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**Research Article**

# Effects of Feeding High- and Low- Forage Diets Containing Different Forage Sources on Rumen Fermentation Characteristics and Blood Parameters in Non-Pregnant Dry Holstein Cows

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## ABSTRACT

This research was conducted to investigate the effects of feeding high and low forage diets with different forage sources on rumen fermentation characteristics and blood parameters of Holstein cows during the dry period. Eight Holstein cows were completely randomized assigned to two groups and repeated measurement was utilized in the analysis. Cows in two treatments were fed with diets with high (F:C = 70:30, 70F; forage source: mixed-sowing whole crop barley and Italian ryegrass silage, BIRG) and low (F:C = 55:45, 55F; forage source: tall fescue hay, TF) forage level. Rumen fluid pH was higher in 70F group. Levels of acetic acid, propionic acid, and butyric acid showed a similar pattern: from the lowest value at 07:30 h to the highest at 10:30 h and then decreased in both groups. The ratio of acetic acid to propionic acid was significantly higher ($p < 0.05$) in 55F group at 09:30 and 10:30 h. Rumen fluid $NH_3$-N concentrations were significantly higher ($p < 0.05$) in 70F group at 09:30 and 10:30 h. Blood urea nitrogen was significantly higher ($p < 0.05$) in 70F group. It was concluded that BIRG based diet with a high forage level had no adverse effects on rumen fermentation, some blood chemical parameters, and immune system in dry Holstein cows and could be used as a forage source instead of imported TF.

(**Key words** : Forage ratio, Rumen fermentation characteristics, Blood parameters, Holstein cows)

## Ⅰ. INTRODUCTION

Supplying domestic self-producing forage for livestock is an important issue in north-east Asian countries. South Korea imports lots of forage for its livestock industry (Her et al., 2010). This costs lots of money and also becomes one of the main reasons for the high price and weak competitiveness of livestock products in the marketplace than imported products. Meanwhile, transportation of forages from distant places may result in decreases and instability of forage quality (Sung, 2000). Seo (2016) reported that the quality of Korean domestic forages was better than imported ones through the results of some comparison experiments. Therefore, replacing imported forages with domestic forages containing high nutritive values is considered to be an effective way to deal with the above concerns (Sung et al., 2012). Korea imports lots of forage hay, such as timothy, tall fescue and alfalfa hay from foreign countries. The imported tall fescue accounted more than 25% of the total tall fescue consumption in South Korea; meanwhile, the price of domestic forages such as whole crop barley, Italian ryegrass, and whole crop rye were about 30-50% cheaper in comparison to the imported forages such as tall fescue, timothy, and alfalfa in recent years (Seo and Yook, 2002; Seo, 2016). Whole crop barley and Italian ryegrass are representative winter forage crops in South Korea (Peng et al., 2016). Whole crop barley is high in energy and annual ryegrass is high in crude protein. Silage made of these winter forage crops was considered as high quality feed source with high feed value, acceptability, and palatability to animals (Mani et al., 2004). Thereafter, silage made by whole crop barley and Italian ryegrass were preferred by farmers to use as feed for livestock instead of imported forages such as tall fescue hay in South Korea in recent years (Peng et al., 2016). The increase of cultivated areas in South Korea of these forage crops could well confirm this trend (Shin et al., 2012; Seo, 2016). The cultivated area of forages was increased double in

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2014 compared to the area in 2007, and the cultivated area of Italian ryegrass and whole crop barley accounted more than half of the total area used for forage cultivation in 2014 in South Korea (Seo, 2016).

Sung et al. (2012) reported that whole crop barley and Italian ryegrass produced in southern areas of Korea shared some similar nutritional values with imported tall fescue hay (TF); meanwhile, there was no reduction of daily gain showed in growing Holstein cows fed with domestic mixed-sowing whole crop barley and Italian ryegrass silage (BIRG) based diet with a high forage to concentrate (F:C) ratio instead of imported TF. Lee et al. (2013) reported an improvement in milk fatty acid composition in lactating Holstein cows fed with BIRG based diet containing forage in a high ratio. In these studies, BIRG based diet showed no negative effects on animals and saved partial concentrate with a high F:C ratio.

End products of rumen microbial fermentation in ruminants fed with different diets results in different productivity performance (Lyle et al., 1981; Na et al., 2003). Indicators of the obtained results may be reflections of rumen fermentation which was not discussed in the previous studies. Soon et al. (2010) reported that domestic BIRG increased the productivity of the Korean black goats resulted from increases in feed intake, nutrient digestibility, and nitrogen retention. However, to the best of the author's knowledge, no research on investigating the effects of feeding domestic BIRG with high F:C ratio instead of imported TF on rumen fermentation characteristics and blood parameters in non-pregnant dry Holstein was reported in South Korea. Thus, this study was initiated for the better understanding of how rumen fermentation end products and blood parameters are influenced by feeding domestic BIRG instead of imported TF with a high F:C ratio in dry Holstein cows.

## Ⅱ. MATERIALS AND METHODS

### 1. Animals, experimental treatments, and study design

The experiment was carried out at an experimental animal farm in Gangwon province, South Korea. Eight rumen fistulated Holstein cows (dry off, average body weight 502.75±31.25 kg) were housed in sheltered dry-lot facilities. All cows were managed and the experimental procedures were done in accordance to the guidelines of the Institutional Animal Care and Use Committee of Kangwon National University. Cows were randomly assigned to two groups to receive the two treatments during the experimental period. The experiment lasted one month with the front twenty seven days for feeding experiment and last three days for collection of rumen fluid and blood samples. Treatments were high- and low- forage diets containing different forage sources. The first diet contained 45% concentrate and 55% forage which was imported TF (low-forage diet, 55F); and the second diet contained 30% concentrate and 70% forage which was domestic BIRG (high-forage diet, 70F). Concentrate used in the experiment was commercial pellet concentrate from the farm.

### 2. Feeding and feed sample measurement

Forage and concentrate were manually mixed each day before morning feeding. The cows were fed twice daily at 08:30 and 17:00 h according to nutrient and energy requirements of dairy non-pregnant dry cows (NRC, 2001). Water was available free of choice for all the animals during the experiment. Feed samples were collected and analyzed for dry matter (DM), ash contents, ether extract (EE), and crude protein (CP) analysis (N × 6.25) according to the AOAC (1990). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured using the filter bag technique (Ankom Technology Corp., Fairport, NY, USA) according to the method of Van Soest et al. (1991). The pH of silage was measured using a digital pH meter (FE20, Mettler-Toledo, Shanghai, China). The chemical compositions of feed resources fed to animals were shown in Table 1. Feed residues were weighed on the following day prior to morning feeding in order to determine daily voluntary feed intake.

### 3. Rumen fluid collection, pH measurements, and samples preparation

After 27 days of adaptation to the diets, for 2 days consecutively, rumen fluid was sampled via a cannula at 07:30

Table 1. Chemical composition of feeds

| Feed | DM[3] (%) | CP | NDF | ADF | EE | NFC | pH |
|---|---|---|---|---|---|---|---|
| TF[1] | 92.23 | 7.22 | 73.77 | 43.67 | 0.39 | 11.37 | - |
| BIRG[2] | 43.87 | 12.99 | 57.62 | 33.31 | 2.05 | 20.00 | 4.84 |
| Concentrate | 93.90 | 16.79 | 31.32 | 19.84 | 3.42 | 39.05 | - |

1 TF, imported tall fescue hay
2 BIRG, domestic mixed-sowing whole crop barley and Italian ryegrass silage
3 DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; NFC, non-fiber carbohydrates

h prior to morning feeding and at 09:30, 10:30, and 14:30 h after morning feeding to measure the rumen pH, $NH_3$-N, and VFAs. The rumen fluid was then squeezed through four layers of surgical gauze within 1 min. Twenty mL of rumen fluid was transferred into a 30 mL tube in duplicate, where the pH was measured immediately with a digital pH meter (FE20, Mettler-Toledo, Shanghai, China). Another 10 mL of rumen fluid was transferred into a 20 mL tube with similar amount of 0.2 N HCl for the stability of $NH_3$-N. Afterwards, this tube was put into the icebox and transferred to a refrigerator in the laboratory for $NH_3$-N measurement. Another extra 10 mL of rumen fluid was stored in a 15 mL tube immediately after rumen fluid collection. Two mL of 25% (w/v) $HPO_3$ and 1mL of saturated $HgCl_2$ was added to the sample, shaken, and transferred into an icebox for quantifying the VFA concentrations through the method of Erwin et al. (1961).

## 4. Rumen fluid $NH_3$-N and VFA measurement

Rumen fluid $NH_3$-N concentrations were measured by 2300 Kjeltec Analyzer Unit (Foss Tecator, Sweden) using sodium tetraborate ($Na_2B_4O_7$) solution as alkaline solution, 0.01 N HCl as acid solution, 1 % boric acid solution containing methyl red, bromocresol green mixed indicator as dye solution, and ammonium sulfate $(NH_4)_2SO_4$ solution as standard solution. One standard solution was used between each ten sample solutions and the data was used for further calculations. Sample solutions were prepared by mixing the same volume of rumen fluid with 0.2 N HCl. The following equation was used for calculation of the $NH_3$-N concentration: $NH_3$-N (mg/100mL) = (observed value of sample ÷ observed value of the standard solution) ×concentration of standard.

Rumen fluid samples prepared for VFA measurement were centrifuged at 3000 rpm for 15 min. One mL supernatant was transferred into a 1.5 mL tube for further analysis. Ruminal VFA was quantified using Shimadzu GC-17A gas chromatograph (Shimadzu, Kyoto, Japan) with a GC capillary column (30 m × 0.32 mmid × 0.25 μM film thickness; Supelco, Bellefonte, PA, USA) and flame ionization detection. Internal standard for determination of VFA was Volatile Acid Standard Mix (Supelco, Bellefonte, PA, USA). The temperature of the GC column oven was increased from 100 to 120°C by 10°C/min and stabilized the temperature for 4 min, increased by 10°C /min to a final temperature of 200°C, and stabilized for 3 min. The injector temperature was 230°C, the detector temperature was 250°C, and the carrier gas was nitrogen ($N_2$) with a 40 mL/min flow rate. The VFA peaks were identified based on comparison of their retention time to those of the standard.

## 5. Blood samples collection, serum chemistry profiles and hematological profiles measurement

Blood samples of all cows were collected by jugular venipuncture in vacutainer tubes at 13:00 h on the first sampling day of each experimental period. Tubes containing EDTA as anticoagulant were used for collection of hematology samples. Tubes without additive were used for blood collection and serum was immediately separated by centrifugation at 1200 rpm for 20 min, and then transported to the laboratory to be stored at -20°C for chemistry profiles analysis including total protein (TP), blood urea nitrogen (BUN), and glucose (GLU) determined via a biochemistry auto-analyzer (CST-240, Dirui Industrial Co., Ltd, Changchun, China). Hematology samples were used for complete blood count including red blood cells (RBC), white blood cells (WBC), hemoglobin, hematocrit, basophil, and eosinophil using Sodium Lauryl Sulfate method with Laser flow cytometry (ABX MINILYSE LMG- France).

Table 2. Feed intake of dry Holstein cows

| | 55F[1] | 70F[2] |
|---|---|---|
| Concentrate intake (kg/d, DM[3]) | 3.76±0.3[b] | 2.72±0.2[a] |
| Forage intake (kg/d, DM) | 4.59±0.3[a] | 6.58±0.4[b] |
| CP intake (kg/d, DM) | 0.96±0.05[a] | 1.31±0.07[b] |
| NDF intake (kg/d, DM) | 4.56±0.2 | 4.64±0.2 |
| ADF intake (kg/d, DM) | 2.75±0.1 | 2.73±0.2 |
| EE intake (kg/d, DM) | 0.15±0.01 | 0.23±0.02 |
| NFC intake (kg/d, DM) | 1.99±0.2[a] | 2.38±0.3[b] |
| NFC/NDF | 0.44±0.02[a] | 0.51±0.03[b] |
| TDN intake (kg/d, DM) | 5.20±0.3 | 4.67±0.2 |

Values are expressed as mean ± SE. Different letters indicate significant differences (a < b, $p < 0.05$).
1 55F, low forage diet containing imported tall fescue hay as forage sources
2 70F, high forage diet containing domestic mixed-sowing whole crop barley and Italian ryegrass silage as forage sources
3 DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; NFC, non-fiber carbohydrate; NFC/NDF, NFC to NDF ratio based on intake; TDN, total digestible nutrients

### 6. Statistical Analysis

*T*-test was used to perform the comparisons of feed and blood chemistry data between two groups by SAS (Statistical Analysis System, version 9.4). A significance level of 0.05 was considered as statistically significant in *t*-test.

Rumen fermentation parameters (pH, $NH_3$, and VFAs) were analyzed by repeated measures model using the MIXED procedure of SAS (Statistical Analysis System, version 9.4). The model included fixed effects (treatment, sampling time, and the interaction of treatment × sampling time) and random effects (cow). The model used for analyzing rumen fermentation data was as follows:

$$Y_{ijk} = \mu + T_i + S_j + C_k + (T \times S)_{ij} + \varepsilon_{ijk}$$

where $Y_{ijk}$ is the mean of each observation trait, $\mu$ is the overall mean, $T_i$ is the effect of treatment i (i=1, 2), $S_j$ is the effect of sampling time j（j =1, 2, 3, 4), $C_k$ is the effect of cow k (k =1, ……,8), $(T \times S)_{ij}$ is the effect of interaction of treatment i and sampling time j, and $\varepsilon_{ijk}$ is the overall error. According to AIC (Akaike's Information Criterion), AR1 of the MIXED procedure of SAS was determined to be the most appropriate covariance structure.

In the results and discussion sections, insignificant effects were not considered; meanwhile, effect of sampling time ($p < 0.05$) was observed in all parameters. Therefore, only means of each parameter between treatments at each sampling time point were presented in tables.

## Ⅲ. RESULTS

### 1. Feed intake

Table 3. Effects of diets on rumen fluid pH in dry Holstein cows

| Time | 55F[1] | 70F[2] | SEM | p-value |
|---|---|---|---|---|
| 7:30[3] | 6.49 | 6.76 | 0.05 | 0.00 |
| 9:30 | 6.31 | 6.49 | 0.06 | 0.01 |
| 10:30 | 6.17 | 6.24 | 0.07 | 0.38 |
| 14:30 | 6.23 | 6.34 | 0.07 | 0.23 |

SEM = standard error of mean.
1 55F, low forage diet containing imported tall fescue hay as forage sources
2 70F, high forage diet containing domestic mixed-sowing whole crop barley and Italian ryegrass silage as forage sources
3 Rumen fluid samples were collected at 07:30, 09:30, 10:30, and 14:30 h on sampling days

Feed intake of dry Holstein cows was presented in Table 2. Forage intake, CP intake, NFC intake, and non-fiber carbohydrate to neutral detergent fiber ratio (NFC/NDF) were significantly higher ($p < 0.05$) in 70F group, while concentrate intake was significantly lower ($p < 0.05$) in 70F group.

## 2. Rumen fluid pH

As presented in Table 3, pH values initially showed a downward trend and then changed to upward at 14:30 h in both groups. Rumen fluid pH values were significantly higher ($p < 0.05$) in 70F group than 55F group at 07:30 and 09:30 h. The highest pH values in both groups were found at 1 hour prior to morning feeding, and then rumen fluid pH showed a downward trend in both groups. Finally, rumen fluid pH value at 14:30 h was higher than the value at 10:30 h, and the descent speed and extent of rumen fluid pH were faster and higher in 70F group.

## 3. Rumen fluid VFA concentrations and acetic acid to propionic acid ratio

Values of acetic acid, propionic acid, and butyric acid showed similar trends, from the lowest value at 07:30 h to the highest value at 10:30 h, and then decreased (Table 4). Acetic acid and propionic acid values had no significant differences ($p > 0.05$) but were higher in 70F group than 55F group at 9:30 and 10:30 h. Meanwhile, values of acetic acid and propionic acid were lower in 70F group than 55F group at 7:30 and 14:30 h. All the butyric acid values were higher in 70F group than 55F group, but only significantly higher at 9:30 and 10:30 h. The ratio of acetic acid to propionic acid had no significant differences ($p > 0.05$) at 7:30 and 14:30 h but the ratios were higher in 70F group than 55F group (Table 4). Significant differences ($p \leq 0.05$) of acetic acid to propionic acid ratios were observed at 09:30 and 10:30 h lower ratios were observed in 70F group.

Table 4. Effects of diets on rumen fluid VFA and acetic acid to propionic acid ratio of dry Holstein cow

| Parameters | Time | 55F[1] | 70F[2] | SEM | p-value |
|---|---|---|---|---|---|
| Acetic acid, mM/L | 7:30[3] | 82.26 | 78.23 | 3.13 | 0.52 |
| | 9:30 | 98.81 | 100.43 | 4.49 | 0.86 |
| | 10:30 | 110.46 | 113.59 | 4.18 | 0.71 |
| | 14:30 | 101.85 | 89.45 | 3.27 | 0.06 |
| Propionic acid, mM/L | 7:30 | 20.39 | 18.95 | 1.00 | 0.52 |
| | 9:30 | 27.98 | 33.41 | 1.69 | 0.11 |
| | 10:30 | 31.14 | 35.35 | 1.36 | 0.14 |
| | 14:30 | 27.02 | 23.82 | 1.31 | 0.24 |
| Butyric acid, mM/L | 7:30 | 12.58 | 13.20 | 0.70 | 0.65 |
| | 9:30 | 17.62 | 25.00 | 1.86 | 0.02 |
| | 10:30 | 20.31 | 26.79 | 1.39 | 0.00 |
| | 14:30 | 17.84 | 19.40 | 1.09 | 0.34 |
| Acetic acid / Propionic acid | 7:30 | 4.09 | 4.20 | 0.14 | 0.69 |
| | 9:30 | 3.55 | 3.02 | 0.09 | 0.00 |
| | 10:30 | 3.57 | 3.21 | 0.09 | 0.05 |
| | 14:30 | 3.82 | 3.83 | 0.10 | 0.96 |

SEM = standard error of mean.

1 55F, low forage diet containing imported tall fescue hay as forage sources

2 70F, high forage diet containing domestic mixed-sowing whole crop barley and Italian ryegrass silage as forage sources

3 Rumen fluid samples were collected at 07:30, 09:30, 10:30, and 14:30 h on sampling days

Table 5. Effects of diets on rumen fluid $NH_3$-N concentrations (mg/100ml) of dry Holstein cows

| Time | 55F[1] | 70F[2] | SEM | p-value |
|---|---|---|---|---|
| 7:30[3] | 4.82 | 5.78 | 0.60 | 0.42 |
| 9:30 | 10.81 | 19.1 | 1.56 | 0.00 |
| 10:30 | 11.25 | 21.81 | 1.67 | <.0001 |
| 14:30 | 3.57 | 4.34 | 0.60 | 0.31 |

SEM = standard error of mean.
1 55F, low forage diet containing imported tall fescue hay as forage sources
2 70F, high forage diet containing domestic mixed-sowing whole crop barley and Italian ryegrass silage as forage sources
3 Rumen fluid samples were collected at 07:30, 09:30, 10:30, and 14:30 h on sampling days

## 4. Rumen fluid $NH_3$-N concentration

Levels of rumen fluid $NH_3$-N of dry Holstein cows showed an increasing trend and reached the highest value at 10:30 h. Rumen fluid $NH_3$-N concentrations were significantly higher ($p < 0.05$) in 70F group than 55F group at 9:30 and 10:30 h (Table 5). Rumen fluid $NH_3$-N levels had no significant differences ($p > 0.05$) but were higher in 70F group at 07:30 and 14:30 h.

## 5. Serum chemistry profiles and hematological profiles

TP and GLU concentrations showed no significant differences ($p > 0.05$) between 55F and 70F groups (Table 6). BUN concentration was significantly higher in 70F group than 55F group ($p < 0.05$). Neutrophil, lymphocyte, monocyte, eosinophil and basophil had no significant differences between 55F and 70F groups. Erythrocyte indices were similar in two treatment groups ($p > 0.05$) (Table 6).

Table 6. Effects of diets on serum chemistry and hematological profiles

| Parameters | | 55F[1] | 70F[2] | SEM | p-value |
|---|---|---|---|---|---|
| Serum chemistry profiles | TP[3] (g/dL) | 6.78 | 6.68 | 0.39 | 0.80 |
| | BUN (mg/dL) | 11.75 | 14.50 | 1.52 | 0.01 |
| | GLU (mg/dL) | 61.75 | 62.50 | 3.26 | 0.86 |
| Erythrocyte indices | RBC (106/μL) | 7.05 | 6.77 | 0.90 | 0.39 |
| | Hb (g/dL) | 11.95 | 11.88 | 1.63 | 0.87 |
| | HCT (%) | 38.95 | 38.83 | 5.06 | 0.94 |
| | MCV (fL) | 55.20 | 57.45 | 1.62 | 0.32 |
| | MCH (pg) | 16.90 | 17.58 | 0.48 | 0.28 |
| | MCHC (g/dL) | 30.65 | 30.53 | 0.31 | 0.64 |
| Leukocytes indices | WBC ($10^3$/μL) | 6.63 | 7.85 | 0.82 | 0.34 |
| | Neutrophil ($10^3$/μL) | 2.61 | 3.27 | 0.33 | 0.17 |
| | Lymphocyte ($10^3$/μL) | 3.37 | 4.24 | 0.58 | 0.30 |
| | Monocyte ($10^3$/μL) | 0.42 | 0.28 | 0.06 | 0.14 |
| | Eosinophil ($10^3$/μL) | 0.21 | 0.06 | 0.10 | 0.21 |
| | Basophil ($10^3$/μL) | 0.02 | 0.02 | 0.01 | 1.00 |

SEM = standard error of mean.
1 55F, low forage diet containing imported tall fescue hay as forage sources
2 70F, high forage diet containing domestic mixed-sowing whole crop barley and Italian ryegrass silage as forage sources
3 TP, total protein; BUN, blood urea nitrogen; GLU, Glucose; RBC, red blood cell; Hb, hemoglobin concentration; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, total white blood cell count

## Ⅳ. DISCUSSION

DM intake was higher in 70F group may be explained by the better palatability of BIRG than TF to dry Holstein cows. It also appears that the 15% difference in forage ratio would not decrease the DM intake, but could increase the DM intake based on the better palatability of BIRG. Higher CP and NFC intake may be explained by the higher forage intake and higher CP and NFC content in BIRG.

Since soluble carbohydrate which is easy to be digested in rumen is not a great portion of forage but in concentrate (Van Soest, 1988; Johnson, 1976). Diet with higher concentrate ratio may generate more VFA and lactic acid (Slyter, 1976) and subsequently a lower rumen fluid pH value in 55F group than 70F group at the same time point. Meanwhile, pH changing trend may also be influenced by other factors such as rumen flora passage rate, microbial protein production or salivary flow to rumen (Sung et al., 2015), in the current study, different forage type may also contribute to the difference of pH in two groups. Preston (1972) reported that higher pH was found in the cattle which were fed with higher forage ratio diet. The results of this study for ruminal pH values were consistent with Preston's research and other previous researches (Cerrillo et al., 1999; Chen et al., 2015). Though the ruminal pH values in these two treatments were different, but all fell in the normal range of 5.5 - 6.8 and both suitable for rumen microorganism activity (Cunningham, 2002). Meanwhile, the ruminal pH values in this research were all above the value 6.0 which was normally defined as a threshold value for acidosis (Nocek, 1997).

Chen et al. (2015) reported that the results of concentration of individual VFA concentration in ruminants fed with diets containing different levels of forage are inconsistent among related experiments. Zinn and Plascencia (1996) reported that no statistically significant differences in pH, acetic acid, propionic acid, and butyric acid were observed between cattle in feedlot fed with different levels of alfalfa as a forage source in the diet. In this study, no significant differences were observed in acetic acid and propionic acid concentrations between two groups. The change of F:C ratio in two groups was not enormous, therefore, rumen microorganism ecosystem of Holstein cows may adapt to the change of feeds well and lead to a similar degradation rate of carbohydrate in two groups. This may be a possible explanation for the insignificant differences of acetic acid concentration and propionic acid concentration between two groups. Several researches (Merchen et al., 1986; Kinser et al., 1988) reported that acetic acid concentration was higher when the diet had a higher ratio of forage. The results of this study were consistent with the previous research in 9:30 and 10:30 h. Ma et al. (2015) reported that higher propionate concentration, higher butyrate concentration, and lower acetate to propionate ratio were observed when dietary NFC/NDF ratio was higher. In this study, the forage source and type were different and the NFC/NDF ratio of BIRG based diet is higher than TF based diet, meanwhile, digestion rate of silage is faster than hay. These reasons may adjust rumen microorganism in a more complex pattern and this may explain the results that higher propionic acid concentrations were observed in 70F group at 9:30 and 10:30 h, and subsequently acetic acid to propionic acid ratio was significantly lower in 70F group at these time points ($p < 0.05$). The significantly higher butyric acid concentration in 70F group at 9:30 and 10:30 h could be explained by the same reasons. Moreover, lower acetic acid to propionic acid ratio in 70F group indicated that cows fed with BIRG based diet showed better propionic acid type fermentation than 55F group. VFA is of paramount importance, providing about 70% of the ruminant's energy supply (France and Dijkstra, 2005). In particular, propionic acid is a substrate for gluconeogenesis and is the main source of glucose in the ruminant (Amaral et al., 1990; Chow and Jesse, 1992). Hence, BIRG based diet was expected to provide more energy for cows than TF based diet.

Nitrogenous compounds in feed are fermented into rumen fluid $NH_3$-N and used for synthesizing microbial proteins by rumen bacteria (Na et al., 2003). Llamas-Lamas and Combs (1991) reported that rumen fluid $NH_3$-N levels were high due to the high CP levels and degradability of the diets. For the reason of extensive proteolysis, which occurs during wilting and ensiling, degradability of silage protein is generally higher than that of hay (McDonald, 1982). In this study, BIRG based diet contained silage as a forage source; meanwhile, the CP intake was higher in 70F group. This may explain the higher values of rumen fluid $NH_3$-N in 70F group after morning

feeding.

Serum chemistry profiles and hematological profiles are important indicators of general health and vitality and the physiological changes of animals (Jain, 1993; Kumar and Pachaura, 2000; Chen et al., 2015). The results of serum chemistry profiles and hematological profiles all fell in the normal ranges (Jain, 1993; Hoff and Duffield, 2003; Morris, 2009). Protein and amino acid catabolism generates BUN. Higher CP intake in 70F group may lead to the higher BUN level in 70F group than 55F group which were 14.50 and 11.75 mg/dL ($p < 0.05$), respectively. No significant differences of hematological profiles between 55F and 70F groups may reflect that BIRG based diet did not suppress the immune system of animals for Holstein cows compared to TF based diet.

## Ⅴ. CONCLUSION

It was concluded that BIRG based diet with a high forage level had no adverse effects on rumen fermentation and blood chemical components (TP, BUN, and GLU) in dry Holstein cows compared to TF based diet with a low forage level. Thereafter, domestic BIRG could be used as a forage source for dry Holstein cows instead of imported TF. The replacement will decrease the cost of forages and save partial concentrate. With the information gained, more studies are necessary to focus on changes in the microbial populations when cows fed with BIRG based diet compared to TF based diet.

## Ⅵ. ACKNOWLEDGEMENT

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## Ⅶ. REFERENCES

Amaral, M.D., Veenhuizen, J.J., Drackley, J.K., Cooely, M.H., McGilliard A.D. and Young, J.W. 1990. Metabolism of propionate, glucose and carbon dioxide as affected by exogenous glucose in dairy cows at energy equilibrium. Journal of Dairy Science. 73:1244-1254.

AOAC 1990. Official methods of analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Cerrillo, M.A., Russellb, J.R. and Crump, M.H. 1999. The effects of hay maturity and forage to concentrate ratio on digestion kinetics in goats. Small Ruminant Research. 32:51-60.

Chen, G.J., Song, S.D., Wang, B.X., Zhang, Z.F., Peng, Z.L., Guo, C.H., Zhong, J.C. and Wang, Y. 2015. Effects of forage:concentrate ratio on growth performance, ruminal fermentation and blood metabolites in housing-feeding yaks. Asian-Australasian Journal of Animal Sciences. 28:1736-1741.

Chow, J.C. and Jesse, E.W. 1992. Interactions between gluconeogenesis and fatty acid oxidation in isolated sheep hepatocytes. Journal of Dairy Science. 75:2142-2148.

Cunningham, J.G. 2002. Textbook of veterinary physiology. WB Saunders. Philadelphia. pp.364-388.

Erwin, E.S., Marco, J. and Emery, E.M. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. Journal of Dairy Science. 44:1768-1770.

France, J. and Dijkstra, J. 2005. Volatile fatty acid production. In: J. Dijkstra, M. Forbes, and J. France (Ed.), Quantitative Aspects of Ruminant Digestion and Metabolism, CABI. Wallingford. UK. pp.157-175.

Her, N., Kim, J., and Yoon, Y. 2010. Perchlorate in dairy milk and milk‐based powdered infant formula in South Korea. Chemosphere. 81(6):732-737.

Hoff, B. and Duffield, T. Nutritional and metabolic profile testing of dairy cows. Available from: http://www.guelphlabservices.com/files/AHL/AHL%20LabNotes/LabNote04.pdf.AccessedSept.1,2016.

Jain, N.C. 1993. Essentials of Veterinary Hematology. Lea and Febiger. Philadelphia. USA. pp.1-417.

Johnson, R.R. 1976. Influence of carbohydrate solubility on non-protein nitrogen utilization in the ruminant. Journal of Animal Science. 43:184-191.

Kinser, A.R., Fahey, G.C., Berger, L.L. and Merchen, N.R. 1988. Low-quality roughages in high-concentrate pelleted diets for sheep: Digestion and metabolism of nitrogen and energy as affected by dietary fiber concentration. Journal of Animal Science. 66:487-500.

Kumar, B. and Pachaura, S.P. 2000. Haematological profile of crossbred dairy cattle to monitor herd health status at medium elevation in central Himalayas. Research in Veterinary Science. 69:141-145.

Lee, B.H., Ghassemi, N.J., Kim, H.S. and Sung, K.I. 2013. Effect of forage feeding level on the milk production characteristics of Holstein lactating cows. Journal of the Korean Society of Grassland and Forage Science. 33:45-51.

Llamas-lamas, G. and Combs, D.K. 1991. Effect of forage to concentrate ratio and intake level on utilization of early vegetative alfalfa silage by dairy cows. Journal of Dairy Science. 74:526-536.

Lyle, R.R., Johnson, R.R., Wilhite, J.V. and Backus, W.R. 1981. Ruminal characteristics in steers as affected by adaptation from forage to all-concentrate diets. Journal of Animal Science. 53:1383 -1390.

Ma, T., Tu, Y., Zhang, N.F., Deng, K.D. and Diao, Q.Y. 2015. Effect of the ratio of non-fibrous carbohydrates to neutral detergent fiber and protein structure on intake, digestibility, rumen fermentation, and nitrogen metabolism in lambs. Asian-Australasian Journal of Animal Science. 28:1419-1426.

Mani, S., Tabil, L.G. and Sokhansanj, S. 2004. Grinding performance and physical properties of wheat and barley straws, corn stover and switchgrass. Biomass Bioenergy. 27:339-352.

McDonald, P. 1982. The effect of conservation processes on the nitrogenous components of forages. Occasional Publication, British Society of Animal Production. 6:41-49.

Merchen, N.R., Firkins, J.L. and Berger, L.L. 1986. Effect of intake and forage level on ruminal turnover rates, bacterial protein synthesis and duodenal amino acid flows in sheep. Journal of Animal Science. 62:216-225.

Morris, D.D. 2009. Alterations in the Erythron/Leukogram. In: B.P. Smith (Ed.), Large Animal Internal Medicin, C.V. Mosby, St. Louis Missouri.US. pp. 400-410.

Na, R., Dong, H., Zhu, Z., Chen, Y. and Xin, H. 2003. Effects of forage type and dietary concentrate to forage ratio on methane emissions and rumen fermentation characteristics of dairy cows in China. Transactions of the ASABE. 56:1115-1122.

NRC 2001. Nutrient requirements of dairy cattle. 7th ed, National Academy Press, Washington, DC.

Nocek, J.E. 1997. Bovine acidosis: Implications on laminitis. Journal of Dairy Science. 80:1005-1028.

Peng, J.L., Kim, M.J., Kim, B.W., and Sung, K.I. 2016. Models for estimating yield of Italian ryegrass in south areas of Korean peninsula and Jeju Island. Journal of the Korean Society of Grassland and Forage Science. 36(3): 223-236.

Preston, T.R. 1972. Molasses as energy source for cattle. World Review of Nutrition and Dietetics. 17:250-311.

Seo, S. and Yook, W.B. 2002. Studies on the forage production and utilization on paddy field in Korea. Proceedings of Intentional symposium on forage production and environment in the 21st century. Seoul. Korea. pp. 93-96.

Seo S. 2016. Forage production, utilization, and animal husbandry in Korea. Proceedings of the 6th Korea‐China‐Japan grassland conference. Jeju, South Korea. pp.5-15.

Slyter, L.L. 1976. Influence of acidosis on rumen function. Journal of Animal Science. 43:910-929.

Soon, H.B., Jung, G.W., Kim, W.H., Lim, Y.C. and Kim, J. D. 2010. The Effect of feeding mixed-sowing winter forage crop and whole crop barley silage on feed intake, nutrient digestibility and blood characteristics in the Korean black goats. Journal of The Korean Society of Grassland and Forage Science, 30(1):49-58.

Sung, K.I. 2000. Quality and evaluation of imported forages. Proceedings of Symposium of the Korean Society of Grassland Sciences. Cheonan. Korea. pp. 19-53.

Sung, K.I., Ghassemi, N.J., Song, Y.H., Kim, S.Y., Lee, B.H. and Kim, W.H. 2012. A comparison of feeding whole crop barley mixed with Italian ryegrass silage versus tall fescue hay for Holstein growing cattle. Proceedings of the XVI International Silage Conference. Helsinki. Finland. pp. 508-509.

Sung, K.I., Ghassemi, N.J., Hong, S.M., Ohh, S.J., Lee, B.H., Peng, J.L., Ji, D.H. and Kim, B.W. 2015. Effects of forage level and chromium-methionine chelate supplementation on performance, carcass characteristics and blood metabolites in Korean native (Hanwoo) steers. Journal of Animal Science and Technology. 57:14-20.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 74:3583-3597.

Van Soest, P.J. 1988. Effect of environment and quality of fibre on the nutritive value of crop residues. Proceedings of a Workshop held at International Livestock Center for Africa: Plant breeding and the nutritive value of crop residues. Addis Ababa. Ethiopia. pp.71-96.

Zinn, R.A. and Plascencia, A. 1996. Effects of forage level on the comparative feeding value of supplemental fat in growing-finishing diets for feedlot cattle. Journal of Animal Science. 74:1194-1201.

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