

# Evaluation of the nutritional value of locally produced forage in Korea using chemical analysis and *in vitro* ruminal fermentation

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**Objective:** The use of locally produced forage (LPF) in cattle production has economic and environmental advantages over imported forage. The objective of this study was to characterize the nutritional value of LPF commonly used in Korea. Differences in ruminal fermentation characteristics were also examined for the LPF species commonly produced from two major production regions: Chungcheong and Jeolla.

**Methods:** Ten LPF (five from each of the two regions) and six of the most widely used imported forages originating from North America were obtained at least three times throughout a year. Each forage species was pooled and analyzed for nutrient content using detailed chemical analysis. Ruminal fermentation characteristics were also determined by *in vitro* anaerobic incubations using strained rumen fluid for 0, 3, 6, 12, 24, and 48 h. At each incubation time, total gas, pH, ammonia, volatile fatty acid (VFA) concentrations, and neutral detergent fiber digestibility were measured. By fitting an exponential model, gas production kinetics were obtained.

**Results:** Significant differences were found in the non-fiber carbohydrate (NFC) content among the forage species and the regions ( $p < 0.01$ ). No nutrient, other than NFC, showed significant differences among the regions. Crude protein, NFC, and acid detergent lignin significantly differed by forage species. The amount of acid detergent insoluble protein tended to differ among the forages. The forages produced in Chungcheong had a higher amount of NFC than that in Jeolla ( $p < 0.05$ ). There were differences in ruminal fermentation of LPF between the two regions and interactions between regions and forage species were also significant ( $p < 0.05$ ). The pH following a 48-h ruminal fermentation was lower in the forages from Chungcheong than from Jeolla ( $p < 0.01$ ), and total VFA concentration was higher in Chungcheong than in Jeolla ( $p = 0.05$ ). This implies that fermentation was more active with the forages from Chungcheong than from Jeolla. Analysis of gas production profiles showed the rate of fermentation differed among forage species ( $p < 0.05$ ).

**Conclusion:** The results of the present study showed that the nutritional values of some LPF (i.e., corn silage and Italian ryegrass) are comparable to those of imported forages widely used in Korea. This study also indicated that the nutritional value of LPF differs by origin, as well as by forage species. Detailed analyses of nutrient composition and digestion kinetics of LPF should be routinely employed to evaluate the correct nutritional value of LPF and to increase their use in the field.

**Keywords:** Digestion Kinetics; *In vitro* Ruminal Fermentation; Locally Produced Forage; Nutrient Analysis; Ruminant

## INTRODUCTION

The use of locally produced forage (LPF) in cattle production has economic and environmental advantages over imported forage. Imported forage is more expensive than LPF (e.g. twice as expensive in Korea) on a dry matter (DM) or total digestible nutrient (TDN) basis owing to the addition of transportation costs [1]. The use of LPF can also increase the sustainability of

cattle production, by reducing its adverse environmental impacts [2]. However, in some Asian countries, including Korea and Japan, where the primary land use is for crop production, ruminant, and especially dairy, production, mostly relies on imported forage. Furthermore, dairy farmers tend to believe that the quality of LPF is poorer compared to imported ones, even though this is not always true [3].

One of the drawbacks of using LPF is that the detailed nutritional information of LPF is rarely available. Knowledge of the nutrient composition of forage is essential for balancing rations in order to maintain animal health and level of production. However, in developing countries, regular feed analyses may not be possible because of the limited numbers of feed laboratories [4]. Further, implementation of new chemical entities in feed analysis may be slow owing to the tendency to conservatively continue to depend on established procedures, in spite of their limitations, as reported by Van Soest [5].

In Korea, the central Chungcheong and southwestern Jeolla regions produce over 70% of the LPF in Korea [6]. The two regions are geographically close, and little difference exists in terms of forage variety [7]. Therefore, it has been assumed that the nutritional value of the forage produced from the two regions is the same within the same forage species. Nevertheless, the presence of regional differences in the nutritional value of LPF should be evaluated, as the quality of forage can vary widely for various reasons [8]. However, differences in the detailed nutrient composition and rate of ruminal fermentation have never been reported for the LPF in Korea.

The present study characterizes the nutritional value of LPF commonly used in Korea using chemical analysis and an *in vitro* fermentation technique. Chemical analysis of commonly used imported forages was also performed, and their nutritional values were compared with LPF. In addition, an *in vitro* analysis was conducted to test whether there are differences in ruminal fermentation characteristics between the same species of LPF from the two major production regions in Korea: Chungcheong and Jeolla.

## MATERIALS AND METHODS

This study involved two fistulated Holstein dry cows maintained at the Center for Animal Science Research, Chungnam National University, Korea. Animal use and protocols were reviewed and approved by the Chungnam National University Animal Research Ethics Committee (CNU-00455).

### Forage samples

A total of 10 LPF (five from each region, central Chungcheong, and southwestern Jeolla), as well as six most commonly used imported forage were used in this study. The LPF were corn silage, Italian ryegrass, sudangrass, and rice straw produced from both regions. Ryegrass, produced only from Chungcheong,

and barley silages, produced only from Jeolla, were included. The imported forages used in this study were alfalfa hay, Timothy, kline hay, ryegrass straw, pine fescue straw, and tall fescue straw, which were imported from North America. The forage samples were purchased at the same time, at least three times a year, and were pooled by each forage species and origin.

The forage samples were dried at 60°C for 96 h and ground using a cyclone mill (Foss Tecator Cyclotec 1093, Foss, Hillerød, Denmark) fitted with a 1 mm screen, prior to chemical analysis and *in vitro* ruminal fermentation. All the forage samples were analyzed for their chemical composition. *In vitro* ruminal fermentation was conducted for the four forage species produced from both regions.

### Chemical analysis

Chemical analysis was carried out on the basis of the CNCPS fractionation scheme [9]. The content of DM (#934.01), crude protein (CP; #976.05), ether extract (EE; #920.39), acid detergent fiber (ADF; #973.18), and ash (#942.05) were determined as described by AOAC International [10]. CP was calculated as 6.25 times the nitrogen content, and total nitrogen measured by the Kjeldahl method using a DK 20 Heating Digester and Semi-Automatic Distillation Unit Model UDK 139 (VELP Scientifica, Usmate, Italy). Neutral detergent fiber was analyzed using a heat-stable amylase, and expressed inclusive of residual ash (aNDF), and acid detergent lignin (ADL) was analyzed, as described by Van Soest et al [11]. Neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) were also determined [12].

Non-fiber carbohydrate (NFC) was calculated by  $100 - CP - EE - ash - (NDF - NDICP)$ , and the TDN of forage samples was estimated based on the equations in National Research Council [13].

### *In vitro* ruminal fermentation and analyses

Before the morning feeding, rumen fluid was collected from two cannulated, non-lactating Holstein cows fed twice daily a ration consisting of 600 g/kg timothy hay and 400 g/kg commercial concentrate mix ( $123 \pm 8.8$  g/kg CP,  $35 \pm 6.4$  g/kg EE,  $265 \pm 6.9$  g/kg NDF, and  $109 \pm 1.2$  g/kg ash), at the Center for Animal Science Research, Chungnam National University, Korea. For a comparison without adaptation to the tested samples, none of the tested forages was fed to the cows. The rumen contents obtained from two cows were mixed and transferred to a thermos bottle, and immediately transported to the laboratory. The rumen content was strained through four layers of cheesecloth and mixed with four times the volumes of *in vitro* solution [14] under strictly anaerobic conditions. A volume of 50 mL of rumen fluid/buffer mixture was transferred into 125-mL serum bottles containing 0.5 g of forage samples under continuous flushing with O<sub>2</sub>-free CO<sub>2</sub> gas. The bottles were sealed with butyl rubber stoppers and aluminum caps and then incubated for 0, 3, 6,

12, 24, 48, and 72 h in an incubator at 39°C.

After each incubation time, total gas production was measured using a pressure transducer (Sun Bee Instruments, Inc., Seoul, Korea), as described by Theodorou et al [15]. pH of the culture fluid was measured using a general-purpose pH meter (Istek Inc., Seoul, Korea). The culture fluid was then centrifuged at 14,000×g for 10 min at 4°C, and some of the supernatant was stored and used for the analyses of volatile fatty acids (VFA) and ammonia concentrations. The remaining undegraded samples and fluid were analyzed for NDF using a modified version of the micro-NDF method proposed by Pell and Schofield [16] for measuring NDF degradability.

For the analysis of VFA [17], 1 mL of supernatant was mixed with 0.2 mL of metaphosphoric acid (250 g/L) in a 2-mL Eppendorf tube and kept at 4°C for 30 min. After re-centrifugation of the mixture at 14,000×g for 10 min at room temperature, the supernatant was injected into a gas chromatograph (HP 6890, Hewlett-Packard, Wilmington, DE, USA) equipped with a flame ionization detector and an SGE BP21 column (SGE, Melbourne, Australia). The temperature of the oven, injector, and detector was set at 150°C, 250°C, and 200°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 124 mL/min. Ammonia concentration was analyzed following the method of Chaney and Marbach [18]. After the supernatant was mixed with phenol color reagent and alkali-hypochlorite reagent, the mixture was incubated in a 37°C water bath for 15 min. The ammonia concentration was determined by measuring the absorbance using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 630 nm.

Gas production profiles during incubation were fitted to a simple exponential model with a single lag [19] using the NLIN procedure of SAS, in order to estimate parameters of gas production in a first order model as:

$$V_T = 0 \quad (0 \leq T \leq L)$$

$$V_T = V_{max} \times \{1 - \text{EXP}[-k_g \times (T - L)]\} \quad (0 \leq T \leq L)$$

where T is time (h), L is discrete lag time (h), EXP is the exponential function,  $k_g$  is the fractional rate of gas production ( $\text{h}^{-1}$ ),  $V_{max}$  is the theoretical maximum gas production (mL) after the asymptote is reached, and  $V_T$  is gas produced at time T (mL).

### Statistical analysis

The data from the chemical analysis and *in vitro* ruminal fermentation study were analyzed using the GLM procedure of SAS. The general linear model for the statistical analysis is as follows:

$$y_{ijk} = \mu + \tau_i + \gamma_j + \tau_i \gamma_j + e_{ijk}$$

where  $y_{ijk}$  is the kth observation in jth region and jth forage species,  $\mu$  is the overall mean,  $\tau_i$  is the fixed effect of the ith region,

$\gamma_j$  is the fixed effect of the jth forage species,  $\tau_i \gamma_j$  is the interaction between region and forage species, and  $e_{ijk}$  is the unexplained random effect.

All the chemical analysis data were analyzed without the interaction term, and the effect of forage species was treated as a block. The purpose of this analysis was not to compare differences in means among Chungcheong, Jeolla, and imported forages, but to identify the most variable chemical components among the forages commonly used in Korea. Following this, the nutrient composition of selected LPF (e.g., corn silage, Italian ryegrass, sudangrass, and rice straw) was compared to test for differences in the nutrient composition between forages and the two regions in Korea. The same linear model without the interaction term was used for this analysis. For the analysis of the *in vitro* ruminal fermentation study, a 2×4 factorial design was used with the region as the first factor and the forage species as the second factor. Each replicate (i.e., the mean of 3 bottles for each treatment at each incubation time) of the triplicate incubations was the experimental unit. When the interaction was not significant (i.e., gas production profiles), the data were re-analyzed without the interaction term.

Pair-wise comparisons of the least square means were also conducted using the PDIF option with Tukey-Kramer adjustment if a significant difference was observed. Statistical significance was declared at  $p < 0.05$ , and a trend was discussed at  $0.05 \leq p < 0.1$ .

## RESULTS

Chemical composition of the forages varied widely by species (Table 1). CP ranged from 39 to 189 g/kg DM, EE from 8 to 32 g/kg DM, ash from 32 to 131 g/kg DM, aNDF from 509 to 814 g/kg DM, ADF from 273 to 644 g/kg DM, ADL from 23 to 84 g/kg DM, NDICP from 14 to 55 g/kg DM, ADICP from 6 to 48 g/kg DM, NFC from 32 to 282 g/kg DM, and  $\text{TDN}_{1x}$  from 421 to 662 g/kg DM. The most significantly variable nutrient among the forages was NFC. There were significant differences in the NFC content between forages and between regions ( $p < 0.01$ ). No other nutrient, other than NFC, showed a significant difference among the forages from Chungcheong, Jeolla, and imported forages. Crude protein, NFC, and ADL significantly differed by forage species. The amount of ADICP tended to be different among the forages.

Statistical analysis of forages produced from both regions (i.e., corn silage, Italian ryegrass, sudangrass, and rice straw) showed significant differences in the chemical composition between Chungcheong and Jeolla (Table 2). The forages produced in Chungcheong had a higher amount of NFC than those in Jeolla ( $p < 0.05$ ). Significant differences were observed in the CP, ADL, NFC, and  $\text{TDN}_{1x}$  content among forages.

*In vitro* incubation using strained rumen fluid exhibited differences in ruminal fermentation of the forages from the two

**Table 1.** Analyzed chemical composition of forages commonly used in Korea (g/kg on DM basis)

| Forages                             | Components (g/kg on DM basis) |       |      |      |      |      |      |       |       |      |                   |
|-------------------------------------|-------------------------------|-------|------|------|------|------|------|-------|-------|------|-------------------|
|                                     | DM (g/kg as fed)              | CP    | EE   | Ash  | aNDF | ADF  | ADL  | NDICP | ADICP | NFC  | TDN <sub>1x</sub> |
| Locally produced forages            |                               |       |      |      |      |      |      |       |       |      |                   |
| Chungcheong, central region         |                               |       |      |      |      |      |      |       |       |      |                   |
| Corn silage                         | 445                           | 89    | 28   | 59   | 601  | 273  | 23   | 24    | 6     | 247  | 662               |
| Italian ryegrass                    | 312                           | 134   | 24   | 91   | 626  | 455  | 49   | 15    | 13    | 140  | 561               |
| Sudangrass                          | 313                           | 39    | 13   | 64   | 739  | 568  | 50   | 14    | 10    | 159  | 541               |
| Rice straw                          | 673                           | 53    | 11   | 79   | 808  | 644  | 81   | 16    | 6     | 65   | 460               |
| Ryegrass                            | 235                           | 86    | 27   | 78   | 756  | 594  | 47   | 21    | 12    | 75   | 549               |
| Jeolla, southern region             |                               |       |      |      |      |      |      |       |       |      |                   |
| Corn silage                         | 207                           | 75    | 15   | 62   | 717  | 468  | 30   | 24    | 17    | 152  | 588               |
| Italian ryegrass                    | 580                           | 134   | 30   | 79   | 726  | 604  | 36   | 30    | 9     | 62   | 585               |
| Sudangrass                          | 369                           | 62    | 18   | 87   | 741  | 586  | 54   | 22    | 11    | 115  | 521               |
| Rice straw                          | 566                           | 44    | 20   | 131  | 791  | 619  | 84   | 18    | 6     | 32   | 421               |
| Barley silage                       | 400                           | 66    | 18   | 63   | 814  | 446  | 42   | 21    | 14    | 62   | 543               |
| Imported forages                    |                               |       |      |      |      |      |      |       |       |      |                   |
| Alfalfa hay                         | 906                           | 189   | 19   | 93   | 509  | 297  | 52   | 55    | 48    | 246  | 561               |
| Timothy hay                         | 903                           | 101   | 20   | 59   | 695  | 497  | 40   | 28    | 12    | 152  | 588               |
| Kline hay                           | 914                           | 85    | 15   | 71   | 577  | 490  | 32   | 30    | 15    | 282  | 616               |
| Ryegrass straw                      | 809                           | 46    | 8    | 53   | 769  | 636  | 47   | 20    | 7     | 143  | 551               |
| Pine fescue straw                   | 856                           | 55    | 14   | 32   | 752  | 589  | 63   | 14    | 9     | 161  | 550               |
| Tall fescue straw                   | 955                           | 64    | 10   | 67   | 743  | 433  | 55   | 26    | 7     | 142  | 534               |
| p-value of the differences in means |                               |       |      |      |      |      |      |       |       |      |                   |
| Forage                              | -                             | 0.02  | 0.70 | 0.49 | 0.14 | 0.19 | 0.04 | 0.11  | 0.08  | 0.03 | 0.11              |
| Region                              | -                             | <0.93 | 0.74 | 0.33 | 0.23 | 0.21 | 0.96 | 0.16  | 0.58  | 0.02 | 0.28              |

DM, dry matter; CP, crude protein; EE, ether extract; aNDF, neutral detergent fiber using a heat stable  $\alpha$ -amylase without sodium sulfite and expressed inclusive of residual ash; ADF, acid detergent fiber; ADL, acid detergent lignin; NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein; NFC, non-fiber carbohydrates; TDN<sub>1x</sub>, total digestible nutrient at 1x maintenance feed intake.

regions (Table 3). More interestingly, interactions between region and forage species were observed in all the measured values. The pH following a 48-h ruminal fermentation was lower for the forages from Chungcheong (6.42) than those from Jeolla (6.45;  $p < 0.01$ ). Consistently, the total VFA concentration was also higher in Chungcheong than in Jeolla (51.5 vs 50.6 mM;  $p = 0.05$ ). These values indicate that fermentation was more

active with the forages from Chungcheong than from Jeolla. With the exception of rice straw, the forages from Chungcheong had a lower pH and higher total VFA concentration than those from Jeolla, which resulted in a significant interaction between region and forage species ( $p < 0.01$ ). Among forages, the reduction in pH was higher in corn silage, as expected. The pH following a 48-h *in vitro* ruminal fermentation was 6.32, 6.44, 6.47, and

**Table 2.** Comparisons of nutrient composition in selected locally produced forages obtained from two different regions in Korea (g/kg on dry matter basis)

| Nutrient          | Region                |                   |      |         | Forage variety   |                   |                   |                  |      |         |
|-------------------|-----------------------|-------------------|------|---------|------------------|-------------------|-------------------|------------------|------|---------|
|                   | Chungcheong (central) | Jeolla (southern) | SEM  | p-value | Corn silage      | IRG               | Sudangrass        | Rice straw       | SEM  | p-value |
| Crude protein     | 79                    | 80                | 5.5  | 0.93    | 84 <sup>ab</sup> | 134 <sup>a</sup>  | 51 <sup>b</sup>   | 49 <sup>b</sup>  | 7.8  | 0.01    |
| Ether extract     | 19                    | 21                | 3.5  | 0.75    | 22               | 27                | 16                | 16               | 5.0  | 0.44    |
| Ash               | 73                    | 90                | 9.9  | 0.33    | 61               | 85                | 105               | 76               | 14.0 | 0.33    |
| aNDF              | 694                   | 744               | 23.8 | 0.23    | 659              | 676               | 740               | 800              | 33.7 | 0.16    |
| ADF               | 485                   | 569               | 37.0 | 0.21    | 371              | 530               | 577               | 632              | 52.3 | 0.12    |
| ADL               | 51                    | 51                | 3.2  | 0.96    | 27 <sup>b</sup>  | 43 <sup>b</sup>   | 52 <sup>ab</sup>  | 83 <sup>a</sup>  | 4.5  | 0.01    |
| NDICP             | 17                    | 24                | 2.4  | 0.16    | 24               | 23                | 18                | 17               | 3.4  | 0.50    |
| ADICP             | 9                     | 11                | 2.3  | 0.58    | 12               | 11                | 11                | 6                | 3.2  | 0.64    |
| NFC               | 153                   | 90                | 10.2 | 0.02    | 200 <sup>a</sup> | 101 <sup>ab</sup> | 137 <sup>ab</sup> | 49 <sup>b</sup>  | 14.5 | 0.02    |
| TDN <sub>1x</sub> | 556                   | 529               | 14.4 | 0.28    | 625 <sup>a</sup> | 573 <sup>ab</sup> | 531 <sup>ab</sup> | 440 <sup>b</sup> | 20.4 | 0.03    |

SEM, standard error of the mean; IRG, Italian ryegrass; aNDF, neutral detergent fiber using a heat stable  $\alpha$ -amylase without sodium sulfite and expressed inclusive of residual ash; ADF, acid detergent fiber; ADL, acid detergent lignin; NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein; NFC, non-fiber carbohydrates; TDN<sub>1x</sub>, total digestible nutrient at 1x maintenance feed intake.

<sup>a,b</sup> Means in the same row that do not have common superscript differ significantly ( $p < 0.05$ ).

**Table 3.** Ruminal fermentation characteristics following a 48-h *in vitro* incubation of forages using strained rumen fluid

| Item                       | Chungcheong, central region |                   |                   |                   | Jeolla, southern region |                   |                    |                     | SEM   | p-value |        |                 |
|----------------------------|-----------------------------|-------------------|-------------------|-------------------|-------------------------|-------------------|--------------------|---------------------|-------|---------|--------|-----------------|
|                            | Corn silage                 | IRG               | Sudan-grass       | Rice straw        | Corn silage             | IRG               | Sudan-grass        | Rice straw          |       | Region  | Forage | Region x forage |
| pH                         | 6.29 <sup>d</sup>           | 6.42 <sup>b</sup> | 6.44 <sup>b</sup> | 6.54 <sup>a</sup> | 6.35 <sup>c</sup>       | 6.45 <sup>b</sup> | 6.50 <sup>a</sup>  | 6.51 <sup>a</sup>   | 0.009 | <0.01   | <0.01  | <0.01           |
| NDFD (g/kg)                | 560 <sup>ab</sup>           | 577 <sup>a</sup>  | 408 <sup>b</sup>  | 236 <sup>c</sup>  | 466 <sup>ab</sup>       | 600 <sup>a</sup>  | 343 <sup>bc</sup>  | 374 <sup>bc</sup>   | 31.7  | 0.977   | <0.01  | <0.01           |
| NH <sub>3</sub> -N (mg/dL) | 15.0 <sup>cd</sup>          | 23.0 <sup>a</sup> | 13.3 <sup>d</sup> | 17.4 <sup>b</sup> | 16.0 <sup>bc</sup>      | 21.1 <sup>a</sup> | 16.5 <sup>bc</sup> | 15.4 <sup>bcd</sup> | 0.49  | 0.839   | <0.01  | <0.01           |
| VFA (mM)                   | 60.9 <sup>a</sup>           | 55.4 <sup>b</sup> | 50.5 <sup>c</sup> | 39.3 <sup>f</sup> | 59.4 <sup>a</sup>       | 54.7 <sup>b</sup> | 46.0 <sup>d</sup>  | 42.3 <sup>e</sup>   | 0.61  | 0.050   | <0.01  | <0.01           |
| Acetate (mmol/mol)         | 624 <sup>e</sup>            | 667 <sup>c</sup>  | 709 <sup>a</sup>  | 701 <sup>a</sup>  | 644 <sup>d</sup>        | 663 <sup>c</sup>  | 676 <sup>b</sup>   | 706 <sup>a</sup>    | 1.8   | 0.047   | <0.01  | <0.01           |
| Propionate (mmol/mol)      | 263 <sup>a</sup>            | 220 <sup>c</sup>  | 196 <sup>d</sup>  | 188 <sup>e</sup>  | 237 <sup>b</sup>        | 224 <sup>c</sup>  | 218 <sup>c</sup>   | 193 <sup>de</sup>   | 1.3   | 0.143   | <0.01  | <0.01           |
| Butyrate (mmol/mol)        | 85 <sup>b</sup>             | 77 <sup>c</sup>   | 79 <sup>c</sup>   | 71 <sup>d</sup>   | 92 <sup>a</sup>         | 69 <sup>d</sup>   | 77 <sup>c</sup>    | 72 <sup>d</sup>     | 0.6   | 0.237   | <0.01  | <0.01           |
| A/P ratio                  | 2.4 <sup>e</sup>            | 3.0 <sup>bc</sup> | 3.6 <sup>a</sup>  | 3.7 <sup>a</sup>  | 2.7 <sup>d</sup>        | 3.0 <sup>c</sup>  | 3.1 <sup>b</sup>   | 3.7 <sup>a</sup>    | 0.02  | <0.01   | <0.01  | <0.01           |

IRG, Italian ryegrass; SEM, standard error of the mean; NDFD, Neutral detergent fiber digestibility; VFA, Volatile fatty acid; A/P ratio, Acetate to propionate ratio.

<sup>a-f</sup> Means in the same row that do not have common superscript differ significantly ( $p < 0.05$ ).

6.53 in corn silage, Italian ryegrass, sudangrass, and rice straw, respectively. Similarly, the total VFA concentration was highest in corn silage (60 mM), followed by Italian ryegrass (55 mM), sudan grass (48 mM), and rice straw (41 mM).

There were significant differences between the two regions, among forage species, and their interactions in the proportion of acetate in total VFA and acetate to propionate ratio (A/P ratio, Table 3). The forages from Chungcheong produced more acetate than those from Jeolla ( $p < 0.05$ ); thus, the A/P ratio was higher in Chungcheong than in Jeolla (3.19 vs 3.11;  $p < 0.01$ ). Compared to Jeolla, the A/P ratio of Chungcheong was higher in sudan-grass and lower in corn silage, which resulted in a significant interaction ( $p < 0.01$ ). Among forages, the A/P ratio was the highest in rice straw (3.7), followed by sudangrass (3.4), Italian ryegrass (3.0), and corn silage (3.7).

NDF digestibility (NDFD) and NH<sub>3</sub>-N concentration differed among the forage species ( $p < 0.01$ ), and there was also a significant interaction between region and forage species ( $p < 0.01$ , Table 3). Among forages, ruminal NDFD after a 48-h incubation was highest for Italian ryegrass (589 g/kg), followed by corn silage (513 g/kg), sudangrass (376 g/kg), and rice straw (305 g/kg). NH<sub>3</sub>-N concentration was highest in Italian ryegrass (22 mg/dL), followed by rice straw (16 mg/dL), corn silage (16 mg/dL), and sudangrass (15 mg/dL). Regarding NDFD values for corn silage and sudangrass were higher in Chungcheong than in Jeolla, but these values were lower for Italian ryegrass and rice straw ( $p < 0.01$ ). Similarly, NH<sub>3</sub>-N concentrations in Italian ryegrass and rice straw were higher in Chungcheong than in Jeolla, but lower in corn silage and sudangrass.

Analysis of gas production profiles showed that the rate of fermentation differed among forage species. No interaction between region and forage species was observed in the *in vitro* ruminal gas production profiles (i.e., theoretical maximum gas production [ $V_{max}$ ] and fractional rate of gas production [ $k_g$ ]); therefore, the data were analyzed with only the main factors (i.e., region and forage species). There was no significant difference between the two regions (Table 4). Mean  $V_{max}$  and  $k_g$  values

were 131 mL and 0.021 h<sup>-1</sup> for the forages from Chungcheong, and 129 mL and 0.021 h<sup>-1</sup> for those from Jeolla, respectively. In contrast, significant differences in  $V_{max}$  and  $k_g$  values among different forage species were observed ( $p < 0.05$ ).  $V_{max}$  value was significantly greater in corn silage compared to other forage species ( $p < 0.05$ ). Corn silage (0.030 h<sup>-1</sup>) and Italian ryegrass (0.028 h<sup>-1</sup>) had significantly greater  $k_g$  values than sudangrass (0.019 h<sup>-1</sup>) and rice straw (0.010 h<sup>-1</sup>). The fractional rate of gas production was greater in sudangrass than in rice straw.

## DISCUSSION

Detailed analysis of chemical composition and *in vitro* fermentation using strained rumen fluid clearly showed that the nutritional characteristics were different between the LPF from Chungcheong and those from Jeolla. To the best of our knowledge, a consistent regional bias in the chemical composition of the forages and a significant interaction between regions and forages

**Table 4.** The fitted parameters of gas production profiles

| Items                       | $V_{max}^{1)}$   | $k_g^{2)}$         |
|-----------------------------|------------------|--------------------|
| Regions                     |                  |                    |
| Chungcheong, central region | 131              | 0.022              |
| Jeolla, southern region     | 129              | 0.021              |
| SEM                         | 4.8              | 0.0008             |
| p-value                     | 0.864            | 0.415              |
| Forages                     |                  |                    |
| Corn silage                 | 151 <sup>a</sup> | 0.030 <sup>a</sup> |
| Italian ryegrass            | 111 <sup>b</sup> | 0.028 <sup>a</sup> |
| Sudangrass                  | 131 <sup>b</sup> | 0.019 <sup>b</sup> |
| Rice straw                  | 127 <sup>b</sup> | 0.010 <sup>c</sup> |
| SEM                         | 6.8              | 0.0011             |
| p-value                     | 0.006            | <0.001             |

SEM, standard error of the mean.

<sup>1)</sup>Theoretical maximum gas production (mL).

<sup>2)</sup>Fractional rate of gas production (h<sup>-1</sup>); Italian ryegrass neutral detergent fiber digestibility.

<sup>a,b,c</sup> Means in the same column that do not have common superscript differ significantly ( $p < 0.05$ ).

have not been previously reported. It has been assumed that the nutritional value of the forages produced from the two regions is the same within the same forage species. The two regions are geographically close, and little difference exists in terms of forage variety and the management practice for forage crop production between the two regions [7].

Analysis of the nutrient composition and ruminal fermentation, however, showed that the forages produced in Chungcheong were nutritionally better than those from Jeolla, in this study. Specifically, the NFC content and total VFA production following ruminal fermentation were greater in the LPF from Chungcheong than from Jeolla. The amount of NFC, mostly sugars and starch, is closely related to the amount of energy that a feed can supply and the microbial protein synthesis in the rumen [13]. The amount of NFC is also positively correlated with the amount of gas produced per DM and total VFA, especially propionate and butyrate [19].

The amount of NFC in crop silage is related to the amount of grain included while producing a silage. If the amount of grain included in a silage is low, the aNDF content, representing the amount of fibrous materials, should increase. LPF from Chungcheong contained 247 g/kg NFC and 601 g/kg aNDF; LPF from Jeolla had 152 g/kg NFC and 717 g/kg aNDF. The NFC and aNDF contents of a forage are also affected by the stage of maturity and supply of nutrient during the growth of a plant. As a plant matures or the nutrient supply is sufficient, NFC decreases, while aNDF increases [8]. All the forages from Chungcheong had a higher amount of NFC and a lower amount of aNDF, respectively, than those from Jeolla, within the same species. As there is little variation in the variety of each forage species, different management of the crop forage production could result in biased nutrient content of the forages between the two regions. Moreover, Chungcheong is cooler in summer and colder in winter, and this may also affect the differences in NFC and aNDF contents and ruminal fermentation of the forages [8].

The chemical composition of the forages varied widely by species, as expected (Table 1). This was consistent with other studies, and some forage nutrient values that are usually analyzed (i.e. CP, EE, ash), were within the range of reported ones [3,20,21]. Rice straw contained the lowest CP, NFC, and TDN<sub>1x</sub>, but the highest ADL, indicating that it is least nutritious among the tested forages. Rice straw also has a large amount of ADL, and reduces the digestibility of NDF and DM [8]. Locally produced corn silage and Italian ryegrass, however, had a relatively large amount of CP, NFC, digestible NDF, and TDN<sub>1x</sub>, and they were comparable with the imported forages widely used for lactating dairy cows (i.e., alfalfa hay, Timothy hay, and Kline hay). Consequently, the production and use of LPF, especially corn silage and Italian ryegrass, needs to be encouraged because their nutritional value is similar to that of imported forages.

Statistical analysis identified a list of nutrients that differed

among the forages: CP, ADL, NFC, and ADICP (Table 1). The coefficient of variation (CV, %), and standard deviation as a percentage of mean, were also calculated. Among the nutrients, ADICP varied the most, followed by NFC, CP, NDICP, EE, and ADL. The CV of ADICP, NFC, CP, NDICP, EE, and ADL were 79.7%, 51.8%, 48.8%, 42.0%, 36.1%, and 33.9%, respectively. The amount of acid detergent insoluble crude protein and NDICP determine the amount of available CP and its degradability, respectively [9]. The large variability in CP, NDICP, and ADICP indicate that the availability of CP varies considerably among the forages, although the amounts of CP, NDICP, and ADICP are correlated. In the present study, the Pearson correlation coefficient between CP and NDICP, between CP and ADICP, and between NDICP and ADICP were 0.76, 0.72, and 0.81, respectively, among the forage. The amount of NFC also varied depending on the forage.

For estimating the NFC content, both NDF and NDICP need to be analyzed. In the proximate analysis, nitrogen-free extract (NFE) using crude fiber (CF) is estimated instead of NFC. It has been shown that CF cannot represent the amount of fiber in forages, and CF and NFE must be replaced by NDF and NFC, respectively [5]. Some recent studies, however, used crude fiber and NFE for comparing the nutritional value of forages [3,20,21]. Van Soest [5] indicated this may be because there is a conservative tendency to continue to depend on established procedures, despite their limitations. Detailed analysis of chemical composition, however, should be performed for balancing rations, in order to maintain animal health and level of production.

The differences in nutrient content among the forages were also reflected in the gas production profiles. Specifically, the fractional rate of gas production successfully differentiated the digestion kinetics among the forages. The reported amount of gas produced by the DM of high-quality hay during *in vitro* fermentation was 0.37 to 0.39 mL/mg [16]. The amount of gas produced by the DM in the present study was 0.26 to 0.41 mL/mg DM, which was mainly due to the wide variation in the quality of forages used. The fractional rates of gas production of corn silage and rice straw in the present study were lower than those reported previously [19], mainly because the nutritional quality of corn silage and rice straw used in this study were lower. The *in vitro* gas production technique has been widely used to evaluate the nutritional value and ruminal fermentation kinetics of forages [22].

The use of LPF is gaining great interest around the world. LPF is cheaper than imported forage. Sung et al [1] compared retail prices of locally produced barley, silage, and Italian ryegrass with those of imported forage and reported that the price of imported forage was 1.3- to 2.1-fold higher on a DM basis and 1.9- to 3.5-fold higher on a TDN basis than that of LPF. Kwon and Woo [23] estimated production costs of major LPF and concluded that the price of LPF is 27% to 49% lower than imported forage; they also indicated that the most significant

portion of the price of imported forage is the cost related with shipping and handling. For example, the production costs of alfalfa and tall fescue straw were 20 and 70 Korean Won (KRW) per kg, respectively, but the retail prices of these forages were 230 and 284 KRW/kg, respectively [23]. Therefore, LPF would be more cost-effective than imported ones.

LPF also has an advantage over imported forages in an environmental aspect. Life cycle assessment studies in Japan showed that the use of LPF could reduce the environmental impact of dairy [2] and beef farming [24]. A dairy farming system using rice silage reduced acidification and eutrophication potentials by 3.6% and 3.3%, respectively, although the global warming potential (GWP) increased by 1.5% because of greenhouse gas emissions from the rice paddy field [2]. The estimated greenhouse gas emissions from transporting 1 kg of feed were 157.9 g CO<sub>2</sub> for imported feed and 2.3 g CO<sub>2</sub> for domestic feed. This implies that the GWP could also be reduced by LPF, if this is not produced in a paddy field. In this line, Tsutsumi and Hikita [24] reported that GWP, acidification potential, and eutrophication potential could be reduced by 11% to 24%, 13% to 22%, and 19% to 24%, respectively, solely by replacing imported forage with LPF.

Despite the economic and environmental benefits, the use of LPF has been limited in Korea, especially in dairy farming. The primary reason for this is the limited production of good quality LPF. Rice straw, low quality roughage mainly used for beef production, contributed to over 60% of the LPF in Korea [7]. The Korean government, however, has made an effort to promote good quality LPF production, by supporting the production of forage crops (e.g., Italian ryegrass, sudangrass, barley silage, and corn silage) in many ways [1]. As a result, forage crop production has been markedly increased recently, and it contributes to approximately 50% of the total LPF in Korea [25]. The use of LPF, however, remains limited. One of the reasons is that dairy farmers tend to believe that the quality of LPF is poorer compared to the imported forage, even though this is not always true [26]. Another, more important problem, is that the detailed nutritional information of LPF is rarely available.

Knowledge about the nutrient composition of forage is essential for balancing ration in order to maintain animal health and level of production. In this regard, modern ration formulation programs, such as National Research Council [13], Cornell Net Carbohydrate, and Protein System [9], require a much more detailed description of the chemical composition and rate of digestion of nutrient pools for each feed ingredient. The Korean feeding standards for dairy cattle have also integrated the feed evaluation system of CNCPS v5 in their most recent update [27]. There is, however, no feed library containing detailed information about LPF. The detailed analysis of the chemical composition and digestion kinetics of LPF, thus, is a pre-requisite to enhance the use of LPF.

In conclusion, the present study performed a detailed analysis

of the chemical composition and ruminal fermentation kinetics of LPF commonly used in Korea, and showed that the nutritional value of some LPF (i.e. corn silage and Italian ryegrass) is comparable to that of imported forage widely used in Korea. This results of the study also indicated that the nutritional value of LPF differs according to the region where they were produced, as well as by forage species. LPF has economic and environmental advantages over imported forage. A detailed analysis of the chemical composition and digestion kinetics of LPF, however, is required, to increase the use of LPF.

## CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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