

Research Article

Effects of Cutting Length on Fermentation Characteristics and Aerobic Stability of Whole Crop Rice Silage

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ABSTRACT

This study was conducted to estimate the effect of different cutting lengths on fermentation characteristics and aerobic stability of whole crop rice (WCR) silage. The WCR was harvested at the yellow ripe stage (43.7%, DM), and then cut at 5 (R05), 10 (R10), and 20 cm (R20) of the theoretical length of cut with no cut WCR (R60). Each forage was ensiled into 20 L mini bucket silo (5 kg) for 150 days in quadruplicates. The cutting lengths were not affected the chemical compositions of WCR silage ($p > 0.05$). The pH ($p < 0.001$) and concentration of ammonia-N ($p = 0.022$) in WCR silage were increased linearly with the increase of cutting length. The concentration of lactate had quadratic effect ($p = 0.007$), which was highest in R20 silage ($p < 0.05$). The concentration of acetate was increased linearly ($p = 0.014$), but the concentration of butyrate was decreased linearly ($p = 0.033$). The lactic acid bacteria count was decreased linearly ($p = 0.017$), and yeast count had quadratic effect ($p = 0.009$), which was the highest in R20 silage ($p < 0.05$). Aerobic stability had strong quadratic effect ($p < 0.001$), which was the highest in R20 silage ($p < 0.05$). In conclusion, R60 silage had highest pH by a linear increase of ammonia-N concentration and led to low aerobic stability. While R20 silage had the lowest pH by high lactate concentration and led to high aerobic stability.

(Key words: Aerobic stability, Cutting length, Fermentation quality, Whole crop rice silage)

I. INTRODUCTION

Forage cutting is one of the key factors for improving the fermentation quality of silage. According to McDonald and Whittenbury (1973), cutting forage before ensiling helps to attach lactic acid bacteria (LAB) into cell walls, which can increase the efficiency of water-soluble carbohydrates utilization for organic acid production and growth. Weise (1968) also reported that forage cutting can increase the density of silages that leads to enhancing the anaerobic condition of silo. The rapid growth of LAB with the anaerobic condition of silo can inhibit a loss of nutrients and the growth of undesirable aerobic bacteria such as yeast, mold, and clostridia (McDonald et al., 1991). Bhandari et al. (2007) demonstrated that the short-length cut of corn silage could increase fermentation quality by increasing concentrations of lactate and volatile fatty acids (VFA) produced by LAB. On other hand, forage cutting was presented beneficial effects on feed intake and digestibility of ruminants (Kaiser et al., 2004).

Bal et al. (2000) also demonstrated that dietary short-length cut of corn silage increased intake, digestibility, and milk production of dairy cow. However, the excessive cutting length of forage could lead to occur the acidosis in ruminants by a high concentration of VFA (Addah et al., 2015). For these reasons, the establishment of optimal cutting length depending on forage type is important for silage fermentation and rumen condition.

Rice (*Oryza sativa* L.) is one of the most important food crops in South Korea, which accounts for about 85% of total grain production, but the consumption of rice decreased dramatically caused by increasing western food consumption in South Korea (An et al., 2021). For this reason, Kim et al. (2008a) suggested that rice could be an alternate feed source for animals. Recently, several rice cultivars for ruminants such as “Nokyang”, “Mogwoo”, “Mogyang”, “Jungmo1029”, “Jungmo1038”, “Kokwoo”, and “Yeongwoo” (Ahn et al., 2018) were developed in South Korea. The optimal harvest stage of whole crop rice (WCR) for making silage is known as the yellow ripe stage (Choi et al., 2010). However, the WCR has

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hollow stems and hard cell walls, which could inhibit the anaerobic condition of silage lead to low-quality silage and the growth of undesirable microbes by the presence of air in silo (Li et al., 2010). And, several studies had investigated the effects of inoculants, harvest stage, or variety to improve the feed value of WCR silage (Choi et al., 2010; Kim et al., 2008a; Kim et al., 2008b). However, limited study has examined the cutting length effect on fermentation characteristics and aerobic stability of WCR silage. Therefore, the present study was conducted that to determine the effect of cutting length on fermentation characteristics and aerobic stability of WCR silage.

II. MATERIALS AND METHODS

1. Silage production

The WCR (Sukwang cultivar) production was conducted at the animal research unit, Gyeongsang National University, Jinju, South Korea. The rice grain was sown at seedbed on April 17th and transplanted at rice paddy on May 25th. The WCR was harvested at yellow ripe stage (43.7%, DM) by the sickle, and cut using the rice cutter at 5 (R05), 10 (R10), and 20 cm (R20) of the theoretical cutting length with no cut WCR (R60) as a control. Each forage was ensiled into 20 L mini bucket silo (5 kg) for 150 days in quadruplicates. The WCR forage and silage were sub-sampled at approximately 500 g to analyses of chemical compositions and *in vitro* digestibility. In addition, 20 g of 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 VFA.

2. Chemical composition 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, 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 (NDF, method 2002.04) and acid detergent fiber (ADF, method 973.18) were determined using an Ankom²⁰⁰ fiber analyzer (Ankom Technology, Macedon, NY, USA). All protocols for the CP, EE, NDF, and ADF analyses were described by AOAC (2005). Hemicellulose (HEMI) was determined by calculating the difference from NDF to ADF (HEMI = NDF - 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).

3. Fermentation characteristic

The pH and the concentration of ammonia-N were measured using the pH meter (SevenEasy, Mettler Toledo, Switzerland) 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) and a column (Metacarb 87H, Varian, CA, USA) described by Adesogan et al. (2004).

4. Microbial enumerations and aerobic stability

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 (10⁴ to 10⁶) 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 CO₂ incubator (Thermo Scientific, USA) at 30°C for 72 h, while the PDA plates were incubated at 30°C for 72 h in a normal incubator (Johnsam Corporation, Korea). Visible colonies were counted from the plates, and the number colony-forming unit (cfu) was expressed per gram of silage. The microbial data were transformed to log₁₀. One kilogram of silage was transferred into the open-top polyethylene containers for the aerobic stability. Two thermocouple wires were placed to the

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center of silage and connected to data loggers (TR-60CH, MORGAN, Hong Kong, China) along with a computer that recorded temperature at every 30 min for 7 days. The silage containers were covered with 2 layers of cheesecloth to prevent drying and contamination by dust. The aerobic stability was indicated by the time required to raise the silage temperature 2°C above the ambient temperature (20°C) as suggested by method of Adesogan et al. (2004).

5. Statistical analysis

All data on the chemical compositions, fermentation characteristics, and microbe counts of the silages were analyzed using PROC ANOVA of SAS (2002). Mean separation was performed using

Table 1. The chemical compositions of whole crop rice just before ensiling (% DM)

	Whole crop rice forage
Dry matter	43.7 ± 0.60
Crude protein	6.39 ± 0.18
Ether extract	2.32 ± 0.02
Crude ash	10.0 ± 0.34
NDF	46.6 ± 0.79
ADF	27.6 ± 0.23
Hemicellulose	19.0 ± 0.94

NDF: neutral detergent fiber, ADF: acid detergent fiber.

a Tukey's test. In addition, orthogonal coefficients for linear, quadratic, and cubic contrast were adjusted to account for the unequal spacing of cutting length (5 cm vs. 10 cm vs. 20 cm vs. 60 cm). Significant differences were declared at $p < 0.05$.

III. RESULTS

1. Chemical compositions and *in vitro* digestibility

Table 1 was shown the chemical compositions of WCR forage before ensiling, the concentrations of DM, CP, EE, CA, NDF, ADF, and HEMI were 43.7%, 6.39%, 2.32%, 10.0%, 46.6%, 27.6%, and 19.0%, respectively. Table 2 was shown the effect of cutting length on chemical compositions and *in vitro* digestibility of WCR silage. The concentrations of DM, CP, EE, CA, NDF, ADF, and HEMI in WCR silage were not affected by cutting length ($p > 0.05$). The IVDMD and IVNDFD were also not affected by cutting length in the present study ($p > 0.05$).

2. Fermentation characteristics

The pH ($p < 0.001$) and concentration of ammonia-N ($p = 0.022$) in WCR silage were increased linearly with the increase of cutting length (Table 3). The concentration of lactate had

Table 2. Effects of different cutting lengths on chemical compositions of whole crop rice silage (% DM)

	Cutting length ¹				SEM	Contrast		
	R05	R10	R20	R60		L	Q	C
Dry matter	45.4	46.1	44.9	45.4	2.184	0.891	0.723	0.495
Crude protein	8.33	8.45	8.10	8.69	0.315	0.093	0.118	0.278
Ether extract	2.65	2.70	2.67	2.53	0.122	0.071	0.566	0.613
Crude ash	8.52	8.45	8.42	8.10	0.279	0.073	0.947	0.398
NDF	48.7	49.8	49.5	46.2	3.405	0.161	0.511	0.716
ADF	28.4	29.8	29.2	27.1	2.004	0.132	0.383	0.414
Hemicellulose	20.3	20.0	20.2	19.1	1.419	0.219	0.734	0.769
IVDMD	59.8	58.6	57.2	58.7	1.828	0.752	0.062	0.932
IVNDFD	31.6	31.2	33.3	33.1	2.444	0.483	0.614	0.647

¹R05, R10, R20, and R60 represent cutting length of whole crop rice silage at 5, 10, 20 cm, and no cut, respectively.

NDF: neutral detergent fiber, ADF: acid detergent fiber, IVDMD: *in vitro* dry matter digestibility, IVNDFD: *in vitro* neutral detergent fiber digestibility, SEM: standard error of the mean, L: linear effect of cutting length, Q: quadratic effect of cutting length, C: cubic effect of cutting length.

Table 3. Effects of different cutting lengths on fermentation characteristics of whole crop rice silage ensiled for 150 days (% DM)

	Cutting length ¹				SEM	Contrast		
	R05	R10	R20	R60		L	Q	C
pH	5.29 ^b	5.31 ^b	5.27 ^b	5.61 ^a	0.075	<0.001	0.082	0.605
Ammonia-N	0.072	0.081	0.077	0.090	0.007	0.022	0.936	0.234
Lactate	0.66 ^b	0.79 ^{ab}	1.08 ^a	0.68 ^{ab}	0.181	0.504	0.007	0.728
Acetate	1.46	1.50	2.07	2.29	0.375	0.014	0.142	0.467
Butyrate	0.51	0.62	0.58	0.32	0.146	0.033	0.273	0.449
Lactate:acetate ratio	0.45	0.53	0.52	0.30	0.167	0.134	0.264	0.659

¹R05, R10, R20, and R60 represent cutting length of whole crop rice silage at 5, 10, 20 cm, and no cut, respectively.

SEM: standard error of the mean, L: linear effect of cutting length, Q: quadratic effect of cutting length, C: cubic effect of cutting length.

^{a, b} Means in the same row with different superscripts differ significantly ($p < 0.05$).

Table 4. Effects of different cutting lengths on microbial counts and aerobic stability of whole crop rice silage ensiled for 150 days (log₁₀ cfu/g)

	Cutting length ¹				SEM	Contrast		
	R05	R10	R20	R60		L	Q	C
Lactic acid bacteria	7.23	7.34	7.20	6.68	0.355	0.017	0.613	0.647
Yeast	7.12 ^{ab}	7.19 ^{ab}	7.31 ^a	6.51 ^b	0.356	0.134	0.009	0.929
Mold	1.51	2.26	3.59	3.24	1.575	0.438	0.342	0.961
Aerobic stability, h	128.0 ^{ab}	144.5 ^{ab}	178.5 ^a	90.9 ^b	26.23	0.008	<0.001	0.150

¹R05, R10, R20, and R60 represent cutting length of whole crop rice silage at 5, 10, 20 cm, and no cut, respectively.

SEM: standard error of the mean, L: linear effect of cutting length, Q: quadratic effect of cutting length, C: cubic effect of cutting length.

^{a, b} Means in the same row with different superscripts differ significantly ($p < 0.05$).

quadratic effect ($p = 0.007$), which was highest concentration in R20 silage ($p < 0.05$). The concentration of acetate was increased linearly ($p = 0.014$) with the increase of cutting length, but the concentration of butyrate was decreased linearly ($p = 0.033$). The lactate to acetate ratio was not affected by cutting length ($p > 0.05$).

3. Microbial counts and aerobic stability

The lactic acid bacteria count was decreased linearly ($p = 0.017$) with the increase of cutting length, and yeast count had quadratic effect ($p = 0.009$), which was highest count in R20 silage ($p < 0.05$) (Table 4). On the other hand, mold count was not affected by cutting length ($p > 0.05$). Aerobic stability had a strong quadratic effect ($p < 0.001$), which was highest in R20 silage ($p < 0.05$).

IV. DISCUSSION

In general, the concentrations of DM, CP, EE, NDF, and ADF in the fresh WCR harvested at the yellow stage were 36.9 - 50.7%, 5.0 - 7.6%, 1.3 - 2.3%, 19.0 - 67.8%, and 11.1 - 51.0%, respectively (Choi and Oh, 2011; Nishino et al., 2007), which the results of the present study were in similar ranges based on those previous studies. Einarson et al. (2004) and Johnson et al. (2003) had also reported similar results with the present study that forage cutting length was not affected the chemical compositions of silages. Even though there are no effects on the chemical compositions of WCR silages in this study, the short cutting length enhanced feed intake and digestibility on ruminants (Bal et al., 2000; Mertens, 1993; Randby et al., 2008). Randby et al. (2008) reported that forage of short length could be increased DM and NDF intake in cows. However, Bal et al. (2000) indicated that feed included silage of short length tended to have a decrease of NDF digestibility. According to

Mertens (1993), NDF digestibility could be decreased by the decrease of the rumen pH and the increase of feed passage rate. No effects on IVDMD and IVNDFD by cutting length in the present study might be caused by the differences of *in vitro* and *in vivo* digestibility, which could be affected by rumination.

McDonald and Whittenbury (1973) reported that forage cutting could affect silage fermentation quality such as pH and concentrations of lactate and ammonia-N. Weise (1968) also reported that short-cut forage was increased the silage density and lead to decrease pH rapidly. Kibe et al. (1981) demonstrated that the concentration of lactate was increased with short cutting length and led to decrease of pH in grass silage. In the present study, the concentration of lactate had a quadratic pattern but pH was decreased linearly with the decrease of cutting length. It was supposed that the change of pH by cutting length was also affected by the concentration of ammonia-N. Ammonia-N is known that one of the main factors to change the pH of silage (Kung and Shaver, 2001). In general, the concentration of ammonia-N can be increased in poorly packed silage which was grown undesirable bacteria such as yeast, mold, and clostridia, and lead to inhibit the decrease of pH in silage, ultimately (McDonald et al., 1991). Bhandari et al. (2007) reported that long-length corn silage had a higher concentration of ammonia-N than short-length corn silage. The present study also showed similar results that the concentration of ammonia-N was increased linearly with increased cutting length, it was supposed the reason for increased pH linearly. The acetate can be produced from heterofermentative LAB and inhibit undesirable bacteria such as yeast and mold, but acetate is not always a positive indicator in silage (Kung and Shaver, 2001). Because the acetate can be also produced from acetic acid bacteria which occur aerobic contamination (Spoelstra et al., 1988). In addition, the optimal pH for growing acetic acid bacteria is known to 5.4 to 6.1 (Krieg, 1984), which is similar to the pH of R60 silage (Table 3). It could partially support the increased acetate concentrations by increasing length of WCR in this study.

Weise (1968) reported that the decrease of cutting length can improve the anaerobic condition of silage by the increase of silage density, and lead to the increase of LAB count. Kibe et al. (1981) also indicated that LAB count increased with decrease of cutting length. The present study also showed a similar result that the LAB count was increased with decrease of cutting length. Yeast is known as the initiator of mold growth in silage (Kung and Shaver, 2001). The growth of yeast can be inhibited

by high concentrations of VFA and ammonia-N (McDonald et al., 1991). In addition, Cao et al. (2014) reported that the growth of yeast was inhibited by high concentrations of acetate and ammonia-N. The present study had also shown a similar result that R60 silage was decreased the growth of yeast by high concentrations of VFA and ammonia-N. Aerobic stability has a relationship with undesirable aerobic bacteria such as yeast, mold, and acetic bacteria (Kung and Shaver, 2001). These undesirable bacteria occurs a heat and other substances such as CO₂ and ethanol (Spoelstra et al., 1988), and are weak in the low pH of silage (Moon, 1983). Several studies demonstrated that the low pH silage had improved aerobic stability by inhibiting undesirable aerobic bacteria (Ashbell et al., 2002; Pahlow and Muck, 2009). In general, a high concentration of lactate is used to the growth of aerobic bacteria in silage exposed to air, and lead to increase the pH rapidly and decrease aerobic stability (McDonald et al., 1991). However, Ashbell et al. (2002) reported that the low pH could be maintained if residual water-soluble carbohydrates are high in silage, and improve aerobic stability. In this study, the higher aerobic stability of R20 silage compare to R60 silage could be partially supported by lower pH (5.27 vs. 5.61).

Therefore, this study could be concluded that R60 silage had a lower aerobic stability caused by highest pH and increased ammonia-N concentration, while R20 silage had a higher aerobic stability caused by lower pH and higher lactate concentration.

V. ACKNOWLEDGMENT

This research was supported (Project No. 319039-03-2-HD020) by IPET (Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries), and Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

VI. REFERENCES

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- (Received : August 25, 2021 | Revised : September 15, 2021 | Accepted : September 15, 2021)