 27.1% vs. 29.2%) than E100D silages. The interaction effects between ensiling period

Table 1. Chemical compositions and *in vitro* digestibility of rye forage before ensiling (% DM)

Item[1]	Fresh rye forage	
	Mean	SE
Dry matter	29.4	0.942
Crude protein	7.43	0.220
Ether extract	3.50	0.360
Crude ash	5.15	0.081
Neutral detergent fiber*	67.4	0.325
Acid detergent fiber	40.6	0.454
Hemicellulose	26.8	0.147
In vitro dry matter digestibility	59.1	0.638
In vitro neutral detergent fiber digestibility	48.2	1.158

*alpha-amylase treated neutral detergent fiber; SE, standard error.

Table 2. Effects of ensiling periods and inoculants on chemical compositions and *in vitro* digestibility of rye silage (% DM)

Item[1]	E50D				E100D				SEM
	CON	LBB	LCH	MIX	CON	LBB	LCH	MIX	
DM	25.5	27.6	27.4	27.5	26.5	26.6	27.6	27.0	0.896
CP	8.37	8.34	8.49	8.31	8.28	8.17	8.30	8.14	0.080
EE	3.51	3.32	3.39	3.34	3.30	3.59	3.27	3.35	0.103
CA	5.50	5.23	5.23	5.39	5.15	5.05	5.14	5.02	0.153
aNDF	70.1^{a}	67.8^{ab}	67.3^{b}	68.8^{ab}	67.5^{ab}	69.8^{ab}	69.4^{ab}	69.9^{ab}	0.921
ADF	43.0^{a}	40.9^{ab}	40.3^{b}	41.1^{ab}	39.1^{b}	40.4^{b}	40.4^{b}	40.7^{b}	0.768
HEMI	27.0^{b}	26.9^{b}	27.0^{b}	27.4^{b}	28.5^{a}	29.4^{a}	29.5^{a}	29.2^{a}	0.460
IVDMD	54.5^{d}	56.7^{bcd}	60.5^{a}	59.6^{ab}	53.5^{d}	54.9^{cd}	58.5^{abc}	53.9^{d}	1.275
IVNDFD	44.3^{b}	45.9^{ab}	49.2^{a}	49.6^{a}	44.2^{b}	45.4^{ab}	49.6^{a}	43.5^{b}	1.365
Contrast	DM	CP	EE	CA	aNDF	ADF	HEMI	IVDMD	IVNDFD
DAY	0.824	0.061	0.841	0.007	0.058	0.004	<0.001	<0.001	0.013
INO	0.051	0.182	0.139	0.341	0.296	0.187	0.235	<0.001	<0.001
DAY×INO	0.350	0.886	0.019	0.459	0.003	0.004	0.150	0.026	0.003

[1]E50D, ensiled for 50 days; E100D, ensiled for 100 days; CON, distilled water (2 mL/kg); LBB, *Lactobacillus brevis* (1.2×10^5 cfu/g); LCH, *Leuconostoc holzapfelii* (1.0×10^5 cfu/g); MIX, mixture of LBB and LCH at 1:1 ratio; DAY, ensiling period effect; INO, inoculant effect; DAY×INO, interaction effect between ensiling period and inoculant; DM, dry matter; CP, crude protein; EE, ether extract; CA, crude ash; aNDF, alpha amylase treated neutral detergent fiber; ADF, acid detergent fiber; HEMI, hemicellulose; IVDMD, *in vitro* DM digestibility; IVNDFD, *in vitro* NDF digestibility; SEM, standard error of the mean.

[a~d] Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 3. Effects of ensiling periods and inoculants on fermentation characteristics of rye silage (% DM)

Item[1]	E50D				E100D				SEM
	CON	LBB	LCH	MIX	CON	LBB	LCH	MIX	
pH	4.83^{a}	3.59^{cd}	3.74^{c}	3.58^{cd}	4.46^{b}	3.48^{d}	3.66^{cd}	3.53^{d}	0.063
Ammonia-N	0.24	0.22	0.23	0.23	0.21	0.23	0.21	0.22	0.030
Lactate	0.49^{c}	3.28^{ab}	2.37^{bc}	2.67^{bc}	0.77^{c}	4.12^{ab}	3.42^{ab}	4.85^{a}	0.632
Acetate	0.37	0.81	0.47	0.49	0.81	0.95	0.75	1.00	0.254
Butyrate*	2.93^{a}	1.75^{abc}	1.14^{bc}	0.90^{c}	2.61^{ab}	1.10^{c}	0.94^{c}	1.39^{abc}	0.463
LA:AC ratio	1.30^{c}	4.05^{ab}	4.13^{ab}	3.20^{b}	0.96^{c}	4.44^{ab}	4.49^{ab}	4.85^{a}	0.533
Contrast	pH	Ammonia-N	Lactate	Acetate	Butyrate	LA:AC ratio			
DAY	<0.001	0.276	0.009	0.011	0.807	0.054			
INO	<0.001	0.374	<0.001	0.252	<0.001	<0.001			
DAY×INO	0.002	0.573	0.186	0.613	0.464	0.059			

[1]E50D, ensiled for 50 days; E100D, ensiled for 100 days; CON, distilled water (2 mL/kg); LBB, *Lactobacillus brevis* (1.2×10^5 cfu/g); LCH, *Leuconostoc holzapfelii* (1.0×10^5 cfu/g); MIX, mixture of LBB and LCH at 1:1 ratio; *butyrate, sum of butyrate and iso-butyrate; LA:AC ratio, lactate to acetate ratio; DAY, ensiling period effect; INO, inoculant effect; DAY×INO, interaction effect between ensiling period and inoculant; SEM, standard error of the mean.

[a~d] Means in the same row with different superscripts differ significantly ($p<0.05$).

and inoculant were shown in the concentrations of aNDF ($p=0.003$) and ADF ($p=0.004$), which CON silage ensiled for 50 days had the highest concentrations of aNDF and ADF ($p<0.05$). The interaction effects between ensiling period and inoculant were also shown in the concentration of IVDMD ($p=0.026$), which LCH silage ensiled for 50 days had the highest concentrations of IVDMD ($p<0.05$). The concentration of IVNDFD had the interaction effects between ensiling period and inoculant ($p=0.003$), which MIX silage ensiled for 50 days and LCH silage ensiled for 100 days had the highest concentrations of IVNDFD, respectively ($p<0.05$).

2. Fermentation characteristics

The interaction effects between ensiling period and inoculant were also shown in the concentration of pH ($p=0.002$), which LBB silage ensiled for 100 days had the lowest concentrations of pH ($p<0.05$) (Table 3). The concentration of lactate was higher in E100D silages ($p=0.009$; 3.29% vs. 2.20%), and MIX

Table 4. Effects of ensiling periods and inoculants on microbial counts of rye silage (log10 cfu/g)

Item[1]	E50D				E100D				SEM
	CON	LBB	LCH	MIX	CON	LBB	LCH	MIX	
LAB	7.09^{a}	6.29^{ab}	6.79^{a}	6.20^{ab}	6.55^{ab}	3.95^{c}	5.41^{b}	5.43^{b}	0.421
Yeast	7.07^{a}	6.50^{abc}	6.88^{ab}	6.10^{abc}	6.44^{abc}	4.21^{d}	5.57^{bcd}	5.09^{cd}	0.523
Mold	ND	ND	ND	ND	ND	ND	ND	ND	N/A
Contrast	LAB				Yeast			Mold	
DAY	<0.001				<0.001			N/A	
INO	<0.001				0.001			N/A	
DAY×INO	0.009				0.078			N/A	

[1]E50D, ensiled for 50 days; E100D, ensiled for 100 days; CON, distilled water (2 mL/kg); LBB, *Lactobacillus brevis* (1.2×10^5 cfu/g); LCH, *Leuconostoc holzapfelii* (1.0×10^5 cfu/g); MIX, mixture of LBB and LCH at 1:1 ratio; DAY, ensiling period effect; INO, inoculant effect; DAY×INO, interaction effect between ensiling period and inoculant; SEM, standard error of the mean; ND, < 4.0 log10 cfu/g; N/A, not available.

[a-d] Means in the same row with different superscripts differ significantly ($p<0.05$).

inoculant had the highest lactate concentration ($p<0.05$). The concentration of acetate was higher in E100D silages ($p=0.011$; 0.88% vs. 0.54%) than in E50D silages. The concentration of butyrate was the highest in CON silages ($p<0.001$). Lactate to acetate ratio was the lowest in CON silages ($p<0.05$).

3. Microbial counts

The interaction effects between ensiling period and inoculant were also shown in the LAB count ($p=0.009$), which LBB silage ensiled for 100 days had the lowest LAB count ($p<0.05$) (Table 4). On the other hand, E100D silages had a lower yeast count ($p<0.001$; 5.33 log10 cfu/g vs. 6.64 log10 cfu/g). Yeast count was highest in CON silage ($p<0.05$), while LBB inoculant inhibited yeast most effectively in silage ensiled for 100 days ($p<0.05$). All silages were not detected mold below at 4.0 log10 cfu/g.

Ⅳ. DISCUSSION

The concentration range of CP, EE, CA, NDF, ADF, and HEMI from rye forage reported in the previous studies was 6.48-10.6%, 1.49-4.40%, 5.13-6.11%, 58.8-74.4%, 35.0-46.2%, and 24.8-48.2%, respectively (Kim et al., 2001; Moon et al., 2014; Kim et al., 2017; Paradhipta et al., 2020), and the results of this study were within the range of the previous studies. Patterson et al. (2021) reported that the change of crude ash could be occurred by soil contamination, generally. However, soil contamination was not detected in the present study, and it means that the fermentation period effect might have occurred from the proportional changes of other nutrients. The concentrations of NDF and ADF were known that could be decreased by the fibrinolytic enzymes (Khota et al., 2016). Li et al. (2019) also demonstrated that LAB-producing fibrinolytic enzymes reduced the concentrations of NDF and ADF. *Leuc. holzapfelii* isolated by Kim et al. (2017) used in the present study was confirmed that can produce fibrinolytic enzymes such as cellulase, xylanase, and esterase. In silages ensiled for 50 days, LCH inoculant which can produce fibrinolytic enzymes decreased concentrations of NDF and ADF than CON, and the results were similar to the results of Li et al. (2019) and Paradhipta et al. (2020). Ultimately, the fibrinolytic enzymes produced by LAB were known that lead to improving the digestibility of the forage. Especially, esterase, one of the fibrinolytic enzymes can increase fiber digestibility in lignified silage, effectively (Paradhipta et al., 2020). Various studies reported that LAB producing esterase can improve fiber digestibilities (Li et al., 2019; Paradhipta et al., 2020), and similar results were also shown in the present study which LCH inoculant improved IVDMD and IVNDFD than CON.

The pH of silage can be decreased rapidly by a high concentration of lactate after ensiling, and the decrease of pH leads to stabilized silage stage which reduces nutrition loss by inhibition of undesirable bacteria (Adesogan et al., 2004). *L. brevis* used in the present study (LBB and MIX inoculants) had confirmed the acidification ability in the previous study

(Kim et al., 2018). In the present study, it also shown the acidification ability with increased lactate concentration (Table 3). A previous study reported that some chemical compositions and nutrients of silages could be changed continually after the stable phase (Der Bedrosian et al., 2012). Grum et al. (1991) reported that the concentration of lactate was increased continually even ensiled for prolonged periods by LAB with high acid-tolerant. In addition, Herrmann et al. (2011) reported that the concentrations of lactate in corn, sorghum hybrid, rye, and triticale silages were increased continually until 90 days. The present study also had shown similar results of lower pH with higher lactate concentration in silages ensiled for 100 days than silages ensiled for 50 days. Herrmann et al. (2011) also reported that the concentration of acetate is increased continually for prolonged periods. The previous studies reported that certain LAB could use lactate anaerobically by limited water-soluble carbohydrates, and lead to increase the concentration of acetate (Hermann et al., 2011; Gou et al., 2017). Der Bedrosian et al. (2012) also showed that acetate concentration of corn silage increased with increase of ensiling period. Similarly, the present study also had shown that the concentration of acetate of silages ensiled for 100 days was higher than silages ensiled for 50 days. In general, Clostridia organisms can convert lactate into butyrate and increase the pH in silage (McDonald et al., 1991; Liu et al., 2020). The high pH silage occurs the growth of undesirable bacteria such as yeast and mold, and leads to the loss of nutrients and aerobic deterioration of silage, ultimately (McDonald et al., 1991). Fortunately, LAB inoculant can inhibit the growth of clostridia and the production of butyrate by the rapid decrease of pH in silage (McDonald et al., 1991). In the present study, CON silage had the highest concentration of butyrate, and it means that CON silage was most contaminated by clostridia. It might be occurred by slow decrease of pH with low concentration of lactate than other treatments.

The LAB, anaerobic bacteria, is an important factor in silage fermentation and can decrease the pH of silage by producing lactate (McDonald et al., 1991). LAB are shown pH resistance until pH 3.5, maximally, but the inhibition of LAB growth could be initiated from pH 4.5 (Yang et al., 2018). Adamberg et al. (2003) also reported that the growth rate of some LAB can decrease 2-3 times or more than 5 times during the pH was decreased from 6.0 to 4.7. The previous study was shown that LAB count was lower in silage applicated with LAB than CON silage by a low pH (Wang et al., 2019). The present study was also shown that the growth of LAB was most inhibited in LBB silage ensiled for 100 days with the lowest pH in silage. Yeast is known that consume water-soluble carbohydrates and lactate, and lead to loss of nutrients and increase of pH (Kung Jr et al., 2018). The increased pH of silage leads to the increase of aerobic undesirable bacteria such as mold, the inhibition of yeast is a key factor to increase aerobic stability in silage (McDonald et al., 1991). Yeast and mold are known that can be inhibited by VFA such as acetate and propionate (Kung Jr et al., 2018). Various studies demonstrated that undesirable bacteria such as yeast and mold are inhibited in silage by high concentrations of acetate and propionate (Lee et al., 2019). In the present study, similar results also had shown that E100D silages were a lower yeast count than E50D silages by increased acetate concentration in silage. Especially, yeast count was most inhibited in LBB silage ensiled for 100 days among all silages. *L. brevis* isolated by Kim et al. (2018) was known that can produce an antifungal substance by *lanC* gene, which inhibits undesirable microbes such as yeast and mold. The lowest yeast count in LBB silage ensiled for 100 days also could be supported by an antifungal substance produced from *L. brevis*.

In conclusion, LCH inoculant improved IVDMD and IVNDFD in lignified rye silage than other treatments. Rye silages ensiled for 100 days had low yeast count with increased concentrations of lactate and acetate. Especially, LBB inoculant inhibited yeast effectively in silage ensiled for 100 days. Therefore, LCH application could help to improve the digestibility of rye silage harvested at delayed stage, while LBB application could effectively inhibit the growth of undesirable microbes.

Ⅴ. ACKNOWLEDGMENT

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