Master's Thesis of Science in Agriculture

Effect of Harvest Dates and Preservation Methods on Forage Quality and β-carotene Content of Rye (*Secale cereale* L.)

수확시기와 제조방법이 호밀의 품질과
베타카로틴 함량에 미치는 영향

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Effect of Harvest Dates and Preservation Methods on Forage Quality and β-carotene Content of Rye (*Secale cereale* L.)

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Abstract

Limited information are available about how forage quality and β-carotene content are affected by various factors on rye in Pyeongchang, Korea. So this experiment was conducted to investigate the effect of harvest dates and different preservation methods on forage quality and β-carotene content. Samples were collected from rye harvested every 5 days, from April 25 to May 31, and also comparisons were done about rye silage wilted for different periods and hay of 3 three stages. As for effect of harvest dates, advancing maturity increased dry matter (DM) content, plant height, DM yield and total digestible nutrients (TDN) yield, but decreased the crude protein (CP), *in vitro* dry matter digestibility (IVDMD) and relative feed value (RFV) significantly ($P < 0.05$). With plant matured, all the 3 parts (leaf, stem and grain) showed decreases of forage quality and the part with lowest quality was stem. β-carotene also decreased by advancing maturity. So for getting higher yield and quality, harvest around blooming stage is proper. For silage, with advancing maturity, DM content, acid detergent fiber (ADF) and neutral detergent fiber (NDF) content increased while CP, IVDMD, TDN, RFV and DM loss decreased ($P < 0.0001$). And wilting raised the DM content and pH value significantly ($P < 0.0001$). Silage harvested at heading stage showed the lowest pH value (4.45), propionic acid (PA) (0.83 g/kg DM), butyric acid (BA) (0 g/kg DM), fungi and yeast (F&Y) population (3.70 log CFU / g of FM), conversely, the highest lactic acid (LA) (9.7 g/kg DM), lactic acid bacteria (LAB) (6.87 log CFU / g of FM), total microorganisms (TM) (7.33 log CFU / g of FM) and Flieg's score (70) ($P < 0.0001$). Wilting elevated LAB and TM

population but had no regular effect on other fermented products. Both of delayed harvest and prolonged wilting decreased β-carotene content. Above all, silage harvested around May 9 (heading) with 24 hours' wilting was preferred for rye silage in Pyeongchang. For rye hay, advancing maturity decreased the DM loss, IVDMD, TDN and RFV, but increased the DM, ADF and NDF content significantly ($P < 0.05$). β-carotene was decreased by delay of hay-making. Consequently, to get lower DM loss and higher hay quality, May 9 (heading) was recommended in Pyeongchang.

Keywords : rye, harvest date, wilting, forage quality, β-carotene content

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List of Abbreviations

AA: Acetic acid

ADF: Acid detergent fiber

BA: Butyric acid

CFU: Colony forming unit

CP: Crude protein

DDM: Digestible dry matter

DM: Dry matter

DMI: Dry matter intake

DW: Distilled water

FM: Fresh matter

F&Y: Fungi and yeast

GLM: General linear model

HPLC: High performance liquid chromatography

IVDMD: *In vitro* dry matter digestibility

LA: Lactic acid

LAB: Lactic acid bacteria

LSD: Least significant difference

LSR: Leaf stem ratio

MRS: De Man, Rogosa and Sharpe agar

NDF: Neutral detergent fiber

NH_3-N: Ammonia nitrogen

PA: Propionic acid

PCA: Plate count agar

PDA: Potato dextrose agar

RFV: Relative feed value

SAS: Statistical analysis software

TDN: Total digestible nutrients

TM: Total microorganisms

WSC: Water soluble carbohydrate

1. Introduction

1.1 Research background

Rye (*Secale cereale* L.) is a grass grown extensively as a cover crop and a forage crop in the world. It can be consumed by dairy cow and beef cattle in the form of green chop, pasture, silage, or hay. As a hardy grain, it is more tolerant of frost and drought than wheat. So even it is less palatable than other forages, it has advantages due to the high tolerance in bad condition and earlier maturity than most other cereal crops. And Rye can be used alone or mixed with other forage crops like clover and ryegrass (David and Laura, 2016).

In livestock industry, forages provide the majority of necessary nutrients in ruminant diets and also play great role in the acquisition of digestion physiology of rumen. Forage quality is a critical factor which affect animal productivity. Ideally, an increase of forage quality fed to animal will improve the animal performance and potential economic profits for farmers. Those are commonly understood that forage quality is determined by various factors. Among all, forage species and cultivar are the most fundamental factor. The greatest differences of forage occur between grasses and legumes and warm season and cool season grasses because of the variations of fiber content and the amount of lignin, the primary inhibitor for fiber digestion. Forage harvested from different stage of maturity also differs in quality. Generally, forage quality decreases while the yield of dry matter increases as the plant matures, so the proper stage of maturity for harvesting should be considered adequately. In grasses and

legumes, leaves are almost the component which has the highest quality. Stems of immature grasses are similar with leaves in forage quality, however, quality decrease faster than leaves with maturity (Minson, 1990). Furthermore, leaves generally contain higher crude protein (CP) and lower fiber than stem (Rotz and Muck, 1994).

For farmers, there are two options for forage preservation: haymaking and ensiling. Hay is more marketable than silage, but harder to mechanize the process. And it is unwise to debate which is better between hay and silage. Farmers can select any preserve method according to their requirements and the weather condition, especially. For example, in South Korea, silage is preferred because haymaking is tough to handle due to the predominantly wet climates during haymaking period.

β-carotene, the most active biological form among the carotenoids, plays a great role in the forage nutrition as a precursor of vitamin A, a critical fat-soluble vitamin. It has great benefit to both human health and livestock. Vitamin A can not be synthesized by animal itself and must be obtained from the cleavage of pro-vitamin carotenoids from dietary supplemented (Theodosiou, Laudet, and Schubert, 2010). Additional supplements of β-carotene can meet the vitamin requirements. As many studies claimed, β-carotene and vitamin A are necessary for reproduction capacity and immune function for dairy cows (Chew et al., 1984; Kume and Toharmat, 2001). But for beef cattle, some other studies showed that levels of β-carotene and vitamin A have influences on beef quality as it has effects on fat stores and fat hardness (Siebert et al., 2006). So the β-carotene level should be considered carefully according to the the target, dairy cow or beef

cattle. β-carotene concentrations in forage is affected by various of factors such as species, harvest times over season, maturity stage at harvest, pre-wilting and preserve method. Numerous of studies documented that β-carotene losses can be less than 20% in well-fermented silages (Carter, 1960; Nozière et al., 2006), while haymaking can loss β-carotene content up to 80-90% (Carter, 1960).

1.2 Aim of research

The aim of this thesis is to investigate the variations of rye in forage quality and β-carotene concentration affected by harvest date and different processing methods. Specifically to say, is how harvest date of progressed maturity stages, different parts of plants, different wilting periods and different processing methods affect forage quality and β-carotene concentrations in forage rye (*Secale cereale* L.).

2. Literature review

2.1 Harvest stage

Harvest of plants from different maturation stages show great difference on quality, quantity and regrowth performance for forage. The proper harvesting stage should be decided under the considerations of species, season, location, climate conditions and other factors which are known to have big effects. It is known that forage yield increase while the forage quality decreases with the maturity of plant. A good example was described by White and Wolf (1996) that, with plants mature, dry matter yield and fiber content of forage plants increase, whereas nonstructural carbohydrates and protein content increase and then followed by a decrease, as shown in Figure 1. The functional material β-carotene, content also varies from different growing stages as proved by Fraser & Bramley (2004). There were many reports suggested that β-carotene concentrations decrease as forage plants mature which is because of the proportion decrease of leaves in plants (Brown, 1953; Olsson et al., 1955; Ballet et al., 2000). And Ballet et al (2000) also concluded that the highest β-carotene content was to be found in the vegetative to boot stages of grasses and legumes, the lowest concentration was after flowering of the plants. Plant shows the best nutritive value at the period of which all the leaves are fully developed. Harvest at too early maturity stage can be difficult to conduct the caring process due to high moisture content and can get lower yield compared to

harvesting at late boot or early heading stage.

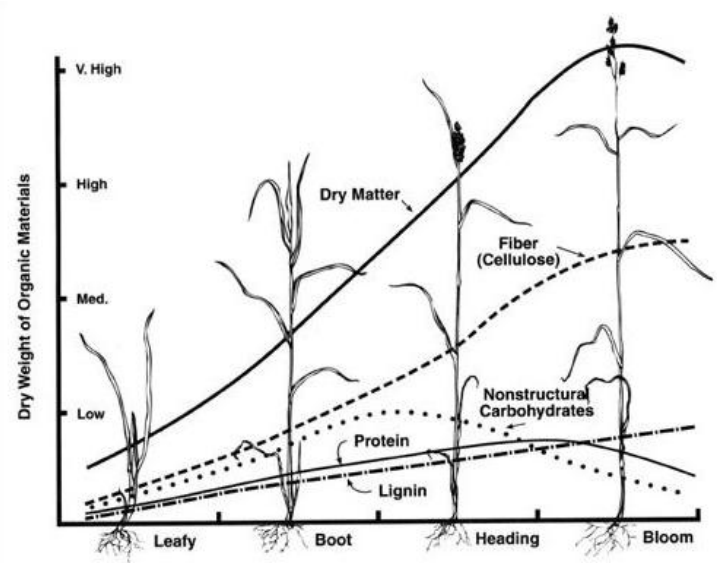

Figure 1. Nutrients content change with plant matures (White, H., & D. Wolf, 1996).

2.2 Different plant parts

Great variations of nutrients content occur among different plant parts. That is widely understood that stem physiologically contains higher structure carbohydrate content then leaf and grain part, while the highest crude protein appears in grain part. And it is claimed that leaf part shows higher relative feed value (RFV) than stem part (Santis et al., 2004). With the reduction of leaf stem ratio (LSR), crude protein content and *in vitro* dry matter digestibility decrease while acid detergent fiber and neutral detergent fiber increase. β-carotene are most abundant in leaves therefore the leaf proportion in plants is considered to have vital effect on β-carotene concentrations in forages (Brown, 1953; Ballet et al., 2000). And the leaf

proportion can be affected by various of factors including species, maturity, climate conditions, season and latitude thus these factors are also known to have effects on β-carotene concentrations (Olsson et al.,1955; Hjarde et al., 1963). However, in all published experiment there is no mention about leave proportion of total plants. And also the highest β-carotene value with maturation are different among studies.

2.3 Wilting conditions

Besides the harvesting stage at maturation and different plant parts, wilting condition is also another crucial factor which has great effect on β-carotene and vitamin content. Wilting is defined as the loss of rigidity of non-woody parts of plants which is known to closely related with solar radiation, ambient temperature and precipitation. Thus the wilting period would be shorter under good weather condition. As Robert (2013) mentioned, wilting in the field to 30% DM will virtually prevent clostridial activity because these bacteria need wet conditions for active growth. One study showed that wheat forage wilted for 20 hours contains lower water soluble carbohydrates content than unwilted wheat (Williams et al., 1995). Wilting of forage prior to ensiling, as a management strategy, is widely used in order to increase dry matter content to a proper level for lactic acid bacteria activity and minimize the effluent pollution caused by silage. It was reported that wilting prior to ensiling reduced the concentration of lactate, acetate, butyrate and propionate significantly, and also increased

acid detergent fiber content (Gordon et al., 1999). As for β-carotene and vitamin A, poor wilting conditions led to the increase of vitamin losses (Carter, 1960). In sunny weather, wilting would decrease β-carotene concentration to a great extent because of the sensitiveness to oxidation (Ballet et al., 2000).

2.4 Different preservation method

There are two options for forages preservation: ensiling and haymaking. The predominant method in a region varies by climate and, to some extent, by tradition, technology available, and use. In ensiling, harvest losses are reduced, but storage losses increase. In haymaking, the largest losses occur during harvesting, with little loss during storage if the crop is sufficiently dry, and there is no big difference for hay quality during storage if it is protected well from precipitation. Hay is more marketable than silage, whereas the handling of silage is more easily mechanized in some wet regions compared with hay because hay is highly depend on appropriate weather condition at harvest time. Some studies showed that ensiling is superior to haymaking when preservation of β-carotene in forages is required (Ballet et al., 2000). Losses of hay can be high up to 80-90% for β-carotene, but that are usually less than 20% in silage with good quality, and vitamin losses increase with poor wilting conditions

(Carter, 1960), but less is known about losses during the ensiling process. Both ensiling conditions and choice of plant species affect losses of β-carotene during ensiling (Beeckman et al., 2010). Nozière et al. (2006) noted that well-fermented silages usually had β-carotene losses of less than 20%, however, Kalač (1983) reported that there was no clear relationship between silage quality and the β-carotene content, and that was also found that large differences between plant species in losses of β-carotene during ensiling.

2.4.1 Ensiling

Across the world, ensiling is a widely adopted method for preserving forages (Wilkinson and Toivonen, 2003). There are two primary mechanisms to preserve a moist crop during ensiling: an anaerobic environment and the fermentation of plant sugars to lactic acid which can produce low pH condition. An anaerobic environment is essential to prevent the growth of aerobic spoilage microorganisms (including fungi, molds, yeasts and other bacteria) because many of these microorganisms can grow even at very low pH but oxygen is required. Therefore, the sealing of silos are critical to achieving and maintaining an anaerobic environment. Any oxygen remaining in the silo after sealing is usually used up rapidly by plant respiration within a few hours.

A low pH reduces the activity of plant enzymes and inhibits growth of

undesirable anaerobic bacteria. Inhibition of clostridial bacteria is most critical to successful silage preservation. These bacteria produce butyric acid and amines from fermentation of sugars or lactic acid or and amino acids, respectively. These kinds of fermentations cause losses of dry matter (DM) and reduce silage intake by ruminants.

Generally, lactic acid bacteria (LAB) already present on the crop produce the low pH by fermenting plant sugars, primarily producing lactic acid, as well as acetic acid, ethanol and other products (Muck, 2010). Beyond lowering pH, the lactic and acetic acids at sufficient levels are themselves inhibitory to undesirable aerobic bacteria and fungi, respectively (Muck et al., 1991). Natural fermentation can be assisted by inoculating the crop with selected LAB or by adding an acid to immediately reduce pH.

2.4.2 Haymaking

Forage is harvested and preserved as either hay or silage. Hay production is defined as the harvest of forage for storage under aerobic conditions. Hay is normally baled at a moisture content below 20% where the forage is stable for long-term storage. Hay can also be baled between 20% and 35% moisture content when air-forced drying or chemical treatments are used to preserve the hay. Forage harvested above 35% moisture content is usually fermented and stored in an anaerobic

environment as silage.

A primary objective in haymaking is to maintain the day matter (DM) yield and nutrient content of standing forage for further use. Hay may be stored up to one year, and perhaps longer. Physical, biological and chemical processes during harvest and storage cause DM and nutrient loss. Good management and proper equipment use can help reduce losses and preserve forage quality.

Other important considerations in hay production include resource use and production cost. Resource inputs include machinery, labor and energy. Due to the high cost of each, these resources must be used efficiently to reduce production costs. As agriculture adjusts to a more global market, the real price of animal products such as milk and meat have been stable or declining for a number of years. Maintaining a sustainable animal industry requires more efficient lower-cost production of forages.

Hay production processes include cutting, conditioning, swath manipulation, packaging, handling, storage and preservation. Each of these processes will be discussed, including their effects on forage quality and resource requirements. Additionally, production costs and the potential economic benefit of various production options will be briefly addressed.

2.5 Storage

2.5.1 Silage storage

During storage of silage, lactic acid bacteria (LAB), enterobacteria, fungi, yeasts and clostridia all play major roles in determining the final quality. Aerobic microbial activity continues immediately after silage sealing since air is present in plant material with a pH of 6-6.5 (Weinberg and Ashbell, 2003). Initially, the LAB populations are low, particularly relative to those of enterobacteria (Lin et al., 1992); however, the LAB populations increase markedly on alfalfa during the first 48h of wilting (Muck, 1989) and in response to chopping (McDonald et al., 1991). Losses during storage consist of fermentation losses and microbial respiration of oxygen entering the silo. Fermentation losses (typically 1% - 4%) are considered unavoidable and are primarily the result of CO_2 production during fermentation of hexoses to acetic acid or ethanol. However, such losses can be reduced by bacterial inoculants. The most significant losses during storage and opening are losses from aerobic microbial respiration. Minimizing a silage's exposure to oxygen minimizes respiration losses. During the emptying process, silage porosity, feedout rate and feedout surface influence respiration losses.

2.5.2 Hay storage

Hay is either stored inside a shelter or outside with varying levels of protection from the weather. During storage, microorganisms, especially fungi, on the hay respire, transforming dry matter to heat, water or gases, which leave the hay, leading to dry matter and nutrient losses even the losses are small or not be detected, sometimes. Respiration of hay contains microbial respiration and plant cell components respiration. Wood and Parker (1971) found that plant respiration is low when moisture content between 200 - 273 g / kg. In dry hay stored under cover, respiration is low, with a 4% to 5% total loss of DM during the storage period. Hay stored outside experiences this loss plus additional weathering loss on exposed hay.

2.6 Microbial activity

2.6.1 Microbial activity during ensiling

During ensiling process, microorganisms including lactic acid bacteria (LAB), fungi and clostridia play great role in the silage fermentation characteristics. When the ensiling start, aerobic microbial activity consume oxygen in plant material rapidly and establish anaerobic environment for LAB growth, at the same time, the anaerobic condition inhibits the plant cell respiration. And clostridia activity is the most undesirable microbial activity which can degrade sugars and organic acids to butyric acid, and

can also degrade protein to ammonia and amine with the bad smell of deterioration, thus decrease the intake by cattle.

2.6.2 Microbial activity during hay preservation

Generally hay is baled after moisture content decreases below 200 g / kg, so respiration of plant cell and activity of plant enzymes are inhibited. So the spontaneous heating and dry matter loss detected in hay with high moisture content are mainly due to microbial respiration (Rotz and Muck, 1994). During forage preservation, population of microbes is resulted greatly by moisture content and the ambient temperature. Rees (1982) mentioned that in moist hay, fungi significantly growth needs at least 70 % of humidity and above 20 ℃ of temperature. And microbial activity usually become more inactive as dry matter content of hay increase. Generally, at the first 10 or 20 days during preservation, there is no big change on bacteria populations.

2.7 β-carotene

β-carotene, known as the most active precursor for vitamin A, is an organic, strongly colored red-orange pigment abundant in plants and fruits. In plants, carotenoids are essential in photosynthesis where they harvest light and protect the plants from free radicals. As described by Asensi-Fabado and Munné-Bosch (2010), β-carotene also is an antioxidant. In addition, various of studies showed that β-carotene plays great role in

reproduction, immune function and health for dairy cows (Chew et al., 1982; Michal et al., 1994; Kume & Toharmat, 2001). According to the study of De Ondarza et al. (2009), supplementation of β-carotene increased milk fat yield in early lactation and mature cows, improved reproduction after 110 d and reduced early embryonic mortality. And that is also proved by Arechiga et at. (1998) that supplementation of β-carotene increased milk and butterfat yield. However, for beef cattle β-carotene is not always beneficial to meat quality, as Arnett and Daniel, (2008) mentioned that removal of Vitamin A enhanced the desirable partitioning of fat away from subcutaneous fat stores to intramuscular fat stores. And small amount of vitamin supplement was associated with a reduction in the proportion of unsaturated fats and an increase in intramuscular fat hardness (Siebert et al., 2006).

3. Materials and Methods

3.1 General information

The experiment was carried out in experimental field of Seoul National University, Pyeongchang Campus (located at 37° 32’ 46.1” N, 128° 26’ 17.9” E, where, average altitude is about 600-700m above sea level, more information are registered as annual mean temperature 11.5℃, average annual precipitation 113.4 mm, average annual wind speed 1.1 m/s, average annual humidity 68.0%, Sin-ri, Pyeongchang, Republic of Korea) from September 30, 2017 to May 31, 2018. More detail meteorological information involved during the experimental period are shown in Figure 2, 3, 4.

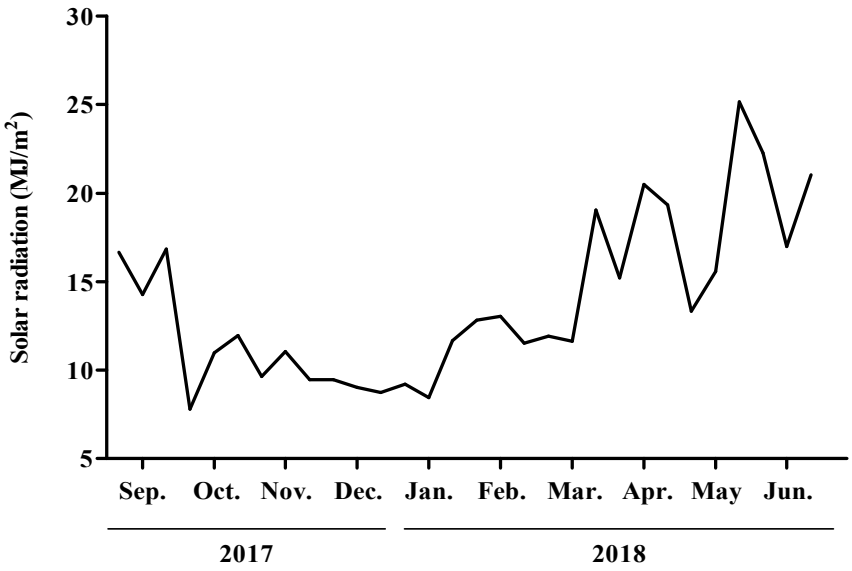

Figure 2. Solar radiation over experiment period.

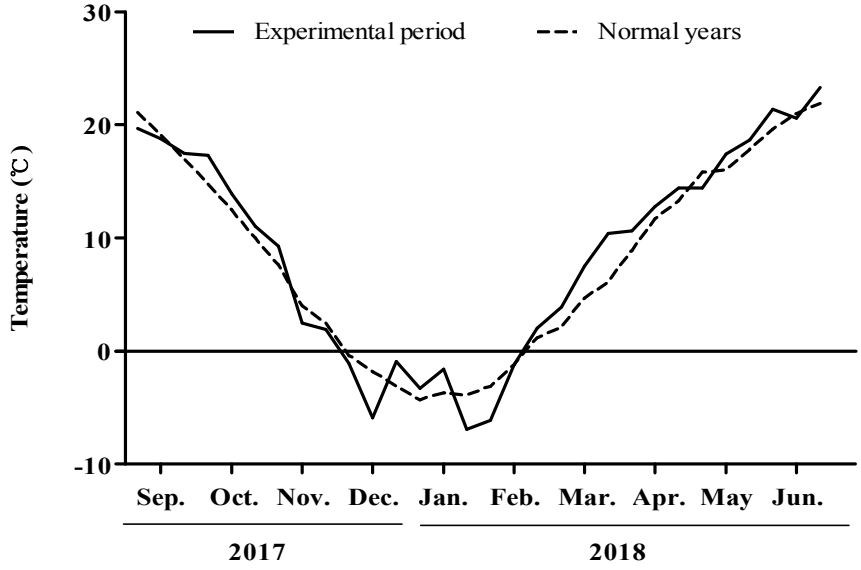

Figure 3. Average temperature comparison between experiment period and normal years.

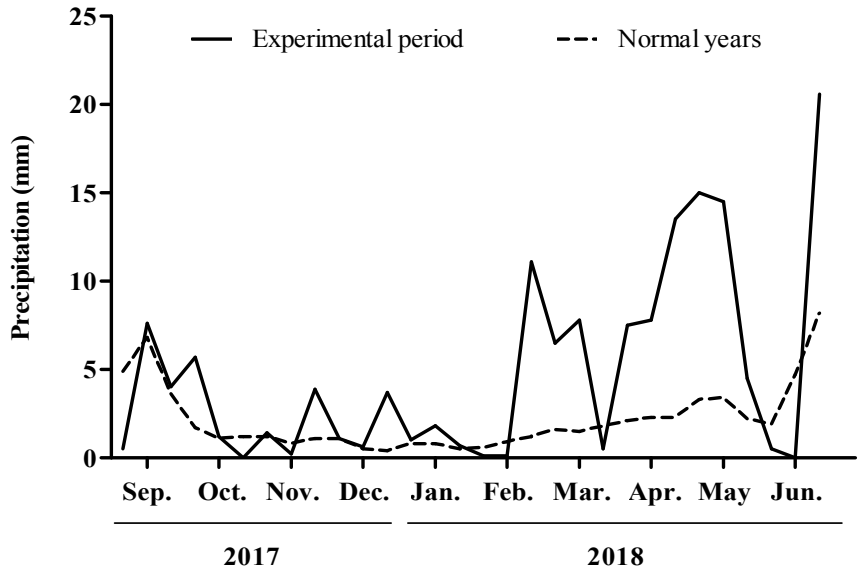

Figure 4. Precipitation comparison between experiment period and normal years.

3.2 Materials preparation

3.2.1 Raw materials preparation

Rye in the experiment was seeded on September 30, 2017 and harvested every 5 days from jointing stage (April 25, 2018) to ripening stage (May 31, 2018), totally 8 times, with mower conditioner leaving a stubble height of about 5cm above soil level. Before harvesting, plant height was recorded by a meter stick. Besides, forage yield was investigated from 1 m^2 (1 m × 1 m) quadrats with 3 replications which were selected randomly and then converted to kg / ha. Around 500 g fresh herbage were separated into stem, leaf (leaf sheath and blade) and grain part every 5 days and the ratio of each part were calculated on dry matter (DM) base. For determination of DM content, around 300g fresh materials were collected and dried in 65℃ air-forced drying oven for 72 hours from each harvest date.

3.2.2 Silage preparation

After been harvested on April 30 (early boot), May 9 (heading) and May 15 (blooming), rye herbage were chopped into 2-3cm length approximately using a fodder chopper (Richi Machinery Co., Ltd, Henan, China). And subsequently followed by treatment of different wilting time,

0 hour (without wilting), 6 hours and 24 hours wilting. The meteorological condition in Pyeongchang during wilting are shown in Table 1 (boldface). Then the chopped rye materials were ensiled into 20L mini silos maximumly without adding any additives and sealed tightly with lids. Three replications were performed for each treatments. And the 27 mini silos were preserved in dark-dried ambient temperature for 60 days before opening. Wet weight of silages were determined to measure DM loss by an electronic scale before and after ensiling.

Table 1. Meteorological condition in Pyeongchang during silage wilting and hay-making.

Date	Average temperature	Highest temperature	Lowest temperature	Precipitation	Average humidity	Insolation duration	Solar radiation	Average wind speed
	℃	℃	℃	mm	%	hr	MJ/m^2	m/s
2018-April-30	**16.9**	**25.9**	**8.0**	**0.0**	**60.3**	**10.9**	**22.6**	**0.8**
2018-May-1	**18.9**	**27.0**	**10.4**	**0.0**	**66.8**	**6.7**	**15.7**	**0.8**
2018-May-2	14.5	18.1	10.4	24.0	90.0	0.0	3.4	1.5
2018-May-3	9.6	15.2	5.4	6.5	75.4	3.4	12.1	2.1
2018-May-4	13.1	20.1	4.8	0.0	53.6	10.8	24.2	2.1
2018-May-5	16.4	23.0	6.3	0.0	47.4	9.2	21.8	1.3
2018-May-6	14.4	16.7	12.1	14.6	92.1	0.1	5.2	0.4
2018-May-7	16.0	22.0	11.9	0.0	77.6	2.2	14.2	0.7
2018-May-8	14.1	17.3	11.2	0.0	62.1	5.2	5.5	2.9
2018-May-9	**13.0**	**20.0**	**8.4**	**0.0**	**62.9**	**10.1**	**4.8**	**2.2**
2018-May-10	**13.8**	**22.7**	**5.8**	**0.0**	**68.6**	**8.3**	**26.3**	**1.1**
2018-May-11	15.7	23.9	7.1	0.0	59.0	8.7	24.8	1.1

(Continued overleaf)

2018-May-12	14.0	15.7	12.1	21.7	93.3	0.0	4.6	0.4
2018-May-13	17.0	22.1	13.4	2.6	79.0	6.6	22.0	1.1
2018-May-14	18.5	26.0	12.5	0.0	73.3	9.4	25.6	1.2
2018-May-15	**20.6**	**30.2**	**13.8**	**0.0**	**66.1**	**7.2**	**24.7**	**0.6**
2018-May-16	**19.2**	**21.3**	**16.6**	**6.5**	**92.3**	**0.0**	**2.3**	**0.3**
2018-May-17	22.7	28.3	20.2	9.1	93.1	1.4	5.6	0.5
2018-May-18	16.4	21.9	10.7	32.7	90.5	0.0	4.0	1.6
2018-May-19	15.4	21.7	9.3	0.0	57.9	12.7	23.4	2.7
2018-May-20	14.9	23.0	5.9	0.0	52.6	5.9	18.8	2.2
2018-May-21	15.7	23.8	5.5	0.0	65.9	10.0	26.8	1.5
2018-May-22	16.9	25.2	10.3	3.7	75.3	4.7	18.7	0.7
2018-May-23	17.9	23.8	12.3	8.0	59.9	11.6	29.0	1.4
2018-May-24	17.6	25.2	8.7	0.0	61.4	11.6	28.0	1.5
2018-May-25	18.2	27.5	10.0	0.0	60.8	8.8	27.6	0.8
Mean	16.2	22.6	10.1	5.0	70.7	6.4	17.0	1.3

Source: Korea Meteorological Administration

3.2.3 Hay preparation

Rye hay were made in triplicate after been harvested on April 30 (early boot), May 9 (heading) and May 15 (blooming) without additives. The harvested rye were spread on ground evenly and tedded twice (9 : 00 and 17 : 00) a day to speed up the moisture evaporation thus shorten the drying process. During the drying process, dry matter content were checked and recorded twice a day (9 : 00 and 17 : 00), after dry matter content increase over 80%, hay were packed into nylon net bag (40cm × 80cm) maximumly (around 2 kg) and preserved in ambient temperature for 70 days. During storage, variation of rye hay temperature (center of nylon net bag) and air temperature were recorded on 17 : 00 every 5 days using a dial probe thermometer with 50mm diameter dial and 300 mm length stainless (Eti Co., Ltd, Easting Cl, UK). After storage, dry matter loss was calculated. May is within rain season in Pyeongchang so it should be mentioned that hay during drying process were moved indoors as long as it rained. The meteorological condition in Pyeongchang during hay-making are shown in Table 1.

3.3 Chemical analysis

3.3.1 Pretreatment for chemical analysis

All fresh samples were collected around 300g and then dried in 65℃ air - forced drying oven for 72 hours for determination of dry matter (DM) content. All the dried samples were subsequently milled by a Willey Mill with 1 mm screen (Thomas Scientific, Inc., New Jersey, USA) into plastic bottles with screw tops and preserved in 4℃ dark-dried storage room prior to analysis.

3.3.2 Detergent fiber analysis

Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured by the method of Van Soest (1991). The American machine "ANKOM 2000 Automated Fiber Analyzer" (Ankom Technologies, Inc., Fairport, NY, USA) was utilized. And in the NDF procedure, heat-stable amylase and sodium sulfite were used.

3.3.3 Crude protein analysis

Crude protein (CP) was determined via Dumas method as described by Jean-Baptiste Dumas, (1884). The instrument "Automatic Elemental Analyzer Euro Vector EA3000" (EVISA Co., Ltd, Milan, Italy) was used.

3.3.4 Calculation of TDN and RFV

Total digestible nutrient (TDN) and relative feed value (RFV) were calculated by the known formula described by Holland et al. (1990). TDN was calculated from ADF value (TDN% = 88.9 - 0.79 × ADF%). And RFV was estimated through digestible dry matter (DDM% = 88.9 - 0.779 × ADF%) and dry matter intake (DMI% = 120 / NDF%) as RFV = (DMI% × DDM%) / 1.29.

3.4 *In vitro* digestibility analysis

For *in vitro* dry matter digestibility (IVDMD) analysis, the two-stage technique (Tilley and Terry, 1963) was used. 0.5 - 0.6 g of ground sample were weighed into F57 filter bags (Ankom Technology, Macedon, NY, USA) and sealed by heat sealer. The filter bags were pre-rinsed by acetone and completely air-dried to remove surfactant that inhibits microbial digestion. Then the sealed samples were placed into the Daisy Incubator digestion jars (Ankom Technologies, Inc., Fairport, NY, USA) with 266 ml of buffer solution B and 1330 ml of buffer solution A (1:5 ratio, v/v), the reagents contained in buffer A and B are shown in table 2. Rumen fluid was collected from two healthy cannulated Holstein steers before morning feed and filtered into 39℃ preheated thermos bottles through four layers of cheesecloth. Immediately, the rumen fluid was moved to laboratory and

filtered one more time by four layers of cheesecloth. Then blended 400ml / jar of fluid with samples and buffer solution in digestion jars and flushed the mixture continuously with CO_2 gas for thirty seconds before securing the screwing cap. All the digestion jars were incubated at 39℃ for 48 hours and sequentially, the NDF procedure was performed to get the *in vitro* dry matter digestibility.

Table 2. Reagent of buffer solution A and B

Buffer Solution A	g / liter
KH_2PO_4	10.0
$MgSO_4 \cdot 7\ H_2O$	0.5
NaCl	0.5
$CaCl_2 \cdot 2H_2O$	0.1
Urea (reagent grade)	0.5
Buffer Solution B	**g / liter**
Na_2CO_3	15.0
$Na_2S \cdot 9H_2O$	1.0

3.5 Fermentation characteristics

All silages were opened after 60 days' fermentation. Silage of upper part was discarded and the sample was collected from the rest part, which was mixed thoroughly and evenly. Two subsamples were retained for further analysis. One subsample was taken about 500 g and dried at 65℃ air - forced drying oven for 72 hours to determine DM content and other chemical composition, including ADF, NDF, CP, IVDMD and WSC. Another subsample was taken about 700 g and stored in - 80 ℃ refrigerator for sequentially determination of silage acidity (pH), organic acid, microorganisms and ammonia nitrogen. The Flieg's score of silages was calculated by the formula below which was presented by Zhang et. al. (2013).

Flieg's score = 220 + (2 × % DM - 15) - 40 × pH.

3.5.1 Acidity(pH)

10 g frozen silage sample was thawed and mixed with 100 ml distilled water in 250 ml conical flask and stored in refrigerator for 24 hours, during which, the conical flasks were shaken by hand every 2 hours. Then the mixture was filtered through filter paper (Whatman No. 6, AVANTEC) and immediately, the filtrates was utilized for silage pH measurement with a pH meter (AB 150, Fisher Scientific International, Inc., Pittsburgh, US).

3.5.2 Organic acid

To get the extract liquid for organic acid measurement, 10 g silage sample was mixed with 100 ml distilled water in 250 ml conical flask and stored in refrigerator with shaking by hand every 2 hours. After 24 hours, filtered through filter paper (Whatman No. 6, AVANTEC) and retained the filtrates in -20 ℃ refrigerator for long term storage. Before analyzing, 1.5 ml of filtrate was taken and centrifuged at 3000 rpm, 4 ℃ for 15 minutes using Centrifuge Smart 15 (Hanil Science Industrial, South Korea). Then the supernatant was used for analysis of lactic acid, acetic acid, propionic acid and butyric acid by high performance liquid chromatography (HPLC, Agilent Technologies, Santa Clara, CA, US). The condition of instrument was shown in Table 3.

Table 3. Instrumental conditions of HPLC for determination of organic acid in rye silage

Column	Agilent Hi-Plex H, 7.7 x 300 mm, 8 μm (p / n PL1170-6830)
Mobile phase	0.005 M H_2SO_4
Gradient	Isocratic
Flow rate	0.7 ml / min
Injection	20 μL
Temperature	60 ℃
Pressure	4.6 MPa (46 bar, 670 psi)
Detector	UV (55 ℃)

3.5.3 Microbial analysis

De Man, Rogosa and Sharpe agar (MRS) medium, plate count agar (PCA) medium and potato dextrose agar (PDA) medium were used for lactic acid bacteria (LAB), total microorganisms (TM), viable fungi and yeast counting, respectively, for silage (as for hay, only fungi and yeast were counted) by spread-plate method (Madigan and Michael et al., 2012). 10 g silage sample was homogenized in 90 ml sterile physiological saline (0.85% NaCl solution) and shaken for 1 hour on incubator. The extract was serially diluted by 0.85% NaCl solution to 10^2 - 10^5 times, then 10 μl of each was inoculated on agar surface and spread evenly by one-off plastic spreader. The MRS and PCA agar plates with samples were incubated at

37 ℃ for 24~48 hours, and 48~72 hours, respectively, for fungi counting, PDA agar plates were incubated at 25 ℃ for 48~72 hours. After incubating, the colony-forming units per gram (CFU / g) of microorganisms were counted on agar plates and calculated according to dilution factor.

3.5.4 Ammonia nitrogen (NH_3-N)

For ammonia nitrogen (NH_3-N) analysis, silage filtrate was used according to a modified phenol-hypochlorite reaction method described by Broderick and Kang (1980). Put 12ml sample into 15 ml centrifuge tube and centrifuged at 3000 rpm for 15 minutes. Then 0.02 ml of supernatant sample was transferred into 25 ml test tube and followed by 1 ml phenol reagent and 1 ml alkali-hypochlorite reagent. After vortex mixed and color reaction in 37 ℃ water bath for 15 minutes, added 8 ml distilled water in and vortex mixed again. Subsequently, the absorbance was detected in 630 nm wavelength for determination of ammonia nitrogen of dry matter, and total nitrogen was calculated by CP / 6.25. A blank was carried out throughout the whole process.

3.5.5 Water soluble carbohydrate (WSC)

Water soluble carbohydrate (WSC) was analysed via modifying the anthrone method proposed by Yemm and Willis, (1954). 0.2g of ground

sample was weighed into labelled Schott bottle with 200ml distilled water, the bottle was capped and followed by shaking for one hour on shaker. Then filtered through filter paper (Whatman No. 1, AVANTEC). 2ml of the filtrate was pipetted into labelled test tubes, rapidly added 10ml of anthrone reagent and mixed by vortex shaking. Loosely screwed cap and placed in the boiling water bath for 20 minutes, then followed by cooled in tap water for 10 minutes. Sequentially, measured the absorbance at 620 nm wavelength in a 1 cm optical cell. The WSC content was calculated by the formula:

$$\text{WSC \%} = G \times D \times E \times 100 \times 0.1 / (W \times \text{Sample lab dry matter \%})$$

Where: W = sample weight (mg), G = mg glucose read from graph, E = Extract volume (200ml), D= Dilution factor.

3.6 Analysis of β-carotene

For β-carotene analysis, 2 g of the ground samples were used. The pretreatment are as follows: put 10 ml of 6 % pyrogallol and sonicate in the samples, reacted for 10 minutes. After sonication, 7 ml of 60 % KOH was added in and mixed evenly by vortexing. Then the mixture was incubated at 80 ℃ water bath for 1 hour and followed by 10 ml of 2 % NaCl and 15 ml of haxane-acetate, the ratio of which was 85: 15. Sequentially, the

mixture was centrifuged at 5000 rpm for 15 minutes. After centrifuging, added 1 ml of chloroform and the β-carotene concentration was detected by HPLC using Agilent 1260 series (Agilent Technologies, CA, USA) . The instrumental conditions were shown below (Table 4.).

Table 4. Instrumental conditions of HPLC for determination of β-carotene.

Column	CAPCELL PAK C18 UG120 S5, 4.6 mm I. D. × 250 mm
Mobile phase	A: acetonitrile: methanol (85: 15)
	B: dichloromethanol
	A (70%), B (30%)
Flow rate	1000 μL / min
Inj. vol.	3.5 μL
Temperature	35 ℃
Pressure	4.9 MPa
Detector	UV 254 nm

3.7 Statistical analysis

Data were analyzed by the general linear model (GLM) procedure of SAS 2002 (version 9.1). The complete random design was used for effect of harvest dates on rye herbage quality and hay quality, and the split plot design was used for effect of plant parts (main plot) and harvest dates (sub plot), harvest dates (main plot) and wilting periods (sub plot). Differences were considered to be significant when $P < 0.05$.

4. Results and Discussions

4.1 Effect of harvest dates

4.1.1 Effect of harvest dates on Agronomic characteristics

The identification of growth stage and the determination of dry matter (DM) content and plant height shown in Table 5. With the progressing maturity of rye, DM content increased significantly. The highest DM content 34.36 % occurred on May 31 (ripening stage). Plant height also showed the similar trend. Factors which have effects on plant height of cereals are various and among them maturity is the most vital factor (De Ruiter et al., 2002). In the current study, plant height increased significantly during study period from 53 cm on April 25 (jointing stage) and 180 cm on May 31 (ripening stage), the similar result was also found by Ku et al., (2018).

Table 5. Growth stages, DM content and plant height of rye according to harvest dates.

Harvest date	Growth Stage	DM (%)	Plant height (cm)
April 25	Jointing	15.50^{e}	53^{h}
April 30	Early Boot	16.46^{e}	75^{g}
May 4	Late Boot	16.49^{e}	89^{f}
May 9	Heading	16.57^{e}	111^{e}
May 15	Blooming	18.99^{d}	141^{d}
May 21	Milking	24.01^{c}	155^{c}
May 25	Dough	26.83^{b}	164^{b}
May 31	Ripening	34.36^{a}	180^{a}
Mean	-	21.15	121
LSD (0.05)	-	1.29	4.55

DM: dry matter, LSD: least significant difference. Means within a column with different superscripts differ ($P < 0.05$).

4.1.2 Effect of harvest dates on yield composition

Yield composition of different growth stage was shown in Table 6. With progressed maturity of rye, yield composition on fresh matter (FM) and dry matter (DM) showed some differences. FM yield increased until blooming stage and followed by a decrease, while DM yield showed the lowest value 3,760 kg / ha and the highest, 10,439 kg / ha on jointing and ripening stage, respectively. Yield of winter rye forage increased quadratically in response to maturity as reported by Kantar et al. (2011). DM yield performance observed in this experiment was more acceptable than that of Hakan et al. (2014), it was might due to the more favourable climatic parameters in the experimental site. However, in Thelen and Leep (2002)' s study, rye harvested on April 30 (early boot) got a yield of 1.7 tons

/ acre, which is close to the result 4,364 kg / ha (1.77 tons / acre) in this experiment. And similar yields were also found on rye harvested on late growth stages of this study and result reported by Tollenarr et al. (1992). As for the crude protein (CP) yield, milking, late boot and dough stages showed higher content than any other stages significantly ($P < 0.05$). Total digestible nutrient (TDN) yield of DM in this study increased from 2,693 kg / ha on April 25 (jointing) to 5,825 kg / ha on May 31 (ripening), during which each advanced stages showed increases markedly than their earlier stages ($P < 0.05$). DM and TDN yield showed highly consistent trend with that of whole crop barley (Kim et al., 2010).

Table 6. Effect of harvest dates on yield composition.

Harvest date	Growth stage	Yield (kg / ha)			
		Fresh matter	Dry matter	CP	TDN
April 25	Jointing	24,333^{d}	3,760^{e}	511^{c}	2707^{h}
April 30	Early Boot	26,500^{cd}	4,364^{e}	508^{c}	2,999^{g}
May 4	Late Boot	34,333^{ab}	5,651^{d}	640^{a}	3,762^{e}
May 9	Heading	36,000^{a}	5,970^{d}	535^{ab}	3,663^{f}
May 15	Blooming	39,000^{a}	7,408^{c}	564^{b}	4,210^{d}
May 21	Milking	38,000^{a}	9,157^{b}	654^{a}	5,218^{c}
May 25	Dough	37,000^{a}	9,880^{ab}	625^{a}	5,603^{b}
May 31	Ripening	30,333^{bc}	10,439^{a}	389^{d}	5,914^{a}
Mean	-	33,187	7,079	533	4,260
LSD(0.05)	-	5109.60	1036.60	40.98	78.68

CP: crude protein, TDN: total digestible nutrient. Means within a column with different superscripts differ ($P < 0.05$).

4.1.3 Effect of harvest dates on forage quality of rye

Azim et al. (1989) reported that DM content would increase with the maturity processes of plant growth. In this experiment, DM content of the whole crop rye increased by 121.68 % (155.00 g / kg on April 25 vs 343.60 g / kg on May 31) with the plant matured, which also showed the same trend with other forage plants such as wheat and corn (Waldren et al., 1979; Yakup et al., 2016). Yield and forage quality varied from harvest date of maturity. Forage production is considered to be a compromise between nutritive yield and forage quality. Previous studies have shown that forage yield increases while forage quality decreases with small grains mature from vegetative stage to reproductive stage (Cherney et al., 1982; Helsel et al., 1987). The same result was found in this experiment, rye harvested on early growth stages (late April or early May) could provide producers with high quality but yields could be very low, while low quality and high yields could be obtained on late stages. Crude protein decreased with progressed maturity until dough development (Kantar et al., 2011). The CP level in this experiment decreased continuously, that was similar to previous studies which were well documented (McCormick et al., 2006; Harmoney and Thompson, 2010; Xie et al., 2012). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) content showed similar trend, which increased from April 25 until May 15 and then kept stable or fluctuated slightly, and the content after May 15 were significantly higher than the earlier stages ($P < 0.05$). The NDF of rye harvested from April 30 (early boot) was 499.83 g / kg of DM, which was in consistent with the 48.6% mentioned by Thelen and Leep, (2002). Water soluble carbohydrate (WSC) content decreased

until May 15 and then followed by a increase, with the lowest value, 80.56 g / kg of DM on the May 15 and the highest, 217.22 g / kg DM was shown on April 25. Generally, there is a continuous rise in starch with advanced development of plant. However, the WSC content showed some differences, it decreased till May 15 and then followed by a increase. Similar observations were reported in some earlier studies (Bhatia et al., 1972; Bhatia et al., 1974). *In vitro* dry matter digestibility of raw rye herbage observed during experimental period went down continuously as the progressing plant maturity, which was in consistent with the observation mentioned by Kim et al. (2010) and Kantar, (2011). The herbage harvested from early stages contained higher moisture and lower structural carbohydrates, like fiber, especially lignin, are not easy to be digested by rumen microbes. The total digestible nutrients (TDN) also showed similar slope with ADF and NDF content, which decreased from jointing to blooming stage and kept steady after blooming stage, as the component contained in fiber is tough to be degraded. And the TDN content contained on early stages were significantly higher than the late stages ($P < 0.05$). As for the relative feed value (RFV) index, rye quality was ranged into 6 grades (shown in Figure 5.) as prime (>151), excellent (150-125), good (124-103), fair (102-87), poor (86-75) and reject (< 74) according to AFGC (1972). The rye forage quality harvested with maturity in this study was defined as prime, excellent, good and fair, respectively, from April 25 (jointing) to May 9 (heading), however, RFV of rye harvested after heading all fell into the grade of poor, as the lowest level 79 appeared on May 15 (blooming) and the highest, 156, appeared on April 25 (jointing) (Table 7.).

Table 7. Effect of harvest dates on chemical composition and feed value

Date	DM	CP	ADF	NDF	WSC	IVDMD	TDN	RFV
	---------- g / kg ----------						%	
April 25	155.00^{e}	135.99^{a}	213.87^{e}	430.23^{g}	217.22^{a}	903.27^{a}	72.01^{a}	156^{a}
April 30	164.63^{e}	116.46^{b}	255.50^{d}	499.83^{f}	206.11^{a}	871.17^{b}	68.72^{b}	128^{b}
May 4	164.93^{e}	113.26^{b}	282.70^{c}	540.13^{e}	196.67^{a}	835.40^{c}	66.57^{c}	115^{c}
May 9	165.67^{e}	89.60^{c}	348.63^{b}	599.93^{d}	102.23^{cd}	825.13^{c}	61.36^{d}	96^{d}
May 15	189.93^{d}	76.11^{d}	405.93^{a}	673.07^{a}	80.56^{d}	715.23^{d}	56.83^{e}	79^{f}
May 21	240.07^{c}	71.45^{d}	404.07^{a}	664.57^{ab}	102.78^{cd}	686.10^{e}	56.98^{e}	80^{ef}
May 25	268.33^{b}	63.24^{e}	407.40^{a}	640.27^{c}	112.22^{c}	632.10^{f}	56.72^{e}	83^{e}
May 31	343.60^{a}	37.26^{f}	408.17^{a}	646.50^{bc}	156.67^{b}	589.77^{g}	56.66^{e}	82^{ef}
Mean	211.52	87.70	340.78	586.82	146.81	757.27	61.98	103
LSD (0.05)	17.61	7.36	11.00	21.61	23.97	16.08	0.87	3.52

DM: dry matter, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber, WSC: water soluble carbohydrate, IVDMD: *in vitro* dry matter digestibility, TDN: total digestible nutrient, RFV: relative feed value, Means within a column with different superscripts differ ($P < 0.05$).

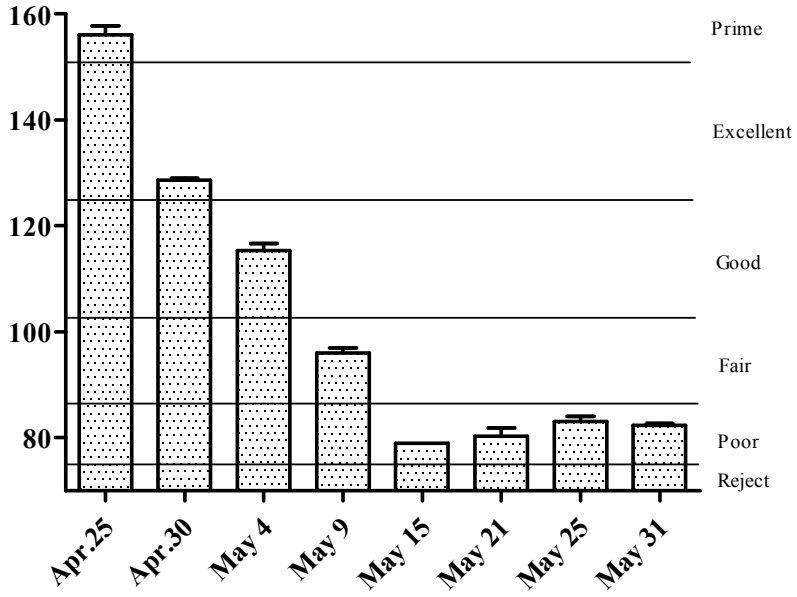

Figure 5. Effect of harvest dates on RFV of rye herbage.

4.1.4 Effect of harvest dates on β-carotene content

Effect of different harvest dates of maturity on β-carotene concentrations were shown in Figure 6. The highest β-carotene concentration 27.14 mg / 100g was on April 25. After April 25, the concentration dramatically decreased and reached its lowest point (0.93 mg / 100g) on May 25. And there was no β-carotene detected on May 31 (ripening). The result in this study was in consistent with the previous studies. The highest β-carotene concentration of forage plant was found on vegetative stage and was lowest after flowering (Ballet et al., 2000). Besides, many researches have found that β-carotene content in forages decrease with plant advancing maturity and that was due to the decreasing of leaf proportion in total plant (Olsson et al., 1955; Ballet et al., 2000).

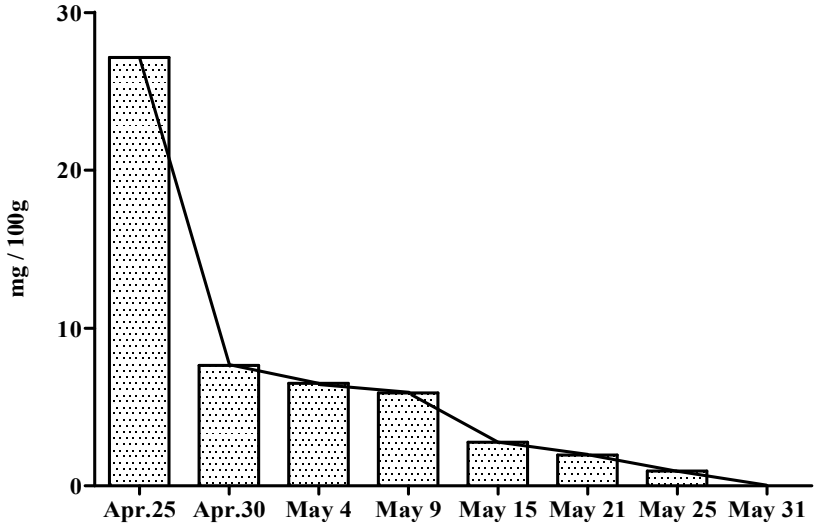

Figure 6. Effect of harvest date on β-carotene concentration of rye herbage.

4.2 Effect of plant parts

4.2.1 Effect of harvest dates on leaf-stem-grain ratio

As the delay of harvest date, the ratio on dry matter of leaf, stem and grain showed some regular changes. On jointing and early boot stage, there were only leaf and stem parts were separated from whole crop rye, within which, the stem part only took 16.19 %, 33.44 % of total, when leaf parts took 83.81 % and 66.56 %, respectively. Grain parts started to develop from late boot stage, and leaf still was the dominant part among the total plant. After heading, leaf ratio decreased, while stem and grain ratio increased and gradually, as the plant elongated and developed, stem became the dominant part on dry matter of total plant. The highest stem ratio and lowest leaf ratio occurred on ripening stage. As plant matured, the

leaf proportion decreased rapidly (Mowat et al., 1965), which was in consistent with the result in this study.

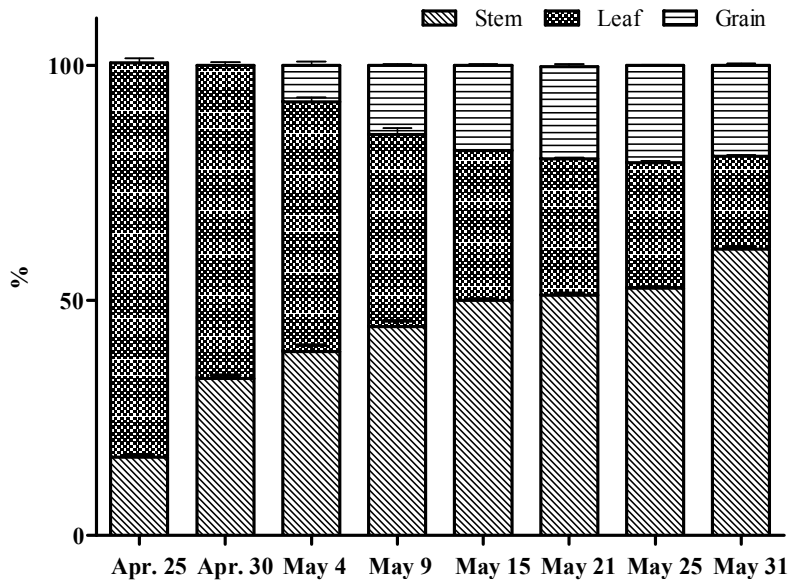

Figure 7. Effect of harvest dates on leaf-stem-grain ratio.

4.2.2 Effect of plant parts and harvest dates on feed value of forage rye

Effect of plant parts and harvest dates on forage quality of rye were shown in Table 8. In this experiment, plant part, harvest date and their interaction affected rye quality significantly ($P < 0.0001$). For all of the three parts, the dry matter (DM) content increased with the delay of harvest date and reached their highest content, 391.5 g / kg, 398.6 g / kg and 427.5 g / kg of FM, respectively, on May 31. Stem part lost its moisture at the most fast speed among the three. And the lowest DM content 139.9 g / kg

occurred on stem part of April 25, when the highest 427.5 g / kg was on grain part of May 31. MacDonald (1946) stated that nutritive composition of leaves and stems decrease as plant progressed maturity. As for CP content, grain contained the highest CP content compared with others while stem contained the lowest ($P < 0.0001$). On each stage, CP content contained in leaf part were 2-3 times as much as that contained in stem. Content in grain decreased during the measured period, for leaf and stem, the highest content was on April 25. Santis et al. (2004) proved that CP, IVDMD and other nutritive value decreased with the reduction of leaf stem ratio.

ADF and NDF value showed similar tendency on three parts, as increased till May 15 (blooming), and after that, followed by a slight fluctuation. Stem contained the highest ADF and NDF content while grain contained the lowest ($P < 0.0001$) compared with other parts. The lowest content of three parts appeared on the early growth stages. With rye matured, IVDMD of the three parts all decreased as a result of the increased fiber content , and grain and leaf were significantly ($P < 0.0001$) higher than stem, which showed as 721.85 g / kg. TDN value showed similar trend with ADF and NDF, the highest value showed on early stages.

The RFV index of grain was the highest on May 4 and decreased until May 31 markedly ($P < 0.0001$), stem and leaf RFV were close on boot stage but stem decreased faster than leaf with prolonged harvesting date. For contents and trend of nutrient contained in leaf, stem and grain, consistent result were observed in several new developed cultivars of whole crop rice (WCR) in Korea as described by Zhao (2018).

Table 8. Effect of plant parts and harvest dates on forage quality of rye

Part	Date	DM	CP	ADF	NDF	IVDMD	TDN	RFV
		----------		g / kg	----------		%	
Leaf	April 25	206.73^{d}	125.74^{a}	215.60^{g}	457.13^{f}	905.70^{a}	71.87^{a}	147^{a}
	April 30	217.20^{cd}	117.79^{b}	224.33^{f}	459.43^{f}	884.43^{b}	71.18^{b}	145^{b}
	May 4	231.90^{c}	99.83^{c}	273.40^{e}	501.80^{e}	829.40^{c}	67.30^{c}	125^{c}
	May 9	243.70^{c}	97.03^{c}	303.93^{d}	552.20^{d}	791.57^{d}	64.89^{d}	110^{d}
	May 15	243.63^{c}	97.93^{c}	328.47^{c}	585.07^{c}	747.00^{e}	62.95^{e}	101^{e}
	May 21	274.13^{b}	88.00^{d}	353.23^{b}	625.40^{b}	720.53^{f}	61.00^{f}	91^{f}
	May 25	289.90^{b}	77.25^{e}	331.07^{c}	580.37^{c}	718.97^{f}	62.75^{e}	101^{e}
	May 31	391.50^{a}	49.11^{f}	362.53^{a}	634.50^{a}	677.43^{g}	60.26^{g}	89^{g}
	Mean	262.34^{B}	94.08^{B}	299.07^{B}	549.49^{B}	784.38^{A}	65.27^{B}	114^{B}
Stem	April 25	139.87^{f}	71.11^{a}	231.53^{g}	443.97^{f}	946.73^{a}	70.61^{a}	149^{a}
	April 30	165.63^{e}	65.20^{b}	264.87^{f}	456.67^{f}	900.80^{b}	67.98^{b}	139^{b}
	May 4	167.10^{e}	45.86^{de}	319.17^{e}	524.77^{e}	842.60^{c}	63.69^{c}	114^{c}
	May 9	147.37^{f}	47.47^{d}	394.40^{d}	624.47^{d}	753.27^{d}	57.74^{d}	87^{d}
	May 15	186.37^{d}	50.31^{c}	457.90^{c}	705.93^{b}	667.50^{e}	52.73^{e}	70^{e}
	May 21	251.53^{c}	44.34^{e}	487.03^{a}	733.50^{a}	605.30^{f}	50.42^{g}	65^{f}
	May 25	285.90^{b}	43.44^{e}	466.30^{b}	704.73^{b}	523.63^{g}	52.06^{f}	69^{e}
	May 31	398.57a	18.70^{f}	451.93^{c}	681.17^{c}	534.97^{g}	53.20^{e}	73^{e}
	Mean	217.79^{C}	48.30^{C}	384.14^{A}	609.40^{A}	721.85^{B}	58.55^{C}	96^{C}
Grain	May 4	196.03^{e}	160.70^{a}	172.30^{e}	424.45^{e}	941.95^{a}	75.29^{a}	165^{a}
	May 9	200.07^{e}	127.58^{b}	257.97^{d}	501.20^{d}	884.97^{b}	68.52^{b}	128^{b}
	May 15	241.87^{d}	109.72^{c}	304.20^{b}	557.23^{b}	799.87^{c}	64.87^{d}	109^{d}
	May 21	302.30^{c}	101.58^{d}	293.77^{c}	529.67^{c}	756.23^{d}	65.69^{c}	116^{c}
	May 25	339.37^{b}	88.08^{e}	308.60^{b}	550.00^{b}	711.87^{e}	64.52^{d}	110^{d}
	May 31	427.50^{a}	49.01^{f}	328.43^{a}	581.70^{a}	643.17^{f}	62.95^{e}	101^{e}
	Mean	284.52^{A}	106.11^{A}	277.54^{C}	524.04^{C}	789.68^{A}	66.97^{A}	121^{A}
Mean	-	252.19	80.72	324.13	564.33	763.09	63.29	109
Probability	P	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001
	D	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001
	P × D	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001

P: plant part, D: date. Within a column, different superscripts in capital letter mains main plot differ, in small letters means sub plot differ ($P < 0.05$).

4.3 Effect of ensiling dates and wilting periods on rye silage

4.3.1 Chemical composition and feed value

Effect of ensiling dates and wilting on chemical composition and feed value of rye silage were shown in Table 9. Fermentation characteristics were affected by maturity stage at harvest of grass silage within each growth cycle (Muck et al., 1991). Furthermore, the advancing maturity of ensiling decreased CP from 137.41 g / kg DM to 86.92 g / kg DM, similar result was also observed by Muck et al. (1991). Similar with CP, the effect of wilting period on ADF and NDF were also significant ($P < 0.05$), as similar value were detected within same maturity stage under treatments of different wilting period. The lowest ADF and NDF content were detected on April 30 (boot) with wilting for 24 hours, and the highest value were 449.27 g / kg and 678.73 g / kg of DM, respectively. About the IVDMD, significant increases were found ($P = 0.0013$) on April 30 (boot) and May 9 (heading). Delay of ensiled date on maturity decreased the digestibility of rye silage, as 807.26 g/ kg on April 30 (boot) and 633.91 g / kg on May 15 (blooming) were observed in this experiment. Similar result was found on Italian ryegrass (IRG) silage wilted for 5 hours by Kim et al. (2018). TDN was decreased significantly ($P < 0.0001$) by delay of ensiling date, as the TDN on April 30 (boot) was the highest and lowest was on May 15 (blooming). RFV was affected by different wilting treatments ($P = 0.0081$) in this experiment, and the effect of ensiling date of stage on RFV was also significant ($P < 0.0001$), as delay of harvesting would decrease the RFV.

Table 9. Effect of ensiling dates and wilting periods on DM content and feed value of rye silage.

Date	Wilting	DM	CP	ADF	NDF	IVDMD	TDN	RFV
			---------- g / kg ----------				%	
April 30 (Boot)	0h	153.43^{c}	143.55^{a}	309.17^{a}	508.73^{a}	784.70^{b}	64.48^{b}	119^{b}
	6h	186.44^{b}	142.51^{a}	283.87^{b}	470.63^{b}	815.07^{a}	66.48^{a}	132^{a}
	24h	236.61^{a}	126.16^{b}	267.37^{b}	476.50^{b}	822.00^{a}	67.78^{a}	133^{a}
	Mean	192.16^{C}	137.41^{A}	286.80^{C}	485.29^{C}	807.26^{A}	66.24^{A}	128^{A}
May 9 (Heading)	0h	160.22^{c}	128.35^{a}	342.30^{a}	572.40^{a}	731.27^{a}	61.86^{a}	101^{a}
	6h	193.77^{b}	129.31^{a}	350.33^{a}	580.03^{a}	749.97^{a}	61.22^{a}	99^{a}
	24h	284.25^{a}	128.70^{a}	343.90^{a}	582.43^{a}	752.90^{a}	61.73^{a}	99^{a}
	Mean	212.75^{B}	128.79^{B}	345.51^{B}	578.29^{B}	744.71^{B}	61.61^{B}	100^{B}
May 15 (Blooming)	0h	163.75^{b}	85.36^{a}	449.27^{a}	675.77^{a}	612.33^{b}	53.41^{b}	74^{b}
	6h	268.80^{a}	90.62^{a}	425.00^{b}	659.73^{b}	656.17^{a}	55.33^{a}	79^{a}
	24h	308.12^{a}	84.80^{a}	429.53^{b}	678.73^{a}	633.23^{ab}	54.97^{a}	76^{ab}
	Mean	246.89^{A}	86.92^{C}	434.60^{A}	671.41^{A}	633.91^{C}	54.57^{C}	76^{C}
Mean	-	217.27	117.71	355.64	578.33	728.63	60.81	101
Probability	D	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001
	W	< .0001	0.0012	0.0016	0.0219	0.0013	0.0016	0.0081
	D × W	0.0087	0.0035	0.0080	0.0098	0.3556	0.0078	0.0042

0h: no wilting before ensiling, 6h: wilted for 6 hours before ensiling, 24h: wilted for 24 hours before ensiling. D: date, W: wilting. Within a column, different superscripts in capital letter mains main plot differ, in small letters means sub plot differ ($P < 0.05$).

4.3.2 Fermentation characteristics

Fermentation characteristics of rye silage were shown in Table 10. Extending of wilting time elevated acidity (pH) value. And it was in consistent with previous studies (Haigh, 1985; Gordon et al., 1999), which described that wilting resulted in a significant increase in grass silage pH ($P < 0.001$). In the current study, lowest pH value was found on May 9 (heading) which was ensiled immediately without wilting. Silage ensiled on May 15 (blooming) showed higher pH value than the earlier stages. However, McDonald (1981) reviewed that with the DM content increase, silage fermentation is restricted and hence pH value is not always reliable parameter for determination of silage quality because with the pH rising, stable conditions may be achieved at much higher pH value (Beck, 1978; McDonald, 1981). DM content of silage were closely related to the DM of raw herbage, as wilting produced significant ($P < 0.0001$) increase of the DM after ensiling. As well as the effect of harvesting date, the silage which was ensiled on May 15 (blooming) was maximized as 246.89 g / kg of FM, and the lowest DM content 153.43 g / kg was on silage ensiled on April 30 (boot) without wilting. The ensiling stage, wilting treatments and the interaction of both influenced the DM content of silage significantly ($P < 0.05$). On April 30 and May 9, wilting significantly decreased the DM loss of silage after ensiling for 90 days. On blooming stage, DM loss of silage wilted for 24 hours were lower significantly ($P < 0.0001$) than 0 hour and 6 hours. Among the three ensiled stages, heading stage showed the lowest DM loss 39.14 g / kg, which was followed by 83 g / kg on April 30 (boot) and 93.31 g / kg on May 15 (blooming). Wilting prior to ensiling increased

the residual WSC content in silage on April 30 (boot), which was similar with the conclusions of Gordon (1981) and Castle & Watson. (1982), but was on the contrary with the results of Wilkinson et al. (1976) and Kim. (1999). Silage wilted for 24 hours contained more WSC than that wilted for 0 hour and 6hours on heading stage, while there was no big difference found among different wilting treatments on blooming stage. NH_3-N proportion to TN is an important factor that can reflect the proteolysis extent after fermentation (Thomas et al., 1980). NH_3-N is produced through decomposing protein by *Clostridium* spp. in fresh materials (Tian et al., 2014). To some extent, NH_3-N content in silage can greatly reflect the silage fermentative quality, as the more it was produced, the worse the silage fermentation was. Wilting decreased NH_3-N / TN ratio on April 30 (boot) and May 9 (heading). Gordon et al. (1999) also reported that wilting prior to ensiling resulted in reduction in NH_3-N (g / kg total N). In this study, the highest content was on May 15 (blooming) without wilting.

Table 10. Effect of ensiling dates and wilting periods on fermentation characteristics of rye silage.

Date	Wilting	pH	DM	DM loss	WSC	NH_3-N / TN
			---------- g / kg ----------			
April 30 (Boot)	0h	4.61^{c}	153.43^{c}	119.91^{a}	37.41^{c}	164.32^{a}
	6h	4.75^{b}	186.44^{b}	83.06^{b}	121.67^{b}	97.55^{b}
	24h	5.00^{a}	236.61^{a}	54.03^{c}	157.78^{a}	88.48^{b}
	Mean	4.79^{B}	192.16^{C}	83.00^{B}	105.62^{A}	116.78^{C}
May 9 (Heading)	0h	4.24^{b}	160.22^{c}	50.06^{a}	21.85^{b}	161.72^{a}
	6h	4.43^{b}	193.77^{b}	45.08^{a}	18.52^{b}	154.68^{a}
	24h	4.78^{a}	284.25^{a}	22.28^{b}	45.00^{a}	124.79^{b}
	Mean	4.45^{C}	212.75^{B}	39.14^{C}	28.46^{B}	147.07^{B}
May 15 (Blooming)	0h	5.28^{c}	163.75^{b}	181.84^{b}	21.48^{a}	316.82^{a}
	6h	5.78^{b}	268.80^{a}	198.17^{a}	25.56^{a}	196.30^{c}
	24h	6.40^{a}	308.12^{a}	93.32^{c}	21.11^{a}	269.16^{b}
	Mean	5.82^{A}	246.89^{A}	157.78^{A}	22.72^{C}	260.76^{A}
Mean	-	5.02	217.27	93.31	52.26	174.87
Probability	D	< .0001	< .0001	< .0001	< .0001	< .0001
	W	< .0001	< .0001	< .0001	< .0001	< .0001
	D × W	0.0006	0.0087	< .0001	< .0001	< .0001

NH_3-N: ammonia nitrogen, TN: total nitrogen. D: date, W: wilting. Within a column, different superscripts in capital letter mains main plot differ, in small letters means sub plot differ ($P < 0.05$).

4.3.3 Organic acid composition

Organic acid composition of silage were shown in Table 11. Lactic acid is the main acid product during silage fermentation. Higher lactic acid content indicates higher efficiency of conversions from WSC to acid. In this experiment, highest lactic acid concentration 9.70 (g / kg DM) were detected on May 9 (heading). It was at variance with conclusion that lactic acid of whole crop barley silage ensiled on milking stage showed the highest value which was published by Kim et al. (2010), it may due to the forage species and the different experimental location. The lowest acetic acid was on April 30 (boot) while the highest was on May 15 (blooming). Enterobacteria and heterofermentative lactic acid bacteria produce the weaker acid, acetic acid, and increase the DM loss (Kim et al., 2001). Silages dominated by acetic acid have higher dry matter loss and might less acceptable for cattles than silages dominated by lactic acid (Bolsen, 1995). The lowest propionic acid appeared on silage of May 9, in addition, wilting (P = 0.3574) and the interaction of date and wilting (P = 0.1632) had no significant effect on propoinic acid. As for butyric acid, it can reflect the extent of the clostridial activity during ensiling and is considered to cause poor fermentation. Silages with high concentration of butyric acid may not readily acceptable by livestock (Bolsen, 1995). Wieringa (1966) claimed that grass silage contained less than 5-10 g / kg DM butyric acid can be considered as good-medium quality silage. In this study, butyric acid of silage ensiled on May 9 (heading) was the lowest when the May 15 (blooming) produced the highest compared with others. 8.39 (g / kg DM) of butyric acid was detected on April 30, and for silage of May 9, there was

no butyric acid detected in all the wilting treatment groups. In the current study, with progressed maturity of ensiling, ratio of LA / AA decreased. As Jones et al. (1992) demonstrated, the LA / AA ratio indicates the relationship of homolactic and heterolactic during fermentation. According to the Flieg's score, all silage made on May 9 (heading) were ranked into good grade, within which, silage wilted for 24 hours showed the highest score. Silage made on April 30 (boot) were determined as average grade, however, the only poor silage was found in silage wilted for 24 hours on May 15 (blooming), which was considered to have a bad fermentation quality. It may due to the bad weather condition, such as rainfall, high humidity and short insolation duration during wilting (Table 1.).

Table 11. Effect of ensiling dates and wilting periods on organic acid composition of rye silage.

Date	Wilting	pH	LA	AA	PA	BA	LA / AA	Flieg's score	Grade
			----------	----------	g /kg	----------			
April 30 (Boot)	0h	4.61^{c}	4.43^{a}	0.55^{b}	3.00^{a}	10.27^{a}	6.85^{a}	51^{a}	Average
	6h	4.75^{b}	1.53^{b}	0.73^{b}	2.27^{a}	10.33^{a}	2.09^{c}	52^{a}	Average
	24h	5.00^{a}	3.80^{a}	1.25^{a}	3.00^{a}	4.57^{b}	3.05^{b}	52^{a}	Average
	Mean	4.79^{B}	3.25^{B}	0.84^{B}	2.76^{B}	8.39^{A}	4.00^{A}	51^{B}	-
May 9 (Heading)	0h	4.24^{b}	8.83^{a}	4.73^{a}	0.67^{a}	0.00^{a}	1.87^{b}	67^{b}	Good
	6h	4.43^{b}	10.20^{a}	4.03^{ab}	0.23^{a}	0.00^{a}	2.59^{ab}	66^{b}	Good
	24h	4.68^{a}	10.07^{a}	3.30^{b}	0.45^{a}	0.00^{a}	3.05a	77^{a}	Good
	Mean	4.45^{C}	9.70^{A}	4.02^{A}	0.83^{C}	0.00^{C}	2.50^{B}	70^{A}	-
May 15 (Blooming)	0h	5.28^{c}	2.15^{a}	5.20^{a}	4.00^{b}	9.00^{a}	0.33^{b}	26^{a}	Fair
	6h	5.78^{b}	2.87^{a}	3.70^{a}	5.53^{a}	6.75^{b}	0.64^{a}	27^{a}	Fair
	24h	6.40^{a}	2.50^{a}	4.60^{a}	4.80^{ab}	0.80^{c}	0.55^{a}	12^{b}	Poor
	Mean	5.82^{A}	2.51^{B}	4.80^{A}	4.78^{A}	5.52^{B}	0.56^{C}	22^{C}	-
Mean	-	5.02	5.15	3.22	2.79	4.65	2.35	48	-
Probability	D	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	-
	W	< .0001	0.3863	0.0546	0.3574	< .0001	< .0001	0.6022	-
	D × W	0.0006	0.0097	0.0529	0.1632	< .0001	< .0001	0.0004	-

LA: lactic acid, AA: acetic acid, PA: propionic acid, BA: butyric acid. D: date, W: wilting. The Flieg's scores (0-100) were ranked into five grades with Poor (0-20), Fair (21-40), Average (41-60), Good (61-80) and Excellent (81-100). Within a column, different superscripts in capital letter mains main plot differ, in small letters means sub plot differ ($P < 0.05$).

4.3.4 Viable count of microbes

Viable count of microbes in this experiment were shown in Table 12. Lactic acid bacteria (LAB) dominate the fermentation during the ensiling process as soon as the anaerobic conditions are established. Some strains of LAB produce bacteriocins which can inhibit the growth of other microbes (Gollop et al., 2005). LAB were elevated by wilting time on all the studied stages in this study. Heading stage showed more viable LAB than other stages ($P < 0.0001$). There was only 3.85 log CFU / g of FM were developed without wilting on April 30 (boot). Fungi and yeast are aerobic microorganisms present in silage that can lead to spoilage during fermentation (Muck, 2010). For fungi and yeast counting, the lowest was found on May 9 (heading), while the highest was on May 15 (blooming), this might due to the less active proteolytic activity in silage on heading stage, so even the LAB number was much acceptable, the silages quality of May 15 were not good because of high fungi and yeast population, which could lead to undesirable fermentation. Population of total microorganism could reflect fermentation quality directly. In this study, silage of May 9 (heading) produced the most microbes, and only 5.63 log CFU / g of FM were produced in the silage of April 30 (boot).

Table 12. Effect of ensiling dates and wilting periods on microbes activities of rye silage.

Date	Wilting	LAB	F&Y	TM
		log CFU / g of FM		
April 30 (Boot)	0h	3.85^{b}	3.95^{c}	4.75^{b}
	6h	6.02^{a}	5.13^{b}	6.06^{a}
	24h	6.03^{a}	5.49^{a}	6.07^{a}
	Mean	5.30^{C}	4.86^{B}	5.63^{C}
May 9 (Heading)	0h	6.44^{c}	5.00^{a}	7.09^{b}
	6h	6.83^{b}	2.92^{b}	6.98^{b}
	24h	7.34^{a}	3.16^{b}	7.92^{a}
	Mean	6.87^{A}	3.70^{C}	7.33^{A}
May 15 (Blooming)	0h	5.97^{c}	5.26^{c}	6.02^{c}
	6h	6.52^{b}	5.95^{b}	6.58^{b}
	24h	7.37^{a}	7.50^{a}	7.62^{a}
	Mean	6.62^{B}	6.24^{A}	6.74^{B}
Mean	-	6.26	4.93	6.57
Probability	D	< .0001	< .0001	< .0001
	W	< .0001	< .0001	0.6022
	D × W	< .0001	< .0001	0.0004

LAB: lactic acid bacteria, F&Y: fungi and yeast, TM: total microorganism, CFU: colony forming units. D: date, W: wilting. Within a column, different superscripts in capital letter mains main plot differ, in small letters means sub plot differ ($P < 0.05$).

4.3.5 β-carotene concentration

As shown in Figure 8., ensiling dates and wilting periods had some regular effects on β-carotene concentration. With the advancing maturity stage of ensiling, β-carotene decreased from April 30 (early boot) to May 15 (blooming stage). Within the same ensiling stage, extension of wilting time also decreased the β-carotene content. Kasangi (2010) mentioned that β-carotene content decreases after silage fermentation, and that was also reported that only less than 20% of β-carotene might be lost in well fermented silage (Nozière et al., 2006). However, in this study, opposite results were observed as β-carotene content of early boot and heading stage after ensiling increased by 80.08 % and 114.51 %, respectively, than that of raw materials. Similarly, increase of β-carotene content after ensiling also were found in some earlier studies (Denter et al., 1981; Angthong et al., 2015; Kwon, 2018). So it maybe possible to synthesize β-carotene during fermentation, but the detail dynamics is not clear, thus further studies are required.

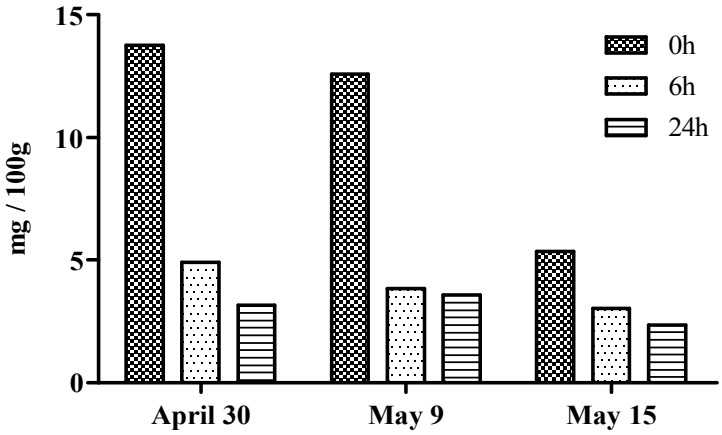

Figure 8. Effect of ensiling dates and wilting on β-carotene concentration.

4.4 Effect of hay-making processing

4.4.1 Temperature change during storage

The temperature fluctuation of rye hay and air were showed as Figure 9. Hay is usually baled at moisture content below 200 g / kg so the activity of plant enzymes are not active, thus, the spontaneous heat and DM loss during preservation are caused by microbial respiration (Hlödversson et al., 1986). Temperature of all the hay made from three stages slightly fluctuated during preservation and air temperature almost kept higher than the three hay. Among the hay made on three harvest dates, hay of May 9 (heading) showed the lowest temperature throughout the preservation, so it could be considered that the most inactive microbial activities occurred on hay from heading stage, which was proved by the viable count of fungi and yeast (Table 13.).

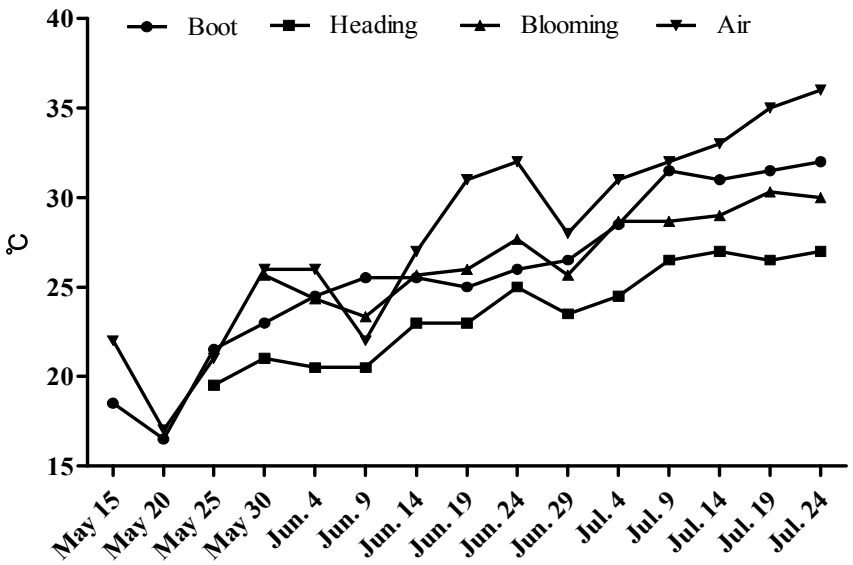

Figure 9. Rye hay and air temperature change during preservation.

4.4.2 Chemical composition and feed value

How to decrease the excessive loss of hay by selecting proper growth stage and maintain the high nutritive value during handling and storage is a great problem. DM loss of hay during preservation is due to the microbial respiration. In this study, DM content were increased by delayed harvest date, as the highest DM was on May 9 (blooming). The delay of harvesting for hay-making decreased DM loss markedly from 167.68 g / kg on April 30 to 17.13 g / kg on May 15. Hay of May 9 (heading) contained higher CP content than the other stages after preservation. ADF and NDF content showed some similar trend as their contents went up with the plant matured when hay were made, the highest and the lowest were on May 15 (blooming) and April 30 (boot), respectively. Consistent result was also found by Mandevu et al. (1999). Conversely, IVDMD content were decreased by the delayed harvest date of rye, hay of May 15 showed the lowest value, as well as TDN. Stone et al. (1960) concluded that with plant matured, digestibility of cool-season grass hays decreased from 63.1 % on vegetative stage to 51.5 % on late bloom stage. The trend of IVDMD in this study was in consistent with the conclusion but the numerical value was higher, it may due to the different physiological condition of experimental cattle. Among the three harvest dates, RFV of hay were significantly different ($P < 0.05$), as progressed maturity of rye hay-making decreased the RFV. Stokes and Prostko (1998) found that RFV decrease sharply with the advancing maturity on alfalfa hay. Generally, microbial activity are inhibited and being low if hays are well-dried. Under the condition where humidity is higher than 70% and temperature is above

20℃, fungi grow significantly in moist hays (Rees, 1982). As demonstrated by Hlödversson and Kaspersson (1986), bacterial populations keep stable within the first 2 or 3 weeks of storage, but the populations of several fungi increase in some moist hays. In this experiment, population of fungi and yeast showed larger amount, 4.91 log CFU / g of FM, on boot stage (April 30) than that of later stages. And there was no statistical difference ($P > 0.05$) of fungi and yeast viable number between rye hay of April 30 and May 15.

Table 13. Effect of hay-making dates on chemical composition and feed value of rye hay.

Date	DM	DM loss	CP	ADF	NDF	IVDMD	TDN	RFV	F&Y
	---------- g / kg ----------						%		log CFU/g
April 30 (Boot)	852.10[b]	167.68[a]	96.50[b]	273.83[c]	514.53[c]	843.20[a]	67.27[a]	122[a]	4.91[a]
May 9 (Heading)	864.60[ab]	37.11[b]	113.75[a]	345.17[b]	601.00[b]	730.43[b]	61.63[b]	96[b]	4.65[b]
May 15 (Blooming)	883.17[a]	17.13[b]	96.30[b]	410.77[a]	675.37[a]	654.33[c]	56.45[c]	78[c]	4.66[b]
Mean	866.62	73.97	102.18	343.26	596.97	742.66	61.78	99	4.74
LSD(0.05)	22.42	33.01	16.32	31.61	23.94	29.00	2.49	6.81	0.23

F&Y: fungi and yeast, Means within a column with different superscripts differ ($P < 0.05$).

4.4.3 β-carotene

With the delay of harvesting, β-carotene content in rye hay decreased from 2.53 to 2.48 mg / 100 g. Compared with raw materials, β-carotene content of hay made from three stages decreased by 66.84%, 57.34% and 10.14% after preservation, respectively. Carter (1960) published that about 80-90% of β-carotene can be lost in hay, which is much higher than the current study, it may because of the different plant species and experimental location. And as Figure 11 showed, hay-making lost more β-carotene than ensiling, so that ensiling is superior to hay-making for β-carotene preservation, as already mentioned by Ballet et al. (2000). For hay-making, harvesting of forage plants at late stages is superior to earlier stages for β-carotene preservation.

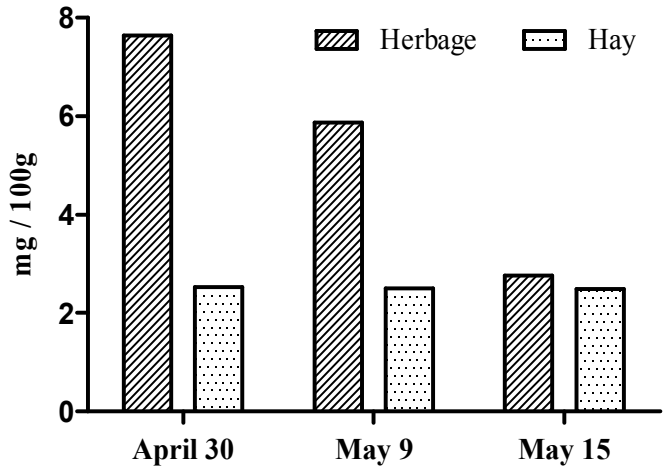

Figure 10. Effect of hay-making date on β-carotene concentration.

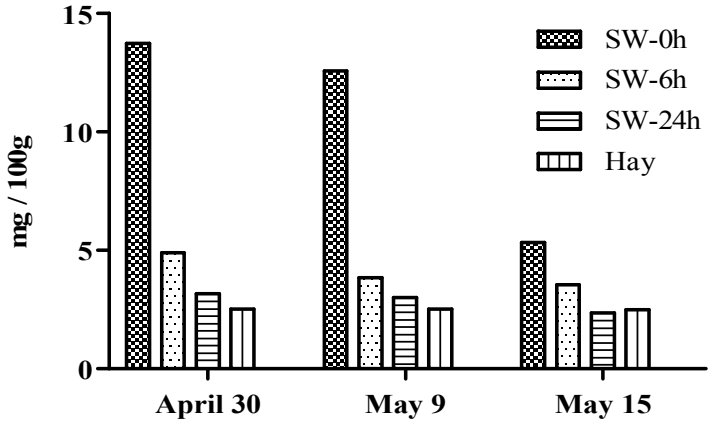

Figure 11. Comparison of β-carotene concentration between rye silage and hay.

SW-0h: silage without wilting, SW-6: silage wilted for 6 hours, SW-24: silage wilted for 24 hours.

5. Conclusion

Several comparisons were conducted in this experiment. DM content, plant height, DM yield and TDN yield increased continuously with the progressed maturity. However, CP content, IVDMD and RFV decreased markedly with the delay of harvesting, while TDN content decreased from April 25 till May 15, then followed by a stable fluctuation. Conversely, ADF and NDF value increased and then fluctuated slightly after blooming stage. For quality of plant parts, stem contained the lowest CP and RFV content, and the highest ADF and NDF content compared with other parts, while the grain showed the higher CP, IVDMD, RFV and lower fiber content than others. With the plant matured, leaf proportion decreased while stem and grain proportion increased, and feed value of all the three parts decreased till blooming stage and followed by a stable phase. β-carotene concentration showed its highest on jointing stage, and then fell down sharply on the sequential stages. In conclusion, harvest around May 15 (blooming) is proper for forage rye if directly consumed by livestock as green chop in Pyeongchang under the consideration of both nutritive yield and forage quality.

For silage, with the delay of harvesting date for ensiling, DM, ADF and NDF content increased, while CP, IVDMD, TDN, RFV and DM loss decreased. Wilting raised the DM content and pH value. Silage made on May 9 (heading) showed lowest pH value, PA, BA concentration and F&Y population, by contraries, the highest LA content, LAB, TM population and also Flieg' s score, which meant the higher fermentative quality than

silages of other stages. Wilting elevated LAB and TM populations but had no regular effect on other fermented products. Both of harvest date and wilting decreased β-carotene concentration. And took all the fermentative characteristics above into consideration, conclusion had been done that ensiled around May 9 (heading) with 24 hours wilting can obtain the highest silage quality for rye in Pyeongchang.

As for hay, delayed harvest date decreased the DM loss, IVDMD, TDN and RFV, but increased the DM, ADF and NDF content. Furthermore, CP content was the highest on May 9 (heading) and F&Y population on May 9 and May 15 were significantly less than April 25 (boot). β-carotene decreased with plant matured when hay-making were conducted. Consequently, to get lower DM loss and higher quality, make hay around May 9 (heading) for rye in Pyeongchang was recommended.

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7. 요약

평창 지역에서 호밀이 다양한 요인에 의해 사료가치와 β-carotene 함량이 어떻게 영향을 받는지에 대한 정보는 많지 않다. 그래서 본 시험은 수확시기와 조제방법이 호밀의 사료가치와 β-carotene 함량에 미치는 영향을 구명하고자 수행되었다. 호밀 시료는 4 월 25 일부터 5 월 31 일까지 매 5 일간 수확하여 수집 되었으며 건초와 사일리지는 3 번의 생육단계 (수잉기, 출수기 및 개화기) 에서 수확하였고, 사일리지는 예건 기간(무처리, 6 시간 및 24 시간) 을 달리하였다. 수확시기에 대한 효과에서 숙기가 진행됨에 따라 건물함량, 초장, 건물수량 및 TDN 수량은 증가하였으나 조단백질 함량, IVDMD 와 RFV 는 유의적으로 감소하였다. 호밀이 성숙함에 따라 모든 식물체부위 (잎, 줄 및 곡실) 에서 사료가치가 감소하였고 줄기의 사료가치가 가장 낮았다. β-carotene 함량은 호밀의 생육이 진행됨에 따라 감소하였다. 따라서 더 높은 수량과 품질을 위해서는 개화기 전후에 수확하는 것이 가장 적절하였다.

사일리지에 있어서는 숙기가 진행됨에 따라 건물함량, ADF 및 NDF 함량은 증가하는 반면 CP, IVDMD, TDN, RFV 및 건물 손실은 유의적으로 감소하였다 ($P<0.0001$). 예건은 건물함량과 pH 값을 유의적으로 증가시켰다 ($P<0.0001$). 출수기에 수확된 사일리지의 pH (4.45), propionic acid (0.83 g/kg DM), butyric acid (BA) 함량 (0 g/kg DM), 곰팡이와 이스트 수 (3.70 log CFU / g of FM) 가 가장 낮았으며, 반대로 lactic acid (LA) (9.7 g/kg DM) 함량, lactic acid bacteria (LAB) 수 (6.87 log CFU / g of FM), 총 미생물수 (TM) (7.33

log CFU / g of FM) 및 Flieg 점수 (70) 는 가장 높았다 ($P < 0.0001$). 예건은 젖산균과 총 미생물수를 증가시켰으나 다른 발효산물에는 일정한 경향을 나타내지 않았다. 진행된 숙기 및 예건시간은 β-carotene 함량을 감소시켰다. 이상의 결과를 종합하여 볼 때 평창지역에서의 호밀 사일리지는 5 월 9 일 (출수기) 경에 수확하여 24 시간 예건하여 조제하는 것이 가장 바람직하다.

건초에 있어는 수확이 지연됨에 따라 건물손실, IVDMD, TDN 및 RFV가 감소하였으며 DM, ADF 및 NDF 함량은 증가되었다 ($P<0.05$). β-carotene 함량은 건초 조제가 지연됨에 따라 감소하였다. 따라서, 낮은 건물손실과 고품질의 건초를 생산하려면 5월 9일 (출수기) 에 수확하는 것이 추천되었다.

주요어: 호밀, 수확시기, 예건, 사료가치, 베타카로틴 함량

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