Master's Thesis of Science in Agriculture

# Effect of Forage Quality on Methane Production and Ruminal Fermentation Characteristics of Cattle

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February 2018

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# Effect of Forage Quality on Methane Production and Ruminal Fermentation Characteristics of Cattle

A thesis
submitted in partial fulfillment of the requirements to the faculty
of Graduate School of International Agricultural Technology
for the Degree of Master of Science in Agriculture

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December 2017

Approved as a qualified thesis
for the Degree of Master of Science in Agriculture
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# Abstract

# Effect of Forage Quality on Methane Production and Ruminal Fermentation Characteristics of Cattle

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The purpose of two experiments was to determine $CH_4$ production and ruminal characteristics of different quality forages fed to cattle. In experiment 1, four Holstein Friesian steers (initial body weight, 237 ± 31 kg) were used in a duplicated 2 x 2 Latin square design. Cattle were fed either timothy hay (Ti) ad libitum or rice straw hay (RS) ad libitum. The average daily OM, NDF and CP intakes (kg DM) for cattle fed Ti were 105 (P = 0.001), 102 (P = 0.002) and 221% (P = 0.001) higher than for cattle fed RS, respectively. Apparent OM and NDF digestibility were not different significantly between forages. Cattle fed Ti produced 85% more $CH_4$ (g/d) than cattle fed RS (54.6 and 29.5, respectively; P = 0.081). When $CH_4$ was eressed as per kg DMI and OMI, there were no significances between diets, however, cattle fed RS emitted significantly more $CH_4$ than Ti, based on kg digested OMI (dOMI) (P = <0.0001) and digested NDFI

(dDNFI) (P = 0.004). Total VFAs of cattle fed two forages were not different, but propionate proportion of VFA was significantly higher (P <.0001) for cattle fed RS, followed by lowered $C_2/C_3$ ratio (P = 0.000). Higher $NH_3$-N concentration was observed (P = 0.002) in rumen fluid of cattle fed Ti than RS.

In experiment 2, four Hanwoo (Korean native beef cattle) steers (initial body weight, 374 ± 40 kg) were used in a duplicated 2 x 2 Latin square design. Cattle were fed either lacerated RG hay ad libitum plus 3.2 kg of concentrate/d as fed basis or lacerated Italian ryegrass silage (IRG-s) ad libitum plus 3.2 kg of concentrate/d as fed basis. The average daily OM, NDF and CP intakes for cattle fed IRG-s were 10% (P = 0.086), 18% (P = 0.059) and 31% (P = 0.038) higher than for cattle fed RS, respectively. Indirect estimates of apparent OM and NDF digestibility of IRG-s (67.4 and 63.5%, respectively) were higher (P = 0.009) than those of RS (61.4 and 55.7%, respectively). Daily $CH_4$ production and per kg DMI, OMI and dOMI for cattle fed IRG-s were not significantly different compared to cattle fed RS. Methane production per kg dNDFI for cattle fed RS showed higher trend (P = 0.071) than that for cattle fed IRG-s. Acetate proportion of total VFA was significantly higher (P = 0.000) for cattle fed IRG-s, while butyrate proportion was significantly higher (P <.0001) for cattle fed RS. However, there was no differences in total VFA and $C_2/C_3$ between treatments. Proportions of isobutyrate and isovalerate were higher (P = 0.003 and P = 0.012, respectively) for cattle fed IRG-s than RS. We concluded that, when there is enough quality difference between forages, high quality forage is expected to have methane mitigation potential in the aspect of dOMI or dNDFI.

**Keywords :** forage quality, Italian ryegrass, methane, rice straw, ruminal fermentation, timothy

***Student Number*:** 2016-20012

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# List of Abbreviations and Formula

**ADF**- Acid detergent fiber

**ADFI-** Acid detergent fiber intake

**$C_2/C_3$ ratio-** Acetate to propionate ratio

**CP-** Crude protein

**CPI-** Crude protein intake

**DM-** Dry matter

**DMI-** Dry matter intake

**dNDFI-** digested neutral detergent fiber intake

**dOMI-** digested organic matter intake

**EE-** Ether extract

**IACUC-** Institutional Animal Care and Use Committee

**IRG-s-** Italian ryegrass silage

**IVDMD-** *In vitro* dry matter digestibility

**IVOMD-** *In vitro* organic matter digestibility

**MCR-** Methane conversion rate

**N-** Nitrogen

**NDF-** Neutral detergent fiber

**NDFI-** Neutral detergent fiber intake

**OM-** Organic matter

**OMI-** Organic matter intake

**RS-** Rice straw hay

**SAS-** Statistical analysis software

**Ti-** Timothy hay

**VFA-** Volatile fatty acids

**$CH_4$-** Methane

**$CO_2$-** Carbon dioxide

**$NH_3$-** Ammonia

**$Cr_2O_3$-** Chromium oxide

# 1. Introduction

According to FAO (2013), ruminants in the world were the most anthropogenic $CH_4$ producing source, and cattle annually emitted 4.6 Gt $CO_2$eq. of total ruminants (5.7 Gt $CO_2$eq.). On the other hand, total greenhouse gas (GHG) emission of Korea in 2014 was 690.6 Mt $CO_2$eq., and agricultural sector contributed 3.1 % (21.3 Mt $CO_2$eq.) of all GHG. Livestock sector produced 8.8 Mt $CO_2$eq. of GHG, and 47.7 % (4.2 Mt $CO_2$eq.) came from enteric fermentation. Dairy cattle and beef cattle including Hanwoo (Korean native beef cattle) mainly contributed to approximately 91.6% (3.8 Mt $CO_2$eq.) of $CH_4$ from enteric fermentation (National Greenhouse Gas Inventory Report of Korea, 2016).

According to Rural development administration (RDA, 2014) in Korea, 588 Mt of forages was supplied in 2014, of which 528 Mt was domestically produced and 60 Mt was imported. Domestic forages consisted of forage crop such as Italian ryegrass, rice straw and pasture, and each items accounted for 57.4 % (303 Mt), 37.7 % (199 Mt) and 4.9 % (26 Mt), respectively. Rice straw is well known for its lower nutritive value than not only imported grass hay such as timothy or tall fescue (Kim, 2006) but also domestically produced Italian ryegrass silage in round bale (Ki et al., 2017). However, rice straw is still an important roughage source for chewing activity during the late fattening stage of Hanwoo steers from 21 to 29 months old as well as reproduction cows.

In order to reduce $CH_4$ emissions from enteric fermentation of ruminant, Korea government have established a policy for substituting low quality forage with high quality forage and increasing the ratio of forage to concentrates from 4:6 to 6:4 (The Republic of Korea, 2013). There is general agreement that high quality forages are highly palatable and more digestible, which leads to faster passage rate, higher intake and therefore higher daily $CH_4$ production. However, when expressed in relation to digested nutrients, replacing low quality forage with high quality forage is expected to have lower methane production, which is attributable to higher digestibility of high quality forage compared with low quality forage (Kurihara et al., 1999; Boadi and Wittenberg, 2001). However, this finding is inconsistent with Pinares-Patiño et al. (2003) and Molano and Clark (2008) who reported that $CH_4$ production was not related to diet chemical composition or digested OM. These controversial observations lead researchers to suggest that a wider range of forage with different quality should be studied before concluding the relationship between forage quality and $CH_4$ production.

To estimate $CH_4$ emission from cattle fed different quality forages, we measured *in vivo* $CH_4$ production based on different nutrient units (DMI, OMI and Digested OMI) between high and low forage. In experiment (Exp.) 1, timothy hay (Ti) imported from U.S considered as relatively high quality forage was compared with rice straw hay (RS) as a main diet. In Exp. 2, Italian ryegrass silage (IRG-s) was compared with RS, both were supplemented with concentrates to meet the maintenance requirements.

# 2. Literature Review

## 2. 1. Necessity of methane mitigation from ruminants in the productivity and environmental aspects

Microbes in the rumen ferment organic matters of feed consumed by ruminants. This process produces free hydrogens, which have a negative effect on the rumen fermentation. In order to prevent this, particular microbe species (represented by methanogen) working as hydrogen scavengers consume free hydrogens, but produce methane as the end product. The ruminant losses 2-15% of energy as methane from the energy it consumed (Johnson et al., 2002). Therefore, many studies have focused on methane reduction from the ruminant to improve feed efficiency.

Besides the productivity aspect, as concern about global warming has been grown internationally, methane has gotten much more attention since methane consists of the largest proportion of non-$CO_2$ greenhouse gases and it has a much shorter lifetime compared to $CO_2$. Since the livestock sector contributes approximately 14.5% of all greenhouse gases (7.1 of 49 Gt $CO_2$eq. year) and enteric fermentation accounts for 39% in this sector (FAO, 2013), it is important to reduce methane production from the ruminant in the environmental aspect as well.

## 2. 2. Factors affecting to the forage quality

The ruminant can degrade the plant cell wall with rumen microbes' assistance and feed for the ruminant could be broadly divided up into the concentrate and forage. Commonly, the concentrate feeds with a high nutrition of several ingredients is commercially saleable for livestock farms. Thus, the fomulation of concentrate is designed to not only offer high productivity, but also uniform product quality. However, forage quality varies greatly since many factors influence forage quality (Ball et al., 2001). The most important factors are plant family, species, maturity, and ratio of leaves and stems. Most plants used as forages belong to two different families, which are grass (Poaceae) and legume (Fabaceae). It is well known that legumes have higher CP content and less fiber composition compared to grasses, therefore easily digestible. This is supported by the study of Durmic et al. (2017), where N concentration of 22 legume species was higher than 23 grass species (24.6 vs. 6.2 g per kg DM), however, grasses consistently had greater NDF and ADF concentrations than legumes. Different species of forage shows a clear distinction of its chemical composition and, within same species, its chemical characteristics have a big difference according to maturity of forages. Compared to fully matured one, forage in their early stage of growth has better nutritional value (Ball et al., 2001). Forage quality also depends on leaf: stem ratio of the plants. In most cases, forages are processed chemically for long-term preservation or improvement of nutritional value.

## 2. 3. Relations between forage IVDMD (or IVOMD) and *in vitro* gas characteristics

Since there are various factors that affect to the forage quality, it is hard to define whether forage has high or low quality. However, among several factors, digestibility is relatively easy to be analyzed by *in vitro* method and it integrally well reflects forage quality. Thus, if the relation between methane production and forage digestibility is established, it is very helpful to set a methane mitigation strategy.

### 2. 3. 1. Total gas production

Characteristics of forages on gas and methane production can be examined by a relatively simple *in vitro* technique. Based on several studies (Jayanegara et al., 2011; Purcell et al., 2011; Meale et al., 2012; Ribeiro et al., 2014), forages which have higher IVDMD (or IVOMD) tend to produce higher total gas *in vitro*. In the study of

Jayanegara et al. (2011), among samples from 27 plants including grass, herb, shrub and tree species, six species which had higher IVOMD (over 69.5%) showed higher total gas production (over 35.1 ml) than other 20 plants except for one sample (*Paspalum dilatatum*). Meale et al. (2012) reported an *in vitro* rumen batch culture study of three grasses, five leguminous shrubs and four non-leguminous shrubs. In this study, forages had higher IVDMD produced more total gas within the same forage families. Ribeiro et al. (2014) studied *Andropogon gayanus* grass harvested at different maturities (56, 84, 112 and 140 days) preserved as hay and silage. With increasing grass maturity, IVDMD decreased linearly, and the maximum potential of gas production linearly decreased in both hay and silage in this study. Purcell et al. (2011) did the same concept of study with perennial ryegrass samples collected at 5 stages of primary growth (12 May, 26 May, 9 June, 23 June, 7 July), and reported the same results with Ribeiro et al. (2014).

### 2. 3. 2. $CH_4$ production per DM incubated

It is well known that forages which have higher gas production and IVDMD tend to produce higher $CH_4$ per DM incubated (Chaves et al., 2006; Jayanegara et al., 2011; Purcell et al., 2011). The six plants, which had higher IVOMD and total gas production in the study of Jayanegara et al (2011) showed higher $CH_4$ production (ml) than the others excluding the same exceptional plant. With increasing IVDMD and total gas production, samples collected in the study of Purcell et al. (2011) linearly produced more $CH_4$ per g DM incubated. Chaves et al. (2006) compared alfalfa and grass sampled from the three different regions. Average IVDMD of alfalfa and grass were 58.6% and 52.8%, respectively and $CH_4$ production per kg DM incubated of alfalfa was significantly higher than that of grass (16.7 vs 13.5 g, respectively).

### 2. 3. 3. $CH_4$ production per digested DM

Although OM in feed having higher IVDMD are easily fermented compared to that having lower IVDMD and it causes high gas and $CH_4$ production per DM incubated,

$CH_4$ production per digested DM did not follow the same trend (Chaves et al., 2006; Purcell., 2011; Meale et al., 2012; Navarro-Villa et al., 2012). In the study of Chaves et al. (2006), $CH_4$ production per DM incubated and digested DM of alfalfa was significantly higher than that of grass. In the study of Purcell et al. (2011), total gas production and $CH_4$ per g DM incubated increased linearly with increasing IVDMD, while $CH_4$ per g digested DM decreased linearly. Navarro-Villa et al. (2012) investigated three types of perennial ryegrass herbages designed to have different extents of fermentation and clostridial activity. In this study, $CH_4$ production per g digested DM had no significant difference among herbages, while total gas and $CH_4$ production per g DM incubated increased significantly with increasing IVDMD.

### 2. 3. 4. Relationship between forage quality and ruminal fermentation

Jayanegara et al. (2011), with the result of *in vitro* incubation of 27 plants species, conducted principal component analysis (PCA). The PCA result closely clustered IVOMD, total VFA, CP, $NH_3$, branched-chain VFA ($C_4$, $C_5$) and $C_3$, and the author considered this cluster as the characteristics of good quality forages. This cluster had a negative relation with a cluster including NDF, ADF, lignin, $C_2$ and $C_2/C_3$. From this point of view, high quality forages are expected to have higher IVDMD and CP, and lower NDF and ADF, which is generally agreed.

It is widely accepted that the type of carbohydrate present in the forage rules $CH_4$ production because it impacts on ruminal pH and the microbial population. (Johnson and Johnson, 1995). High quality forages composed high proportion of soluble carbohydrate are expected to promote the production of propionate in the rumen (Russell, 1998), lower ruminal pH and inhibit methanogen growth (Van Kessel and Russell, 1996). For the same reason, low quality forages containing high portion of cell wall produce relatively less propionate, which makes more hydrogen available finally utilized by methanogens for $CH_4$ formation. Consequently, high quality forages are expected to produce lower $CH_4$ production per digested DM *in vitro*, even though some studies did not satisfy this expectation.

## 2. 4. Relationship between forage quality and *in vivo* methane production

### 2. 4. 1. General relationship between DMI and $CH_4$ production

Shibata et al. (1992) reported that DMI and $CH_4$ production could be explained by linear regression with high coefficient of determination ($r^2$) when DMI was below 1.5, and between 1.5 and 2.5 maintenance level ($r^2$ = 0.899 and 0.928, respectively). The author mentioned that this may be attributed to increased concentrate proportion that promote a high propionate and decreased mean retention time with high levels of feeding. However, when DMI was over 2.5 maintenance level, $CH_4$ production could not be explained by DMI ($r^2$ = 0.016) and they obtained highly significant regression equation expressed as a quadratic form ($r^2$ = 0.934). Kurihara et al. (2001) investigated $CH_4$ production of cattle fed two tropical forage diets and high concentrate diets. In case of the two tropical forage diets, DMI was a major determination of variations in $CH_4$ emissions ($r^2$ = 0.981), however, DMI and $CH_4$ production had no significant relationship (P = 0.09) with high concentrate diets.

### 2. 4. 2. Relationship between DMI and $CH_4$ production with respect to forage quality

There is a lack of studies investigating the effect of forage quality on $CH_4$ production *in vivo*. Searchable studies (Moss et al., 1994; Kurihara et al., 1998; Boadi & Wittenberg., 2001; Hart et al., 2009; Muñoz et al., 2016; Pesta et al., 2016) indicated that DMI of cattle fed high quality forages were significantly higher than those fed low quality forages. This may be attributed to increased passage rate of high quality forage due to its high digestibility. In these experiments, $CH_4$ production were increased in proportion to the increase in DMI. One exceptional study (Muñoz et al., 2016) revealed almost same amount of $CH_4$ production (323 and 321 g per day, respectively) from dairy cattle fed high and low quality forages (15.5 and 13.9 kg intake per day, respectively). When expressed per kg DMI, this study showed that $CH_4$ production of cattle fed high quality forage was lower than cattle fed low quality forage (21.3 and 23.2 g per kg DMI, respectively; $P = 0.05$). Compared with other studies, extra 1.8 kg of concentrate per day was offered to every cattle in this study.

### 2. 4. 3. Relationship between digested OMI (or DMI) and $CH_4$ production with respect to forage quality

When $CH_4$ was expressed as digested OMI or digested DMI, animals fed high quality forages emitted less $CH_4$ than low quality forages (Moss et al., 1994; Kurihara et al., 1998; Boadi & Wittenberg., 2001; Muñoz et al., 2016), but the study of Hart et al. (2009) showed no significant difference (29.3 and 32.1 g per kg DDMI, respectively; $P = 0.24$). Pesta et al. (2016) also represented that steers fed diet containing high quality forage showed numerically higher value compared to diet containing low quality forage (16.1 and 14.9 liter per lb dOMI, respectively). In this report, the diet with lower nutritional value had higher digestibility of OM (with no significance), NDF and ADF (with significance) and the author mentioned that digestibility for low quality forage was greater than anticipated.

## 2. 5. Forage quality and $CH_4$ mitigation strategy

Kurihara et al. (1998) reported that cattle fed Rhodes grass (high quality forage) gained 500g of live weight per day, whereas cattle fed Angleton grass (low quality forage) experienced loss of live-weight. Muñoz et al. (2016) stated cattle grazed high quality pasture produced less $CH_4$ per kg of milk yield ($P = 0.05$), and tended to produce less $CH_4$ per kg of ECM (energy corrected milk) yield ($P = 0.07$), compared to cattle grazed low quality pasture. In conclusion, feeding high quality forages instead of low quality forages can be one of methane mitigation strategy since less amount of $CH_4$ would be produced to meet the same demand of animal products, or it is possible to offer more animal products with emitting equal amount of $CH_4$.

The hypothesis was that feeding high quality forages could be a methane mitigation strategy in cattle rearing condition in Korea. Therefore, we estimated $CH_4$ production of cattle fed 1) Rice straw hay (RS) and Timothy hay (Ti) alone, 2) Rice straw hay with concentrate and Italian ryegrass silage (IRG-s) with concentrate. In both studies, RS was used as control feed since it is generally considered as typical low quality forage of Korea.

# 3. Materials and Methods

The *in vivo* experiments and following analyses were conducted at Animal Farm and Laboratory of Animal Metabolism in Seoul National University, Pyeongchang, Republic of Korea. All animals used in this study were cared in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Seoul National University, Republic of Korea. The concentrate and forages were purchased from a domestic feed company and domestic farms, respectively. All chemicals were obtained from Sigma-Aldrich Co. (St. Louis, MO).

## 3. 1. Animals and Experimental Design

Both experiments (1 & 2) were conducted as a duplicated 2 x 2 Latin square design. Four Holstein Friesian steers (initial body weight, 237 ± 31 kg) were used in Exp. 1, and four Bos Taurus coreanae (Hanwoo, Korean native beef cattle) steers (initial body weight, 374 ± 40 kg) were used in Exp. 2.

Exp. 1 consisted of two consecutive periods of 24 days and 14 days were used for diet adaptation. The rest 10 days of experiments include 2 days of $CH_4$ measurement, 4 days of digestibility analysis and 1 day for rumen fluid sampling. In Exp. 1, cattle were placed in metabolism cages throughout the whole experiment.

In Exp. 2, cattle were equally divided into two pens equipped with calan doors, and cattle located in the same pen had the same diet in order to eliminate feed competition within the same pen. The experiment was divided into two periods of 28 days including 21 days for diet adaptation in the pen, each 2 days of $CH_4$ and digestibility measurements and 1 day for rumen fluid sampling. Cattle were located in metabolism cages after diet adaptation. Body weight was measured at the start and the end of each period of Exp.

## 3. 2. Experimental Diets and Feeding

In Exp. 1, RS and Ti were fed to cattle to investigate the effects of low and high quality forages on $CH_4$ production. Rice straw locally cultivated was purchased from domestic farm and Ti was imported from U.S.. Forages were fed ad libitum without concentrate supplement. In Exp. 2, RS and IRG-s were chosen since these two forages are commonly cultivated locally and used for feeding Hanwoo in Korea. Rice straw and Italian ryegrass were harvested on May and October in 2016, immediately processed to make RS and IRG-s. Before fed to animals, both RS and IRG-s were lacerated by the TMR mixer and then fed ad libitum with 3.2 kg of concentrate/d, as fed basis, to meet the maintenance requirement and to prevent possible weight loss of animals. The concentrate was offered 20 min earlier than forages for cattle to consume the entire quantity offered. Experimental forage and concentrate were fed to animals twice a day in equal amount at 0900 and 1800 h.

In both experiments, animals were fed 110 % of forage intake of the previous day and residual amount of forage were recorded every day. Animals can freely access to water and mineral block for 24 hours throughout the experimental period. Feed samples were collected every week and kept in the cold store for dry matter calculation and chemical analyzing.

**Table 1. Ingredient composition of concentrate**

| Ingredient Composition (%) | |
|---|---|
| Broken corn | 6.11 |
| Wheat | 7.00 |
| Wheat bran | 10.50 |
| Rice bran | 9.80 |
| Salt | 0.36 |
| Molasses | 3.80 |
| Ammonium chloride | 0.50 |
| Urea | 0.46 |
| Alfalfa | 5.00 |
| DDGS[a] | 15.00 |
| Soybean hull | 5.82 |
| Amaferm | 0.05 |
| Corn gluten meal | 19.92 |
| Limestone | 2.48 |
| Palm kernel meal | 13.00 |
| Mineral-vitamin mixture[b] | 0.20 |
| Total | 100.00 |

[a] Dried Distrillers Grains with Solubles

[b] Provided following nutrients per kg of additive (Grobic-DC, Bayer Health Care, Leverkusen, Germany): Vit. A, 2,650,000 IU; Vit. $D_3$, 530,000 IU; Vit. E, 1,050 IU; Niacin, 10,000 mg; Mn, 4,400 mg; Zn, 4,400 mg; Fe, 13,200 mg; Cu, 2,200 mg; I, 440 mg; Co, 440 mg

## 3. 3. Methane Emissions Measurement

Animals were placed in four chambers for $CH_4$ emission measurement on days 14 – 15 of Exp.1 and on days 22 – 25 days of Exp. 2. In Exp. 1, $CH_4$ was directly measured after the adaptation periods since animals were placed in chambers from the start. However, first two days of Exp. 2 were spent for adaptation of animals to chambers and actual gas sampling was performed following two days. Adaptation period was settled to prevent inaccurate results, which could occur by the decrease in feed intake.

In 2nd period of both experiments, animals occupied the same chamber where they had been located in order to do more clear comparison between periods. The indirect open circuit respiratory chamber (137 cm wide x 256 cm deep x 200 cm tall) was made up of steel frame and polycarbonate sheet and equipped with feeder, waterer, air conditioner (Busung Co. Ltd., India, model ALFFIZ-WBCAI-015H) and dehumidifier (Dryer Korea, model DK-C-150E). During gas sampling period, temperature and humidity was maintained at 18 ℃ and 50 %, respectively. A flow meter (Teledyne Technologies Inc., USA, model LS-3D) was used to flow gases into a gas analysis system at a constant rate for sampling period. The gas analysis system was composed of gas sampling pump (B.S technolab INC., Korea), tunable diode LASER $CH_4$ gas analyzer (KINSCO Technology. Co. Ltd., Korea, model Airwell+7), data acquisition and analysis unit. This system was calibrated before beginning of the Exp. with measurements of chamber recovery rate using a standard calibration $CH_4$ gas mixture (Air Korea, 25% mol/mol). Recovery rate was measured again after the Exp. to identify any error occurred during analyzing. $CH_4$ concentration inside and outside of the chamber was recorded every 14 min in the system which is summed to estimate the 24-h emission value. This was corrected again based on the recovery rate of each chamber to get final $CH_4$ emission value.

### 3. 4. Digestion Trial and Rumen Sampling

In Exp. 1, apparent digestibility was measured by total collection method. Feces were scraped into stainless steel boxes, which was weighed in advance, on days 18 – 21. Animals were equipped with urine collector to prevent mixture of urine into feces. Boxes containing feces were weighed every 24 hours and total weights of feces were estimated. Based on the weight, 10 % of the total feces was sampled every day and four-day samples were thoroughly mixed. Then, 10 % of feces was sampled again and frozen at -20℃ for further analysis.

In Exp. 2, indirect estimates of apparent digestibility were measured to study the effect of forage quality using chromic oxide ($Cr_2O_3$) as an external marker. Twice a day, 8g of chromic oxide was mixed with concentrate, which was offered on top of roughage and there was no residual of concentrate throughout the experiment. Fecal samples were collected on days 26 – 27 directly from the rectum of each cattle. In order to reduce fluctuation of Cr concentration, 8 times of sample collection were fixed which were 8:30, 10:30, 13:30, 16:30 on a day 26; 8:30, 12:00, 15:00, 18:00 on a day 27. Collected samples were frozen at -20℃ for further analysis.

In both experiments, rumen fluid was sampled three times, before feeding and after 1.5 and 3 hours after feeding using a collection tube and filtered by 4 layers of cheese cloth. pH was measured using a pH meter (model AG 8603; Seven Easy pH, Mettler-Toledo, Schwerzenbach, Switzerland) immediately after filtering and stored at -20℃ for analyzing ammonia ($NH_3$)-N and volatile fatty acid.

## 3. 5. Chemical Analysis

Feed and feces samples were placed in convection dry oven (model VS -1202D9, Vision Scientific Co., Ltd., Korea) at 65℃ for 48 hours and ground to pass through a 1mm sieve (Thomas Scientific Model 4, New Jersey, USA). Crude protein (CP), ash and Cr were analyzed by the same method explained in AOAC (Method 990.03, 942.05 and 990.09, respectively; AOAC International, 2007). The neutral detergent fiber (NDF) content was estimated using the method of Van Soest (1991) and contents of acid detergent fiber (ADF) was determined according to Van Soest (1973). The bomb calorimeter (Shimzdzu CA-3, shimadzu corporation, Japan) was used to estimate the gross energy (GE) of feed and feces.

Gas chromatography equipped with flame ionization detector and a FFAP CB column (25 m x 0.32mm, 0.3 µm, Agilent Technologies, Santa Clara, CA, USA) was used to measure volatile fatty acids. In the pre-treatment step, a 5.0 mL aliquot of rumen fluid was mixed with 0.05 mL saturated $HgCl_2$, 1.0 mL 25% $HPO_3$ and 0.2 mL 2% pivalic acid (Erwin et al. 1961). Concentration of $NH_3$-N was measured by a modified colorimetric method (Chaney and Marbach, 1962).

## 3. 6. Statistical Analysis

The data for feed intake, diet digestibility and $CH_4$ emissions were analyzed using SAS PROC MIXED (version 9.4). The pH, ammonia-N, and volatile fatty acid were analyzed as repeated measures assuming an AR(1) covariance structure (Littell et al., 1998). Experimental diets were considered as a fixed effect. Replication, animals nested within replication, and period nested within replication were considered as a random effect. Statistical differences were considered significance at $P < 0.05$ and a trend at $0.05 \leq P < 0.10$.

# 4. Results and Discussions

## 4. 1. Experiment 1. Rice straw hay vs. Timothy hay

### 4. 1. 1. Chemical composition of diets

Dry matter (g/kg) of Ti and RS were 929 and 795, respectively (Table 7). RS showed almost same DM value between Exp. 1 and 2. There was 8.1% OM difference between two forages in Exp. 1 since RS had higher ash content (g/kg DM) than Ti (142 and 61, respectively). Although it was expected that RS would have higher NDF and ADF concentration than those for Ti but Ti showed 5.3% and 4.2% higher NDF and ADF, respectively. In addition, there was only 2.8% of difference in CP content between two forages. When non fiber carbohydrate (NFC) was calculated as followed: NFC = 100 – (NDF + CP + EE + ash ), Ti and RS had almost same NFC content (127 and 128 g per kg DM, respectively).

We did not find remarkable differences in overall chemical composition between RS and Ti. The quality of forage varies with management, season, maturity stage, leaf to stem ratio and fertilization, and quality loss can occur during curing for hay making due to plant respiration, rainfall and curing damage (Ball et al., 2001). Collins (1988) reported that timothy leaf had 3.2 times as much CP as stem (29 and 9 g/kg DM, respectively) and nearly 1.5 times NDF concentration of stem (491 and 725 g/kg DM, respectively). These data support that timothy in this experiment had either quality loss during curing or higher proportion of stem.

**Table 2. Chemical composition of rice straw hay (RS) and timothy hay (Ti)**

| | RS | Ti |
|---|---|---|
| Dry matter (g/kg) | 795 | 929 |
| Organic matter (g/kg DM) | 858 | 939 |
| Neutral-detergent fiber (g/kg DM) | 646 | 699 |
| Acid-detergent fiber (g/kg DM) | 381 | 423 |
| Crude protein (g/kg DM) | 40 | 68 |
| Ether extract (g/kg DM) | 44 | 45 |
| Ash (g/kg DM) | 142 | 61 |
| Gross energy (cal/g DM) | 3924 | 4430 |

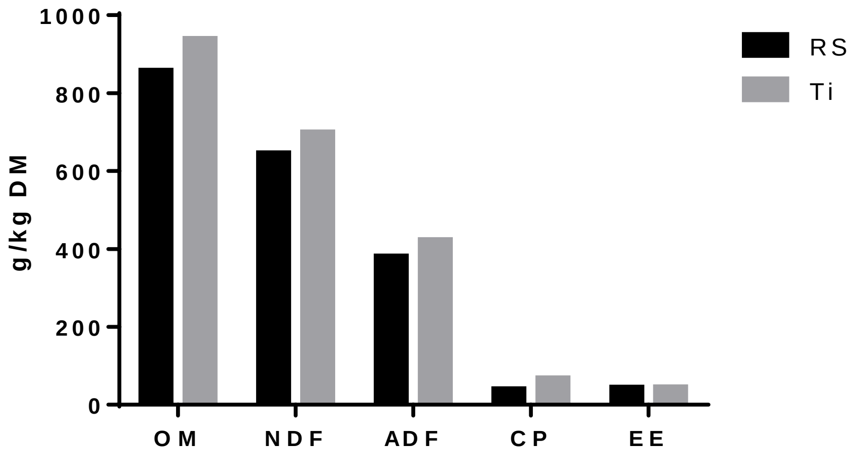



**Figure 1. Chemical composition of rice straw hay (RS) and timothy hay (Ti)**

### 4. 1. 2. Nutrient intake and digestibility

In Exp. 1, experimental animals were supplied either RS or Ti without concentrate supplement to investigate the effect of sole forage quality on methane production. Table 3 showed that DM and nutrient intakes amounts of timothy were more than two times of those of RS ($P < 0.01$), and CP intake was three time more than RS ($P = 0.001$). Dry matter intake of Ti for Holstein steers was to meet around 0.8 kg daily gain but DMI of RS was insufficient to meet the maintenance energy.

In general, the higher the quality and palatability, the more forage will be consumed (Ball et al., 2001). The poor quality forage remained in rumen for longer time, causing low nutrient intake and animal performance (Baumont, 1996). Likewise, high nutrient intake of good quality forage resulted in increased fermentable substrate including both structural and non-structural carbohydrates (Moe and Tyrrell, 1979), which can be explained by the decrease in the mean retention time (Thornton and Minson, 1972). Nutrients intake of steers in this Exp. indicated that Ti was highly palatable rather than RS even though both forages showed similar chemical composition.

**Table 3. Nutrient intake of cattle fed rice straw hay (RS) and timothy hay (Ti)**

| | RS | Ti | SEM | P-value |
|---|---|---|---|---|
| Dry matter (kg) | $2.56^{b}$ | $4.79^{a}$ | 0.208 | 0.002 |
| Organic matter (kg) | $2.20^{b}$ | $4.50^{a}$ | 0.201 | 0.001 |
| Neutral-detergent fiber (kg) | $1.65^{b}$ | $3.34^{a}$ | 0.149 | 0.002 |
| Acid-detergent fiber (kg) | $0.976^{b}$ | $2.02^{a}$ | 0.091 | 0.001 |
| Crude protein (kg) | $0.102^{b}$ | $0.327^{a}$ | 0.017 | 0.001 |

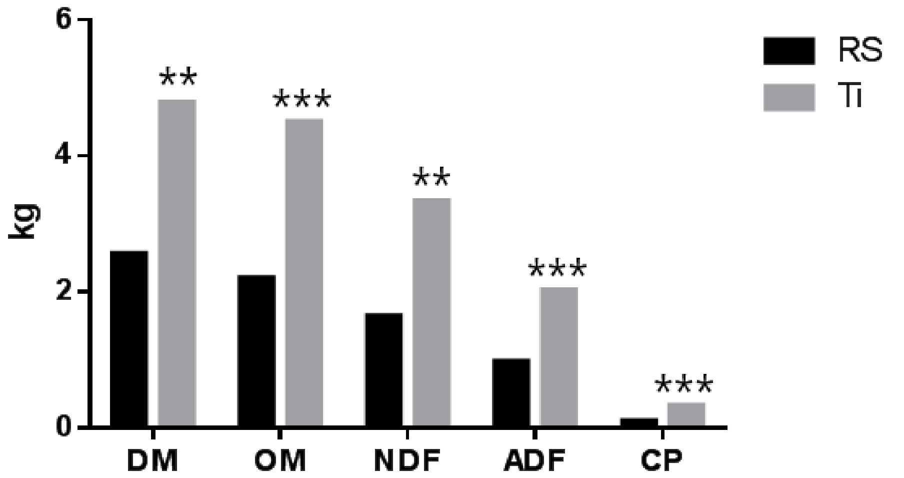



**Figure 2. Daily nutrient intake of cattle fed rice straw hay (RS) and timothy hay (Ti)**

Dry matter digestibility of two diets showed 10 % difference (P < 0.001) (Table 4), it may be attributed to the difference in ash content. It was supported by the results that there were no significant differences of OM and NDF digestibility after correction for ash content between two forages. Another explanation for similar OM digestibility between RS and Ti can be found in undernutrition of steers fed RS. Chilliard et al. (1998) described the adaptive mechanism of digestion and metabolism in underfed ruminants. A decrease in intake results an increase in nutrient digestibility due to an improvement in the extent of ruminal digestion. This is mainly caused by a longer retention of feed particles in the rumen and the contact time between microbes and feed particles. However, according to the review article by Doreau et al. (2003), the reverse relationship between OM digestibility and intake was observed at the intake levels higher than maintenance, based on 317 diets given to sheep. However, for forage-based diets from the above review, very different responses of OM and fiber digestibility were observed. In some cases, the same trend like over maintenance occurred. In some particular cases, a reduction in digestibility occurred by fasting or insufficient filling of the rumen, especially with low quality forage. Relatively small difference in digestibility between RS and Ti compared with IRG-s in this experiment affected $CH_4$ production per nutrient intake or digested nutrient intake.

**Table 4. Apparent digestibility of rice straw hay (RS) and timothy hay (Ti)**

| | RS | Ti | SEM | P-value |
|---|---|---|---|---|
| Dry matter (%) | 44.5$^{b}$ | 53.9$^{a}$ | 0.000 | <.0001 |
| Organic matter (%) | 51.1 | 55.9 | 2.03 | 0.259 |
| Neutral-detergent fiber (%) | 57.7 | 57.8 | 1.20 | 0.929 |
| Acid-detergent fiber (%) | 59.4$^{b}$ | 59.2$^{a}$ | 0.003 | 0.019 |

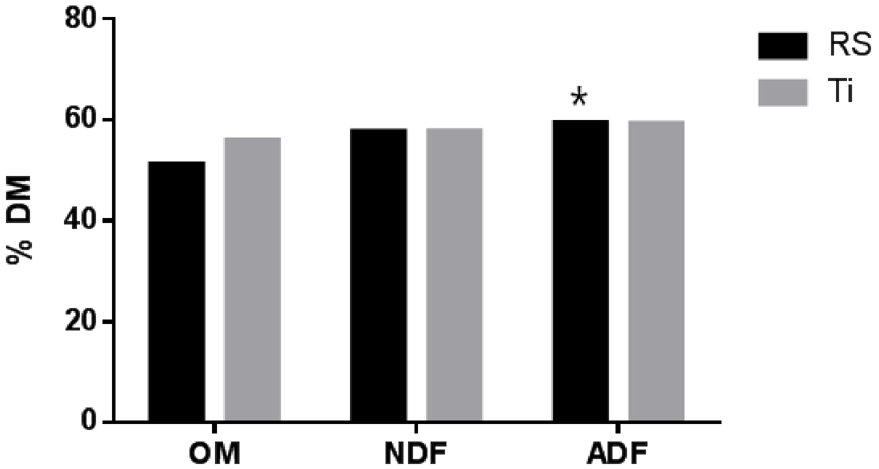



**Figure 3. Apparent digestibility of rice straw hay (RS) and timothy hay (Ti)**

### 4. 1. 3. Methane production from enteric fermentation

Daily $CH_4$ production for cattle fed Ti was higher than for cattle fed RS since DMI of Ti was greater than that for cattle fed RS (Table 5). However, there was no significant difference ($P = 0.081$). When $CH_4$ was expressed per kg DMI, cattle fed two diets emitted almost same $CH_4$ between Ti and RS. This result could be explained by the high relationship between DMI and $CH_4$ with feeding below 2.5 maintenance level (Shibata et al., 1992), and feeding forages without concentrate (Kurihara et al., 1999). On the other hand, there is evidence that increasing the feed intake decreased the $CH_4$ production per DMI or $CH_4$ conversion rate (MCR) (Blaxter and Clapperton, 1965; Hammond et al., 2013; Chaokaur et al., 2015), which was explained by the decrease in the mean rumen retention time. This consequently decreased the extent of rumen fermentation compared to low intake levels (Thornton and Minson, 1972; Hammond et al., 2014; Huhtanen et al., 2016). In this experiment, however, feed intake did not affect $CH_4$ amount per OMI or NDFI because forages were not same species and RS digestibility was improved by a longer retention time of particles in the rumen.

There were significant differences in $CH_4$ amount per unit of digested OMI and NDFI, which was in agreement with previous studies (Moss et al., 1994; Kurihara et al., 1999; Boadi & Wittenberg., 2001; Muñoz et al., 2016). In an experiment comparing tropical mature Angleton grass and immature Rhodes grass, distinct different responses in nutrients digestibility, energy partition and $CH_4$ production were observed (Kurihara et al., 1999). Voluntary OMI for cattle fed Angleton grass was half of that of cattle fed Rhodes grass (3.1 and 6.3 kg/d) and cattle fed Angleton grass experienced BW loss, which is same with RS treatment in the present experiment. However, the total tract digestibility of OM and NDF of Angleton grass were significantly lower than those of Rhodes grass but RS was not the case in this experiment. The results of Kurihara et al. (1999) indicated that improving forage quality is one practical means of reducing $CH_4$ production per digested OMI. The present Exp. 1 showed the same result that steers fed

Ti emited lower $CH_4$ per unit of dOMI, however, higher $CH_4$ production per dNDFI. This opposite results in $CH_4$ production per digested nutrients must be interpreted cautiously. It can be controversial that replacing low quality forage with high quality forage is expected to have lower methane production in terms of unit of digested nutrients.

**Table 5. Methane production by cattle fed rice straw hay (RS) and timothy hay (Ti)**

| | Treatment | | SEM | P-value |
|---|---|---|---|---|
| | RS (n = 4) | Ti (n = 4) | | |
| $CH_4$(g/d) | 29.5 | 54.6 | 3.19 | 0.081 |
| $CH_4$/DMI (g/kg DM) | 11.5 | 11.6 | 1.17 | 0.951 |
| $CH_4$/OMI (g/kg DM) | 13.4 | 12.3 | 1.33 | 0.475 |
| $CH_4$/dOMI (g/kg DM) | $26.8^{a}$ | $26.0^{b}$ | 0.000 | <.0001 |
| $CH_4$/NDFI (g/kg DM) | 17.8 | 16.6 | 1.77 | 0.540 |
| $CH_4$/dNDFI (g/kg DM) | $31.4^{b}$ | $33.2^{a}$ | 0.010 | 0.004 |
| MCR (%) | 3.10 | 3.23 | 0.317 | 0.709 |

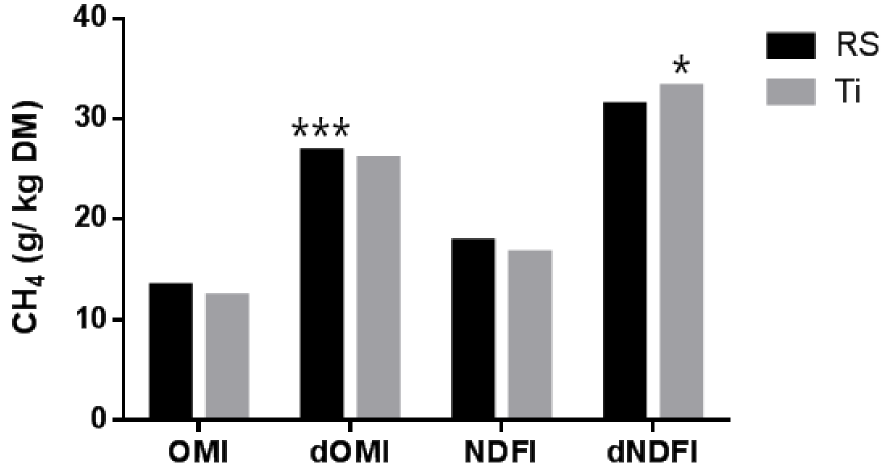



**Figure 4. Methane production per nutrient units by cattle fed rice straw (RS) and timothy hay (Ti)**

### 4. 1. 4. Ruminal fermentation characteristics

When values for concentrations of each volatile fatty acid (VFA) and total VFA at each sampling times were considered (Table 6), there were several interesting results. The most interesting one is the same rate of total VFA production for 3 hours after feeding even though there were two times of intake differences between treatments. This result is not consistent with the experiment using one grass hay given in restricted amounts at three intake levels; maintenance requirement, 60% and 20% of maintenance (Michalet-Doreau and Doreau, 2001), which showed that total VFA production was reduced significantly by decrease in feed intake. The other experiment carried out at levels of intake higher than maintenance (Benchaar et al., 2001), where increase in daily DMI was accompanied by an acceleration of total VFA production rate.

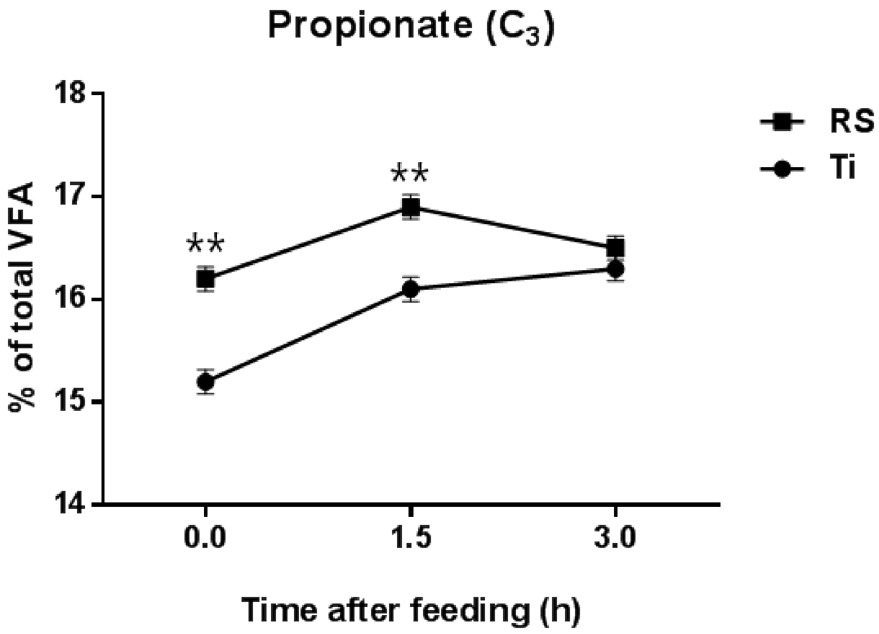
Another one is the significant differences in propionate percentage at 0 and 1.5 hours after feeding between two treatments. These total VFA production and propionate proportion may be a reflection of the fermentation characteristics before morning feeding, not due to early phase of the degradation of ingested forage because two forages were degraded relatively slow. The high proportion of propionate for steers fed only RS compared to Ti can not be explained by the general theory of ruminal fermentation of nutrients. It can be assumed that RS were degraded more slowly but steadily until the next feeding, however, fermentation of IRG-s was relatively fast in the rumen after 3hours from morning feeding and used relatively more amount of NFC as fermentation substrate. It was noted already that NFC contents of two forages were same. Indeed, the ruminal degradation rate of silage nutrients would be both extensive and vary rapid (Kim et al., 1999).

### 4. 1. 5. Conclusion of Exp. 1

The decision to select Ti as high quality forage, which imported from U.S., was taken with the aim of comparing clearly with RS (low quality forage) in Exp 1. However, chemical composition of Ti was not the quality level of what we expected since it had higher NDF content than RS and there was only small difference of CP between forages. It caused Ti to have relatively lower NFC proportion compared with RS. Although Ti could not satisfy the general characteristics of high quality forage, cattle consumed considerably more Ti than RS. This may be attributable to relatively soft texture of Ti, in addition to its higher digestibility. Two forages showed significance in DM digestibility, but there was no statistical difference in OM and NDF digestibility. High consumption of Ti would cause fast passage rate of organic matters, which hindered enough degradation by the bacteria, while RS might have relatively long retention time, or it was necessary to be sufficiently fermented under nutrient-poor condition since intake of RS was substantially lower. Despite of unexpected nutritional composition of Ti, $CH_4$ per digested OMI of Ti was significantly lower, but $CH_4$ per digested NDFI was higher than RS.

**Table 6. Ruminal fermentation characteristics of cattle fed rice straw hay (RS) and timothy hay (Ti)**

| Treatment | RS | | | Ti | | | SEM | P-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time interval | 0 hr | 1.5 hrs | 3 hrs | 0 hr | 1.5 hrs | 3 hrs | | Feed | 0 hr | 1.5 hrs | 3 hrs |
| pH | 8.02 | 7.88 | 7.97 | 7.93 | 7.87 | 7.96 | 0.024 | 0.148 | 0.064 | 0.866 | 0.737 |
| Total VFA (mM) | 60.2 | 62.4 | 61.4 | 59.6 | 60.6 | 58.0 | 4.49 | 0.667 | 0.894 | 0.723 | 0.497 |
| Acetate (%) | 76.9 | 75.3 | 75.8 | 77.9 | 77.1 | 76.8 | 0.873 | 0.167 | 0.274 | 0.068 | 0.283 |
| Propionate (%) | 16.2 | 16.9 | 16.5 | 15.2 | 16.1 | 16.3 | 0.117 | <.0001 | 0.003 | 0.006 | 0.375 |
| iso-Butyrate (%) | 0.642 | 0.595 | 0.593 | 0.788 | 0.709 | 0.693 | 0.119 | 0.325 | 0.243 | 0.358 | 0.417 |
| Butyrate (%) | 5.14 | 5.97 | 5.81 | 4.68 | 4.82 | 4.90 | 0.443 | 0.077 | 0.330 | 0.023 | 0.063 |
| iso-Valerate (%) | 0.566 | 0.499 | 0.482 | 0.968 | 0.812 | 0.773 | 0.238 | 0.179 | 0.117 | 0.216 | 0.247 |
| Valerate (%) | 0.614 | 0.681 | 0.791 | 0.459 | 0.501 | 0.553 | 0.229 | 0.418 | 0.513 | 0.452 | 0.322 |
| C2/C3 | 4.76 | 4.45 | 4.60 | 5.12 | 4.81 | 4.73 | 0.063 | 0.000 | 0.004 | 0.004 | 0.240 |
| $NH_3$-N (mg/L) | 3.26 | 3.49 | 3.27 | 5.61 | 7.20 | 5.52 | 0.73 | 0.002 | 0.009 | 0.000 | 0.011 |



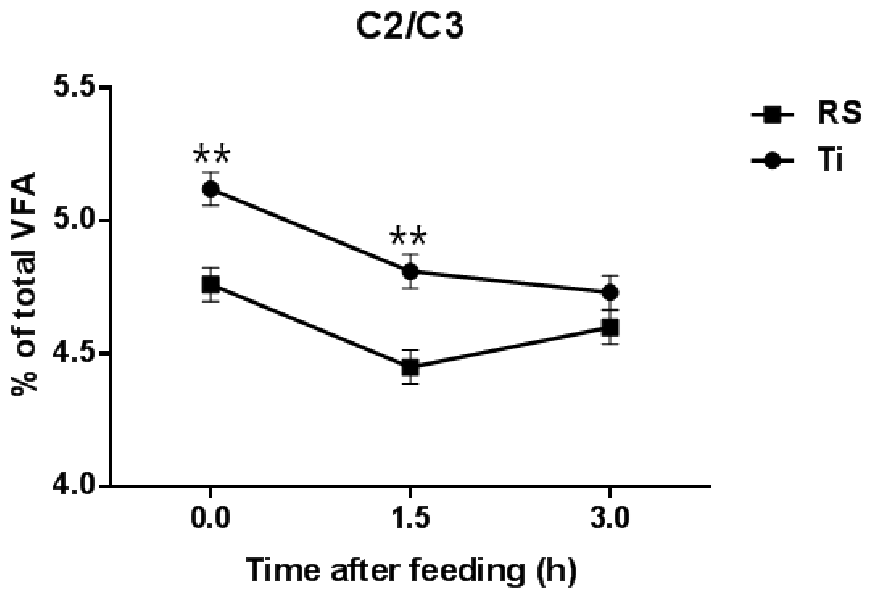




**Fig 5. Effect of rice straw hay (RS) and timothy hay (Ti) on Propionate ($C_3$) and $C_2/C_3$ ratio**

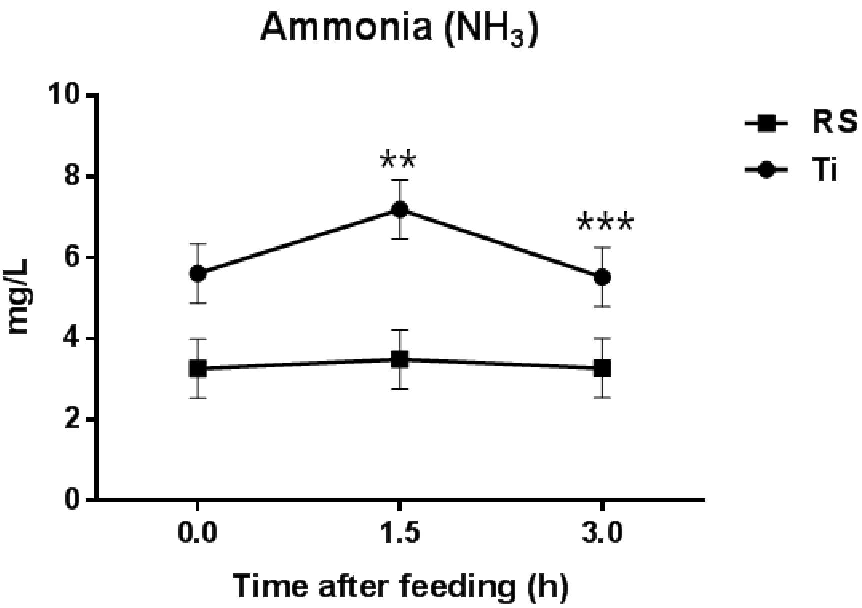



**Fig 6. Effect of rice straw hay (RS) and timothy hay (Ti) on ruminal ammonia concentration**

## 4. 2. Experiment 2. Rice straw hay vs. Italian ryegrass silage

### 4. 2. 1. Chemical composition of diets

There was a great DM difference between IRG-s and RS (585 and 805 g/kg, respectively) due to different preservation processing of forage (Table 7). However, OM (g/kg DM) was similar between two forages (891 and 880, respectively) and CP (g/kg DM) content of IRG-s was two times more than RS, which accorded with general characteristics of the high quality forage. On the other hand, in terms of NDF content (g/kg DM), RS showed around 8% lower value when compared to IRG-s and this is inconsistent with the result of Ki et al. (2017) that the mean value of NDF contents of locally produced RS used in Korea were numerically higher (800 and 676 g/kg) than that of IRG-s. These data showed that NDF concentration of IRG-s in this experiment was similar with that of locally produced ones, but RS in this experiment was 27% lower value than when compared to locally produced RS. An incidental point worth noting is that, in Exp. 1 and 2, NDF concentrations of RS were not high level when compared to imported Ti and domestically produced IRG-s. This indicated that RS quality was relatively good while IRG-s had relatively not much good quality which showed wilted silage characteristics of high pH (5.8), low lactic acid (2.7 % DM) and relatively high butyric acid (2.0 % DM) concentrations in this experiment.

**Table 7. Chemical composition of diets in Exp. 2**

| | RS[a] | IRG-s[b] | Concentrate |
|---|---|---|---|
| Dry matter (g/kg) | 805 | 585 | 907 |
| Organic matter (g/kg DM) | 880 | 891 | 920 |
| Neutral-detergent fiber (g/kg DM) | 586 | 641 | 326 |
| Acid-detergent fiber (g/kg DM) | 361 | 410 | 157 |
| Crude protein (g/kg DM) | 43 | 106 | 197 |
| Ether extract (g/kg DM) | 13 | 22 | 53 |
| Ash (g/kg DM) | 120 | 109 | 80 |
| Gross energy (cal/g DM) | 3880 | 4231 | 4441 |

[a] Rice straw hay, [b] Italian ryegrass silage

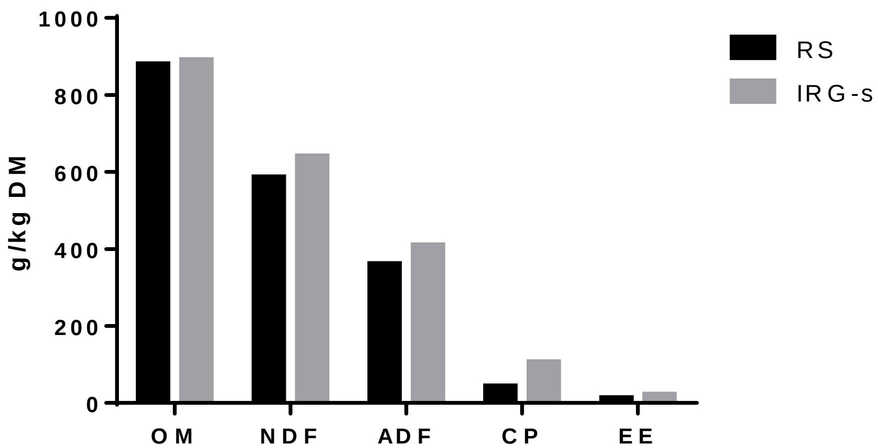



**Figure 7. Chemical composition of**

**rice straw hay (RS) and Italian ryegrass silage (IRG-s)**

### 4. 2. 2. Nutrient intake and digestibility

Cattle fed RS had 0.55 kg less DMI than cattle fed IRG-s without significant differences (Table 8). When compared with Exp. 1 (Table 3), the intake difference between forages of this experiment were much less. Small difference of DMI and other nutrient intakes may be explained by high water content of IRG-s, which made cattle feel quickly satiated, thus cattle may not be able to consume more IRG-s than expected. Additionally, offered 3.6 kg of concentrate reduced the overall consumption of IRG-s because cattle could meet energy requirements for maintenance from concentrate supplement. In addition, higher NDF and ADF values of IRG-s (Table 7) may contribute to this small gap of intakes between treatments, since typically the more NDF and ADF a forage contains, the less forage intake will be (Ball et al., 2001). Similar nutrient intakes in this experiment might be an important point to clarify the $CH_4$ production between different qualities of forages in a different way, compared to Exp. 1.

In the study of Kurihara et al. (1999) to investigate the effect of sole forage, cattle fed Rhodes grass (high quality forage) had almost twice DMI (7.07 and 3.58 kg, respectively) than Angleton grass (low quality forage). However, Muñoz et al. (2016) reported less difference in DMI from dairy cows with average body weight of 554 kg, when cattle were offered additional 1.8 kg/d of concentrate supplement, although *Lolium perenne* was pregrazed by different herbage masses (2,200 and 5,000 kg of DM / ha) to make quality disparity of regrowth forage with different structural carbohydrate content.

IRG-s showed significantly greater digestibility values than RS. There were differences in 6 % of OM digestibility and 7.8 % of NDF and ADF digestibility between two diets (Table 9). This indicated that IRG-s was in accordance with general property of relatively high quality forage in terms of digestibility compared to RS.

**Table 8. Nutrient intake of cattle fed rice straw hay (RS) and Italian ryegrass silage (IRG-s) including concentrate**

| | RS | IRG-s | SEM | P-value |
|---|---|---|---|---|
| Dry matter (kg) | 6.38 | 6.93 | 0.253 | 0.118 |
| Organic matter (kg) | 5.70 | 6.25 | 0.218 | 0.086 |
| Neutral-detergent fiber (kg) | 2.98 | 3.53 | 0.185 | 0.059 |
| Acid-detergent fiber (kg) | $1.73^{b}$ | $2.13^{a}$ | 0.123 | 0.047 |
| Crude protein (kg) | $0.708^{b}$ | $0.926^{a}$ | 0.062 | 0.038 |

**Table 9. Apparent digestibility of rice straw hay (RS) and Italian ryegrass silage (IRG-s) including concentrate**

| | RS | IRG-s | SEM | P-value |
|---|---|---|---|---|
| Dry matter (%) | $55.4^{b}$ | $64.6^{a}$ | 3.00 | 0.002 |
| Organic matter (%) | $61.4^{b}$ | $67.4^{a}$ | 1.21 | 0.009 |
| Neutral-detergent fiber (%) | $55.7^{b}$ | $63.5^{a}$ | 1.35 | 0.009 |
| Acid-detergent fiber (%) | $55.4^{b}$ | $63.2^{a}$ | 1.35 | 0.014 |

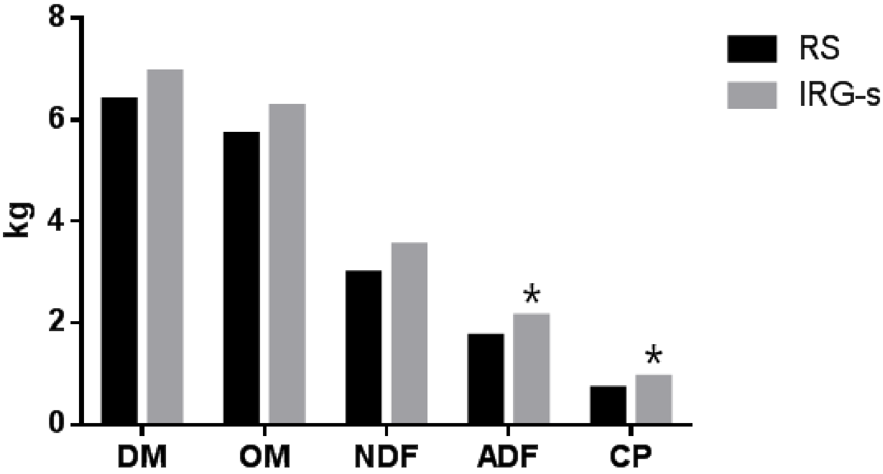



**Figure 8. Nutrient intake of cattle fed rice straw hay (RS) and Italian ryegrass silage (IRG-s) including concentrate**

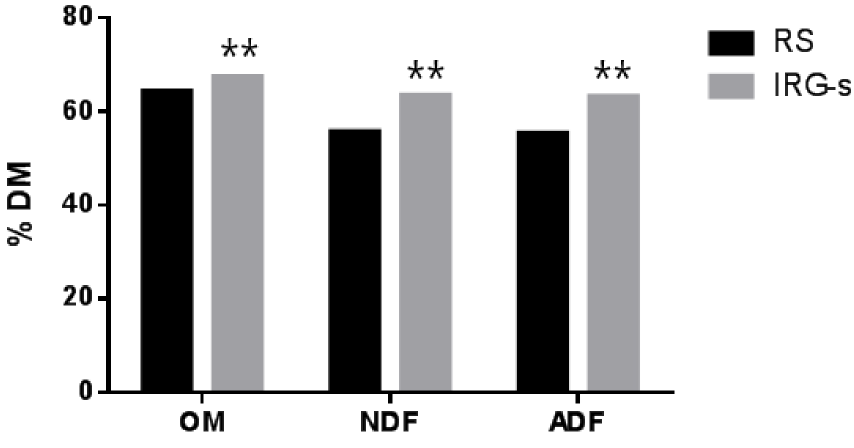



**Figure 9. Apparent digestibility of rice straw hay (RS) and Italian ryegrass silage (IRG-s) including concentrate**

### 4. 2. 3. Methane production from enteric fermentation

Daily $CH_4$ production (g/d) was higher in cattle fed IRG-s since DMI value was higher, however, there was no significance between two diets. Similarly, $CH_4$ conversion rate (MCR) and $CH_4$ production per kg DMI, OMI and NDFI had no significant differences between cattle fed IRG-s and RS, but IRG-s had slightly higher numerical values. In general, there is a strong relationship between DMI and enteric $CH_4$ production per animal (Kurihara et al., 1999; Shibata et al., 1992; Shibata et al., 2010; Charmley et al., 2016). Increase in DMI increases $CH_4$ production, as a greater DMI provides a greater intake of fermentable substrate, including both structural and nonstructural carbohydrates (Moe and Tyrrell, 1979). However, RS and IRG-s barely affect to nutrient intakes and daily methane production or nutrient intakes in the present study.

Despite steers fed IRG-s had around 7% higher digestibility than RS, there was no difference in $CH_4$ production per digested OMI and IRG-s had a decreasing trend ($P = 0.071$) of $CH_4$ production per digested NDFI in this experiment. Based on published studies (Moss et al., 1994; Kurihara et al., 1999; Boadi & Wittenberg., 2001; Muñoz et al., 2016), which investigated $CH_4$ emission characteristics from different forages, high quality forage always had lower $CH_4$ production per digested OMI or DMI. Hart et al. (2009) reported, however, there was no significant difference between high and low quality diets (29.3 and 32.1 g per digested DMI; $P = 0.24$). Similarly, there was no difference between early grass silage and late grass silage when $CH_4$ production was expressed by kg NDF digested irrespective of significant difference in chemical composition and digestibility (Brask et al., 2013).

**Table 10. Methane production by cattle fed rice straw hay (RS) and Italian ryegrass silage (IRS-s)**

| | Treatment | | | |
|---|---|---|---|---|
| | RS (n = 4) | IRG-s (n = 4) | SEM | P-value |
| $CH_4$(g/d) | 57.8 | 68.2 | 5.98 | 0.134 |
| $CH_4$/DMI (g/kg DM) | 9.08 | 9.85 | 0.641 | 0.313 |
| $CH_4$/OMI (g/kg DM) | 10.1 | 10.9 | 0.698 | 0.322 |
| $CH_4$/dOMI (g/kg DM) | 16.5 | 16.1 | 0.876 | 0.706 |
| $CH_4$/NDFI (g/kg DM) | 19.4 | 19.4 | 1.15 | 1.00 |
| $CH_4$/dNDFI (g/kg DM) | 35.0 | 30.5 | 1.64 | 0.071 |
| MCR (%) | 2.93 | 3.03 | 0.199 | 0.633 |

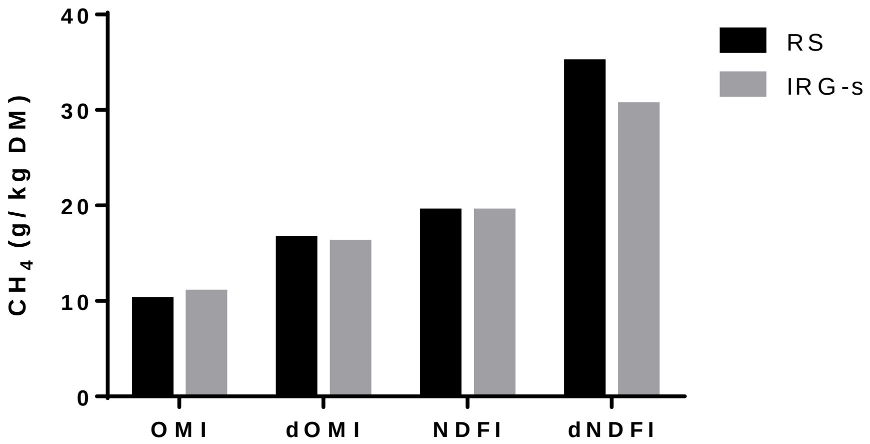



**Figure 10. Methane production per nutrient units by cattle fed rice straw hay (RS) and Italian ryegrass silage (IRS-s)**

### 4. 2. 4. Ruminal fermentation characteristics

Acetate proportion of total VFA was significantly higher in IRG-s, while butyrate proportion was significantly higher in RS treatment (Table 11). Howerer, there were no significant differences in propionate proportion and $C_2/C_3$ ratio between two diets. Steers were given same amount of concentrate and total DM intake were not different between treatments. It indicates that different actete and propionate proportions between treatments were attributed to the different fermentation characteristics of different forage. However, the reason for the differences in those VFA profile are not clear.

Proportion of both iso-butyrate and iso-valerate was significantly higher in cattle fed IRG-s. This finding may be attributable to relatively high CP content of IRG-s since iso-butyrate and iso-valerate are produced from valine and leucine, respectively, by microbial attack (Allison, 1977).

**Table 11. Ruminal fermentation characteristics of cattle fed rice straw hay (RS) and Italian ryegrass silage (IRS-s)**

| Treatment | RS | | | IRG-s | | | SEM | P-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time interval | 0 hr | 1.5 hrs | 3 hrs | 0 hr | 1.5 hrs | 3 hrs | | Feed | 0 hr | 1.5 hrs | 3 hrs |
| pH | 7.40 | 7.16 | 7.08 | 7.47 | 7.10 | 7.16 | 0.104 | 0.771 | 0.678 | 0.714 | 0.573 |
| Total VFA (mM) | 65.9 | 88.4 | 86.2 | 60.6 | 93.7 | 86.9 | 3.15 | 0.950 | 0.394 | 0.406 | 0.904 |
| Acetate (%) | 52.0 | 46.5 | 47.7 | 53.7 | 47.4 | 49.4 | 0.284 | 0.000 | 0.015 | 0.160 | 0.013 |
| Propionate (%) | 21.3 | 24.5 | 23.8 | 22.2 | 25.2 | 23.8 | 0.628 | 0.411 | 0.190 | 0.328 | 0.962 |
| iso-Butyrate (%) | 1.55 | 1.55 | 1.47 | 2.11 | 2.02 | 1.86 | 0.132 | 0.003 | 0.001 | 0.005 | 0.015 |
| Butyrate (%) | 22.9 | 24.4 | 24.3 | 19.0 | 21.6 | 21.6 | 0.561 | <.0001 | <.0001 | 0.001 | 0.001 |
| iso-Valerate (%) | 1.25 | 1.35 | 1.22 | 1.79 | 1.75 | 1.40 | 0.131 | 0.012 | 0.003 | 0.020 | 0.261 |
| Valerate (%) | 0.929 | 1.62 | 1.59 | 1.18 | 2.03 | 1.97 | 0.121 | 0.012 | 0.143 | 0.022 | 0.035 |
| $C_2/C_3$ | 2.47 | 1.90 | 2.02 | 2.42 | 1.88 | 2.08 | 0.052 | 0.980 | 0.529 | 0.812 | 0.418 |
| $NH_3$-N (mg/L) | 2.93 | 15.9 | 11.0 | 3.49 | 17.7 | 12.3 | 2.25 | 0.591 | 0.867 | 0.579 | 0.692 |

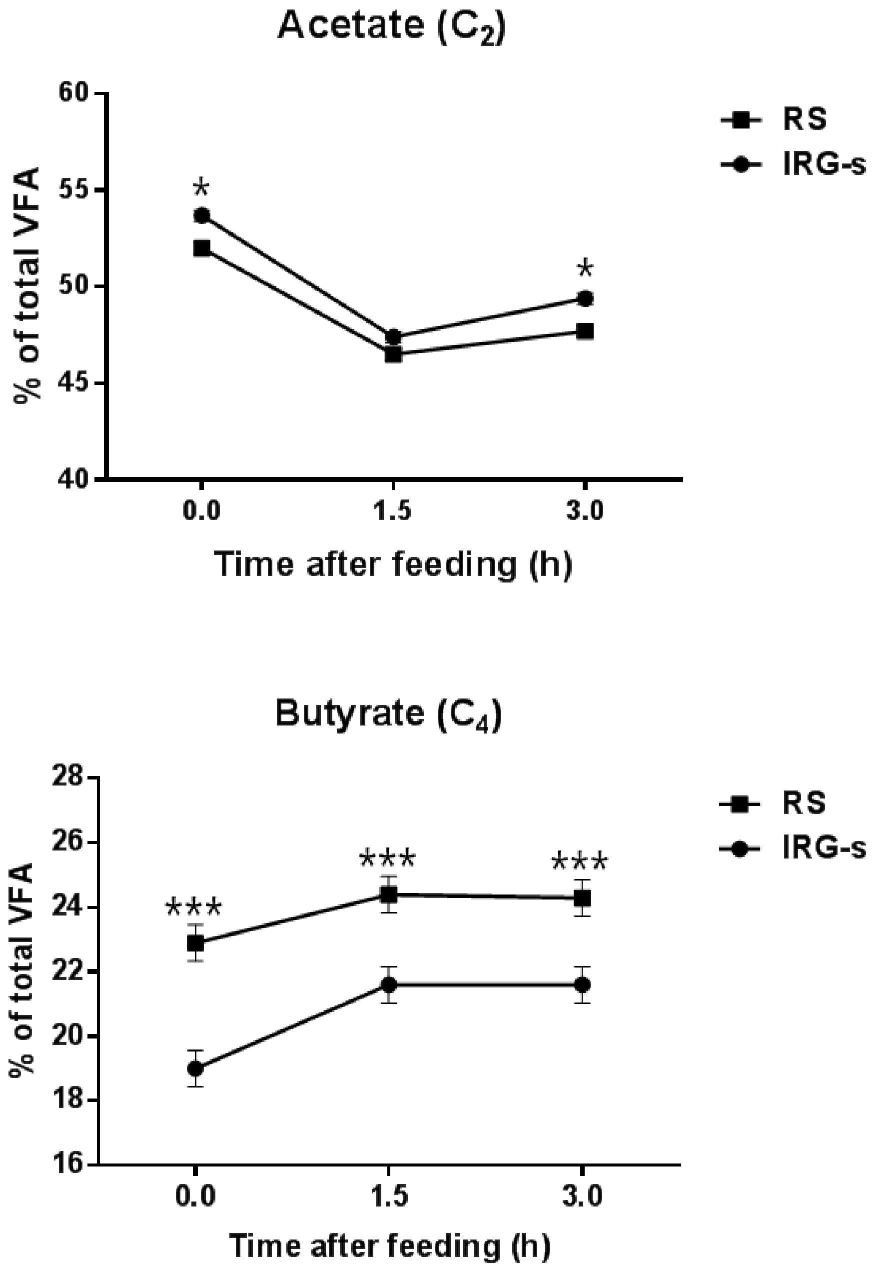



**Fig 11. Effect of rice straw hay (RS) and Italian ryegrass silage (IRG-s) on Acetate ($C_2$) and Butyrate ($C_4$)**

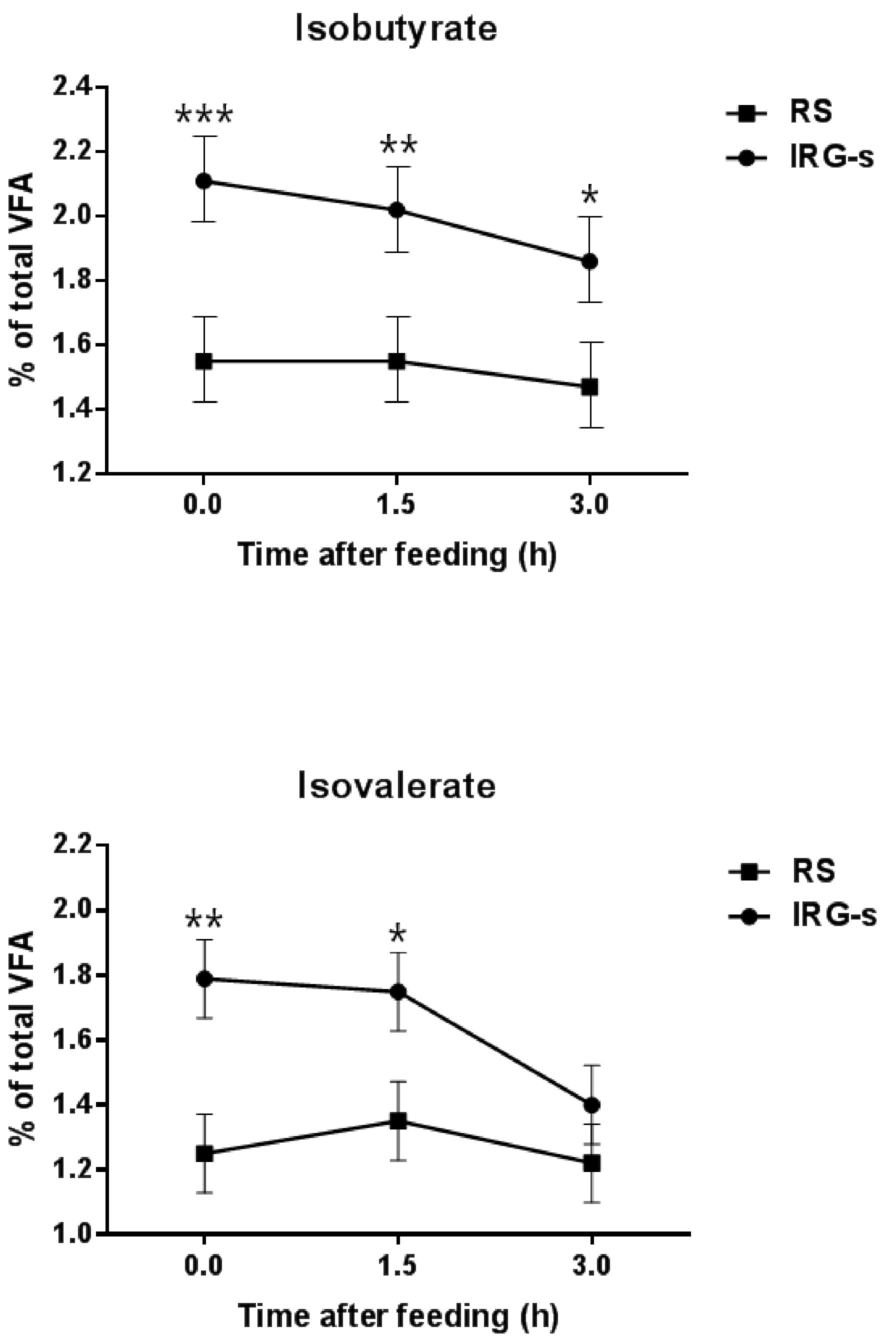



**Fig 12. Effect of rice straw hay (RS) and Italian ryegrass silage (IRG-s) on isobutyrate and isovalerate**

### 4. 2. 5. Conclusion of Exp. 2

The intention in Exp. 2 was that IRGs and RS would represent general characteristics of different quality of forages produced in Korea and show significant different $CH_4$ production per dOMI or dNDFI. Even though the nutrient intakes and digestibilities of IRGs were significantly higher than RS, however, there was only a tendency ($P < 0.10$) for a decrease in the $CH_4$ production per kg dNDFI for steers fed IRGs when compared to RS.

## 5. Conclusion

Although nutrient compositions of experimental forages did not show their typical quality in both Exp. 1 and 2, there was significant difference of intake in Exp 1, while only small difference was observed in Exp 2. Ti showed low $CH_4$ per kg dOMI but high value per kg dNDFI compared to RS in Exp. 1, and IRGs did not show any difference in $CH_4$ per kg dOMI but tendency for low $CH_4$ production per kg dNDFI. These data means that two experiments may not have provided an adequate test of the hypothesis; however, it clearly highlights the need for further research using different forage quality in terms of chemical composition, nutrient intake and rumen degradability.

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# 7. 요약

지구온난화로 인해 각 산업분야마다 온실가스를 줄이고자 하는 다양한 노력들이 이루어지고 있다. 축산 분야 또한 예외가 아닌데, 축산 분야 내에서는 반추동물의 장내 발효로 인한 메탄 발생을 가축생산성을 유지한 채로 어떻게 저감할 수 있을지에 대한 활발한 연구가 진행되고 있다. 대한민국은 양질의 조사료 공급 확대로 메탄을 줄이겠다는 정책을 수립하고 있지만, 국내 연구는 전무하고 국외에서도 아직 많은 연구가 되지 않았다. 따라서, 본 연구는 조사료 품질과 반추위 장내 메탄 발생량과의 관계를 구명하기 위해서 두 차례의 실험을 수행하였다. 실험 1에서는 4마리의 홀스타인 거세우를 대상으로 볏짚 건초와 티모시 건초를 무제한으로 급여하여 메탄 발생량을 측정하였다. 조사료 섭취량에 있어서는 티모시 건초가 볏짚 건초에 비해 모든 영양단위에서 유의적으로 높았으나, 둘 사이에 유의적인 소화율 차이는 없었다. 본 실험에서 주목하였던 가소화 영양소 섭취량 당 메탄발생량은 티모시 건초가 가소화 유기물 당 유의적으로 낮은 메탄을 발생시켰으나 그 차이가 0.8g으로 적었고, 가소화 섬유소 당 메탄 발생량에서는 오히려 티모시 건초가 볏짚 건초에 비해 더 높은 메탄을 발생시켰다. 우리는 이러한 결과가 티모시 건초의 예상보다 낮은 품질과 볏짚 건초의 유지 에너지 이하의 섭취량에 있었다고 보고 두 번째 실험을 진행하였다.

실험 2에서는 4마리의 한우 거세우를 대상으로 볏짚 건초와 이탈리안 라이그라스 사일리지를 조사료로 무제한 급여하고 원물 기준 3.2kg의 농후

사료를 추가로 급여하여 실험동물의 유지에너지를 만족시켜주고자 하였다. 추가적인 농후사료의 급여와 사일리지의 높은 수분함량으로 인하여 볏짚 건초와 이탈리안 라이그라스 사일리지의 섭취량 차이는 실험1과 비교하였을 때 크지 않았으나, 소화율은 모든 영양단위에서 이탈리안 라이그라스 사일리지가 유의적으로 높았다. 가소화 유기물 당 메탄발생량은 이탈리안 라이그라스 사일리지가 볏짚 건초에 비해 0.4g 적었으나 유의적인 차이는 아니었다. 가소화 섬유소 당 메탄발생량에서는 이탈리안 라이그라스 사일리지가 30.5g의 메탄을 발생시켜 볏짚 건초 (35 g) 대비 13% 정도 낮은 경향성을 보였다. 따라서, 본 연구를 통해 양질의 조사료를 급여함으로써 가소화 섬유소당 메탄저감효과를 기대할 수 있다는 결론은 도출할 수 있었다. 또한 실험 2에서의 볏짚 건초의 품질이 평균 이상이었던 것을 감안할 때, 품질 차이가 극명한 두 조사료를 대상으로 한 추가 실험을 진행할 경우 가소화 영양소 섭취량 당 메탄 발생량에서 더욱 확연한 차이가 기대된다. 조사료 품질과 메탄 발생량과의 관계를 조사한 연구는 국내에서 처음으로 이루어져 의미가 있으며 온실가스 감축사업 등의 정책들을 뒷받침할 수 있는 자료로 이용될 수 있을 것이라 기대하고 있다.

주요어: 조사료 품질, 이탈리안 라이그라스, 메탄, 볏짚, 반추위 발효, 티모시

학번: 2016-20012

# 8. Acknowledgement

우여곡절 끝에 입학하게 된 서울대학교 평창캠퍼스에서의 석사과정을 무사히 마칠 수 있어서 감사합니다. 이곳에서의 석사과정은 비단 지식적인 부분만을 배운 것이 아니라 새로운 감정 그리고 상황들을 마주하며 조금 더 지혜로워질 수 있는 시간이었습니다. 저의 부족함을 이해하고 옆에서 도와주신 많은 분들이 있었기에 이런 의미 있는 시간이 가능했다고 생각합니다.

먼저, 쉽지만은 않은 석사과정이었지만 가족과 같은 마음으로 한결같이 바르게 지도하여 주신 김경훈 교수님, 진심으로 감사드립니다. 그리고 학업과 힘든 실험들을 함께한 동물에너지대사학 실험실 구성원들, 특별히 이창현 연구원과 바라니에게 감사의 말을 전합니다.

논문 심사를 담당해 주신 김종근, 강상기 교수님을 포함하여, 많은 가르침을 주신 국제농업기술대학원의 모든 교수님들께도 감사드립니다. 앞으로 국제농업기술대학원의 이름을 빛낼 수 있도록 더욱 노력하겠습니다.

춥고 외로울 수도 있었던 평창 캠퍼스에서의 생활에 즐거움을 주고 어려울 때는 힘이 되어준 모든 학생, 연구원들에게 감사합니다. 특히, 이곳에서 가장 많은 시간을 함께한 은비, 오대, 학진, 정욱에게 고맙다는 말을 전하고 싶습니다. 마지막으로 이곳에서 좋은 인연이 되어준 시원아, 고맙다!

혼자서도 대학생활 잘 해내고 있는 대견한 동생과 언제나 제 삶을 지지해주시고 지원해주시는 부모님 감사합니다. 항상 건강하세요.