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Effects of Rice Straw, Annual Ryegrass Straw and Klein Grass Hay on Disappearance Rates of Nutrients in the Rumen and CH₄ Emission in Hanwoo Cows

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The objective of this study was to investigate the effects of rice straw, annual ryegrass straw, and Klein grass hay on ruminal disappearance characteristics and CH₄ emission in Hanwoo cows. Two Hanwoo cows (358±10.61 kg) fistulated at the rumen were treated with two replications of each treatment during the 63-day experimental period. In accordance with TDN (total digestible nutrients) of the Korean Feeding Standard for Hanwoo (NISA, 2007), concentrates and forage (6:4 ratio) were provided twice a day, with a free access to mineral block and water. Using the *in situ* method with a nylon bag, disappearance rates in the rumen of DM, CP, NDF, and ADF were measured. CH₄ emissions were measured hourly using a hood-type chamber. Disappearance rates in the rumen of DM, CP, NDF, and ADF were higher in Klein grass hay, rice straw, and annual ryegrass straw, in that order, with significantly higher ($p < 0.01$) in Klein grass hay than in rice straw and annual ryegrass. The average hourly CH₄ emissions were as follows; annual ryegrass straw (169.89±6.94 mg/hr), rice straw (139.92±18.89 mg/hr), and Klein grass hay (128.20±4.91 mg/hr), significantly higher ($p < 0.01$) in annual ryegrass straw than in Klein grass hay. These results suggest that rice straw is a useful feed resource for ruminant production, which is mostly utilized as a domestic feed. However, imported roughage such as annual ryegrass straw showed lower disappearance and higher CH₄ emission than rice straw.

Key words: Annual ryegrass straw, CH₄, Hanwoo cow, Klein grass hay, rice straw

INTRODUCTION

The self-sufficiency rate of the forages in the recent years in Korea is just at around 84% (Woo, 2010), with grasses and forage crops occupying around 38% and rice straw around 46% (Han *et al.*, 2009). Therefore, around 16% of feeds are pasture plants that are used for livestock (Woo, 2010). However, it was reported that the content of crude protein in the imported forages domestically used is lower than that proposed in the standard tables of feed composition in Korea published by the National Institute of Animal Science (NIAS, 2007) (Lee and Lee, 2000; Lee *et al.*, 2001). Further it is expected that there could be imported forages that have more or less inferior quality than rice straw that uses most of the domestic lands. It is necessary to evaluate the feed's value for the imported forages. However, it is difficult to evaluate the nutrient composition at fields (Kim *et al.*, 2005).

Forage is essential for ruminants. Ruminants digest the ingested feeds by the microorganism in the rumen, using them as energy. The gases that are produced while ruminants digest feeds are known to be CO₂, CH₄, N₂O, and O₂. CH₄ is produced by the action of methanogen in

the rumen and gives rise to around 2–12% energy loss (Kim, 1999). Since discharged CH₄ causes a strong greenhouse effect, there have been many researches such as effects of methane inhibitors on ruminal fermentation characteristics and methane production (Oh *et al.*, 1998), effects of halogenated compounds on *in vitro* fermentation characteristics in the rumen and methane emissions (Czerkawski *et al.*, 1966), effect of monensin on methane and heat productions of steers fed lucerne hay (O'Kelly and Spiers, 1992), evaluation on the greenhouse gas emission according to the intake levels of total mixed rations of Hanwoo cow (Kim *et al.*, 2011), and effect of green tea, garlic, and may flower powder supplementation on greenhouse gas emission in Hanwoo cow (Kim, 2012) to reduce this greenhouse effect. These various preceding researches were performed with the major target to reduce CH₄ production by feeding animals with additives in the feeds. However, researches about CH₄ produced from Hanwoo through different types of forages are lacking.

This study was conducted to investigate the effects of rice straw used most in Korea, annual ryegrass (Italian ryegrass) and Klein grass hay among imported forages on the disappearance rate of nutrients in the rumen and CH₄ production in Hanwoo cows.

MATERIALS AND METHODS

Animals, experimental period, and experimental site

Two heads of Hanwoo cow (average body weight: 358±10.61 kg) fitted with rumen cannula were used for

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the experiment. The experiment was performed in the Ruminants Laboratory of Kangwon National University from February 1 till April 14, 2012 for a period totaling 63 days. The preliminary experiment for 30 days was conducted to adapt the animals to the metabolism cage. This was followed by the *in situ* test, and the animals were fed rice straw, annual ryegrass straw and Klein grass hay in the main experiment with four replications.

Feeds for experiment and growing management

Dried rice straw, annual ryegrass straw (Italian ryegrass straw), and Klein grass hay were used as feeds for the animals. Restricted feeding was performed with concentrates and forage at the mixture ratio of 6:4 according to the total digestible nutrients (TDN) required for maintaining a mature cow as in the Korean feeding standard from the National Institute of Animal Science (NIAS, 2007) as shown in Table 1.

Feeds were given as a batch with 3 kg of concentrates and 1.6 kg of rice straw, or 2.8 kg of concentrates and 1.8 kg of annual ryegrass straw, or 2.8 kg of concentrates and 1.86 kg of Klein grass hay twice a day (at 08:30 and 17:30), while the animals had free access to water and mineral blocks. Feeds for the experiment were analyzed by the methods of AOAC (1995), while NDF and ADF were analyzed by the methods of Goering and Van Soest (1970). The general compositions of the feeds are shown

Table 1. Maintenance nutritional requirement of Hanwoo cows

Item	TDN(kg)		DMI (kg)	CP (%)
	standard	feeding		
Rice straw	2.78	0.70	1.6	16.2
Concentrates		2.11	3	
Annual ryegrass straw	2.78	0.84	1.8	13.3
Concentrates		1.97	2.8	
Klein grass hay	2.78	0.84	1.86	20
Concentrates		1.97	2.8	

Table 2. Chemical composition of experimental diets (DM basis)

Chemical composition (%)	Concentrates	Rice straw	Annual ryegrass straw	Klein grass hay
Moisture	10.94	7.37	7.65	6.37
Crude protein	14.59	4.31	1.81	8.51
Crude fiber	3.88	27.98	35.81	31.94
Ether extract	3.26	1.84	1.06	0.85
Crude ash	6.39	8.36	4.36	7.83
NDF	23.83	66.06	68.66	67.41
ADF	10.51	41.33	47.77	43.00
NFE ¹⁾	49.56	50.14	49.31	44.50
TDN ²⁾	70.26	26.84	25.90	41.69

¹⁾ Nitrogen-free extract.

²⁾ Calculated values (Weiss *et al.*, 1992).

in Table 2.

General composition analysis results showed that the CP contents from annual ryegrass straw and Klein grass hay were in the range of 1.81–8.51% which was of similar level with the preceding report that the CP contents from imported hay was mostly lower than 7–10% (Lee *et al.*, 2001; Lee and Lee, 2000). Rice straw that is produced and used most in Korea, annual ryegrass straw with lower CP and TDN as compared with those in the rice straw, and Klein grass hay having higher CP and TDN among the imported forages were chosen as test feeds by referring to the general composition analysis table.

Analyses

In situ Measurements

In situ digestion experiment with the ruminants was carried out using a nylon bag having a porosity of 50 μm (Ankom technology) according to the methods of Ørskov and McDonald (1979). Ten grams of powder-form concentrates were put into the nylon bag, while annual ryegrass straw and Klein grass hay were cut into around 3–5 cm lengths by scissors and five grams of these cut grasses were put into the nylon bag. Two replications were made for the experiment with concentrates, rice straw, annual ryegrass straw, and Klein grass hay. The nylon bags with feeds were put through the cannula into the rumen. This was followed by collecting nylon bags at 0, 6, 12, 24, 48, and 72 hours after feeding to investigate the disappearance rate of nutrients in the rumen. Collected nylon bags were washed with a running tap water till clear water was running and then dried in the forced convective drying oven at 65°C for 72 hours. After that, the residue was collected and weighed to calculate the DM, CP, NDF, and ADF disappearance rates (%) using the equation below.

$$1) \text{ DM disappearance rate (\%)} \\ = \frac{\text{Sample DM(g)} - \text{Residue DM (g)}}{\text{Sample DM (g)}} \times 100$$

$$2) \text{ CP disappearance rate (\%)} \\ = \frac{\text{Sample CP(g)} - \text{Residue CP (g)}}{\text{Sample CP (g)}} \times 100$$

$$3) \text{ NDF disappearance rate (\%)} \\ = \frac{\text{Sample NDF(g)} - \text{Residue NDF (g)}}{\text{Sample NDF (g)}} \times 100$$

$$4) \text{ ADF disappearance rate (\%)} \\ = \frac{\text{Sample ADF(g)} - \text{Residue ADF (g)}}{\text{Sample ADF (g)}} \times 100$$

CH₄ Measurement

CH₄ production was measured with a hood-type chamber. The metabolism cage was set to 230 cm (width) \times 205 cm (height) \times 140 cm (length), which was then occupied by one cow with its head placed inside the chamber sealed with a transparent acryl having a size of 100 cm (width) \times 139 cm (height) \times 92 cm (length).

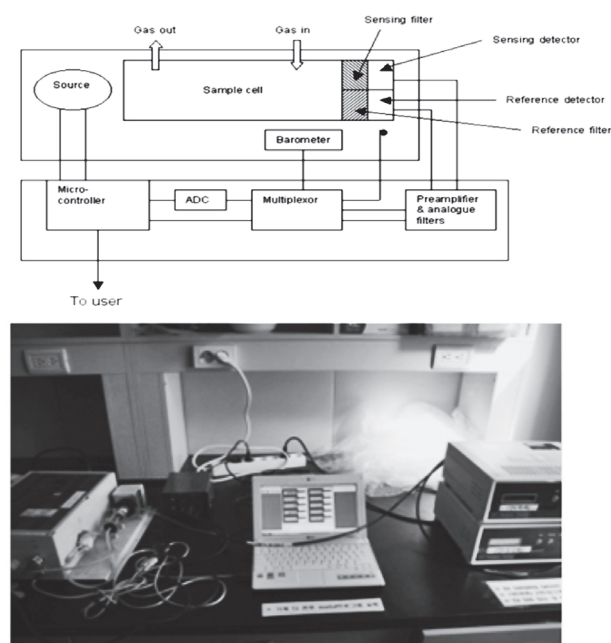


Fig. 1. Apparatus for measuring greenhouse gases and gas monitoring system (Mamos-300, Australia).

With a transparent acryl plate, all the activities and states such as the conditions and feed intake of the animals could be observed during greenhouse gas measurements. The animals have gone through an adaptation period of two weeks and another two weeks of preliminary experiment inside a hood-type chamber before switching to the main experiment, with an adaptation period of four days for the rice straw, annual ryegrass straw, and Klein grass hay, respectively. The measurement was carried out for two days. Greenhouse gas was measured using a multiple gases detector (mamos-300, Australia) with the help of a non-dispersive infrared absorption (NDIR) sensor as shown in Figure 1.

Statistical Analyses

All the results obtained during the experiment were analyzed using the General Liner Model (GLM) from Statistical Analysis System (SAS, ver. 9.2). Significance test was carried out for the treatment interval using the Duncan's multiple range tests.

Y_{ij} : The measurement value for the i th forage and the j th animal

μ : The average of the total

G_i : The effect of the i th forages ($i = 1, 2,$ and 3)

e_{ij} : The uncertainty error

RESULTS AND DISCUSSION

Comparison of *in situ* disappearance rates for rice straw, annual ryegrass straw, and Klein grass hay DM disappearance rate

The DM disappearance rates measured in the rumen of the animals are presented in Table 3 and Figure 2.

DM disappearance rates in the rumen of rice straw, annual ryegrass straw, and Klein grass hay increased as the fermentation progressed. The DM disappearance of

Klein grass hay was significantly higher ($p < 0.01$) compared with rice straw and annual ryegrass straw. This higher disappearance of DM might be due to the increased number of protein degrading microorganisms in the rumen, because protein contents in the gramineous forages are high (Choi, 2007). Though there were no significant differences ($p > 0.05$), the rice straw tended to show a slightly higher DM disappearance rate than the annual ryegrass straw.

The DM disappearance rate of the forages in the rumen at the interval of 24 hours was found highest in the initial 24 hours, followed by the third 24 hours (48 ~ 72 hours). When compared with the results in the preceding studies (Ørskov and McDonald, 1979), the DM disappearance rate of the forages, especially that of the rice straw was found to be low. It might be because the forage was crushed in a Wiley mill with a screen having mesh at around 1–2 mm, cutting into around 3–5 cm lengths instead of putting the crushed forages into the nylon bag ultimately reduced the disappearance of DM and the activation of microorganisms. Further, since the rice straw was cut instead of being crushed in the Wiley mill, the amount of DM escaping through the pore of the nylon bag might be relatively low.

Table 3. Effects of experimental diets on *in situ* dry matter disappearance in Hanwoo cows

Time (hr)	Treatment		
	Rice straw	Annual ryegrass straw	Klein grass hay
0 ¹⁾	8.16±1.21 ^{2b)}	6.96±0.96 ^{b)}	15.11±1.63 ^{a)}
6	12.28±0.71 ^{b)}	9.55±0.35 ^{b)}	21.88±0.57 ^{a)}
12	15.79±1.39 ^{b)}	10.86±0.91 ^{b)}	29.43±4.62 ^{a)}
24	18.17±2.13 ^{b)}	14.17±1.51 ^{b)}	36.06±0.98 ^{a)}
48	21.12±0.40 ^{b)}	17.65±0.13 ^{b)}	38.50±0.89 ^{a)}
72	31.63±5.03 ^{b)}	29.96±7.16 ^{b)}	48.43±5.89 ^{a)}

¹⁾ Values at 0 hr show the amount that passed through the pores of the nylon bag by only washing with running tap water.

²⁾ Values are mean ± standard deviation.

^{a,b)} Means with different letters in the same row are significantly different ($p < 0.01$).

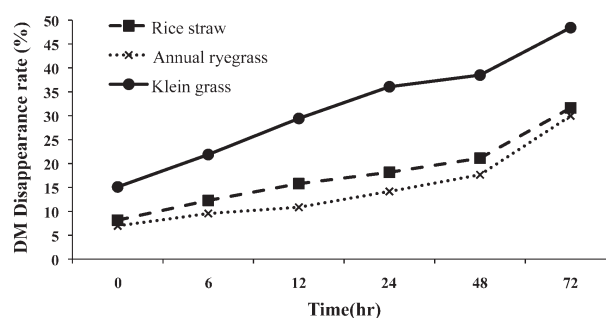


Fig. 2. Effects of experimental diets on *in situ* DM disappearance in Hanwoo cows.

CP Disappearance Rate

The CP disappearance rates in the rumen according to the fermentation duration are shown in Table 4 and Figure 3.

Table 4. Effects of experimental diets on *in situ* crude protein disappearance in Hanwoo cows

Time(hr)	Treatment		
	Rice straw	Annual ryegrass straw	Klein grass hay
0 ¹⁾	12.80±1.15 ^{2)b}	10.97±0.92 ^b	17.04±1.59 ^a
6	16.89±0.67 ^b	12.02±0.34 ^b	21.50±0.57 ^a
12	25.82±1.64 ^b	23.79±0.97 ^b	33.68±6.06 ^a
24	26.88±2.53 ^b	25.00±1.77 ^b	39.47±1.35 ^a
48	33.60±0.46 ^b	30.08±2.50 ^b	49.29±1.34 ^a
72	41.47±6.22 ^b	37.55±10.40 ^b	54.61±10.60 ^a

¹⁾ Values at 0 hr show the amount that passed through the pores of the nylon bag by only washing with running tap water.

²⁾ Values are mean ± standard deviation.

^{a,b} Means with different letters in the same row are significantly different (p<0.01).

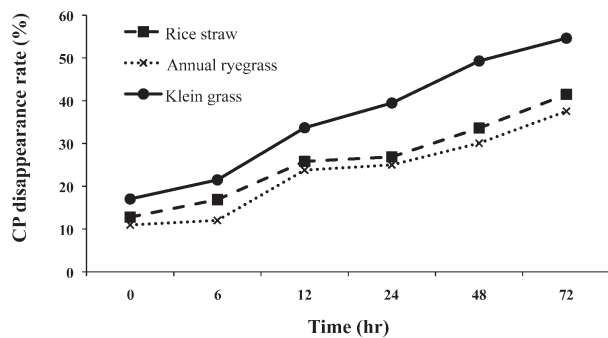


Fig. 3. Effects of experimental diets on *in situ* CP disappearance in Hanwoo cows.

The CP disappearance rate of the Klein grass hay was significantly higher (p<0.01) than that of the rice straw and the annual ryegrass straw. This result was different from the results reported by Han *et al.* (2009) that CP was mostly very low in the straws than in the hays. However, it implies that CP contents can be differently included in the forages according to the collection location of the herbage (Wood and Tanner, 1985), species seed combination (Peel and Green, 1984), preparation method of the forages, harvest stage, and distribution states (Lee *et al.*, 2001). Though there was no significant difference (p>0.05) in the CP contents between the rice straw and the annual ryegrass, the CP disappearance rate of rice straw tended to be slightly higher than the annual ryegrass straw. CP disappearance rates of rice straw, annual ryegrass straw, and Klein grass hay gradually increased as the fermentation time progressed in the rumen. Further, CP disappearance rates of rice straw, annual ryegrass straw, and Klein grass hay showed large increases from 6 to 12 hours of the fermentation in the rumen. This result was different from that by Seok

(2009). Yet, this might be due to the effect of the washing method (Kempton, 1980) and types of the sample. The highest rate of CP disappearance was found in the Klein grass hay among the forages tested, whilst the lowest CP disappearance was found from the annual ryegrass straw. Klein grass hay showed a higher CP disappearance rate from the beginning of the experiment, which might be due to its high CP contents that could be dissolved independently from the disappearance action by the microorganism in the rumen.

Disappearance Rates of NDF and ADF

Tables 5 and 6 show the disappearance rates of NDF and ADF according to the fermentation time in the rumen, respectively. Figures 4 and 5 present the changes in the disappearance rates of NDF and ADF during each measurement time.

NDF disappearance rate was significantly higher (p<0.01) in Klein grass hay in the rumen. Further, there was no significant difference (p>0.05) in the NDF disappearance rate between rice straw and annual ryegrass

Table 5. Effects of experimental diets on *in situ* NDF disappearance in Hanwoo cows

Time(hr)	Treatment		
	Rice straw	Annual ryegrass straw	Klein grass hay
0 ¹⁾	6.13±1.24 ^{2)b}	1.09±1.02 ^b	4.78±1.83 ^a
6	10.72±0.72 ^b	4.40±0.97 ^b	16.93±0.60 ^a
12	12.72±1.45 ^b	4.57±0.37 ^b	21.27±5.15 ^a
24	17.74±2.14 ^b	10.29±1.58 ^b	34.82±1.00 ^a
48	23.00±0.39 ^b	20.26±0.13 ^b	41.89±0.84 ^a
72	37.22±4.62 ^b	36.23±6.52 ^b	53.17±5.35 ^a

¹⁾ Values at 0 hr show the amount that passed through the pores of the nylon bag by only washing with running tap water.

²⁾ Values are mean ± standard deviation.

^{a,b} Means with different letters in the same row are significantly different (p<0.01).

Table 6. Effects of experimental diets on *in situ* ADF disappearance in Hanwoo cows

Time(hr)	Treatment		
	Rice straw	Annual ryegrass straw	Klein grass hay
0 ¹⁾	3.51±1.27 ^{2)b}	1.18±1.02 ^b	2.68±1.87 ^a
6	6.11±0.76 ^b	5.97±0.37 ^b	13.27±0.63 ^a
12	11.81±1.46 ^b	7.19±0.94 ^b	23.48±5.01 ^a
24	14.45±2.22 ^b	13.10±1.53 ^b	30.12±1.07 ^a
48	17.80±0.41 ^b	20.24±0.13 ^b	38.57±0.89 ^a
72	32.82±4.95 ^b	34.44±6.71 ^b	52.42±5.43 ^a

¹⁾ Values at 0 hr show the amount that passed through the pores of the nylon bag by only washing with running tap water.

²⁾ Values are mean ± standard deviation.

^{a,b} Means with different letters in the same row are significantly different (p<0.01).

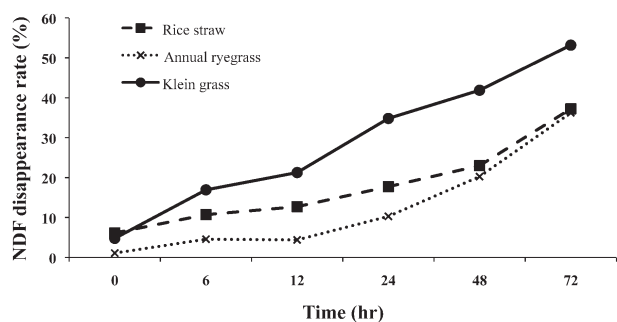


Fig. 4. Effects of experimental diets on *in situ* NDF disappearance in Hanwoo cows.

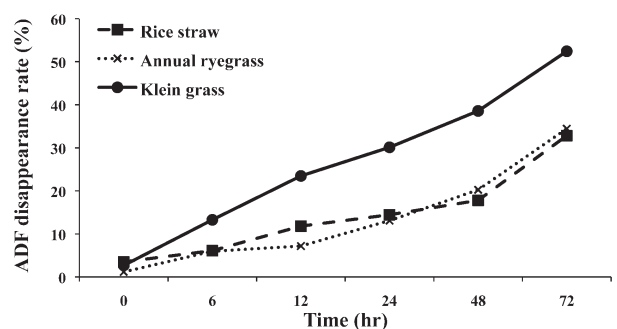


Fig. 5. Effects of experimental diets on *in situ* ADF disappearance in Hanwoo cows.

straw. However, a little higher NDF disappearance rate tended to be observed in the rice straw in all the time intervals than in the annual ryegrass straw. The NDF disappearance rate occurring at zero hour was a disappearing from the pore of the nylon bag. This result was consistent with the preceding study (Kim *et al.*, 2005) that the NDF content increased as the CP and the components inside the cell were decreased.

The NDF disappearance rates of the rice straw, annual ryegrass straw, and Klein grass hay gradually increased as the fermentation progressed in the rumen. Moreover, the NDF disappearance rate of Klein grass hay showed a higher increase than that of rice straw and annual ryegrass straw. Similar NDF disappearance rates were observed between annual ryegrass and rice straw.

The ADF disappearance was significantly higher ($p < 0.01$) in Klein grass hay than in the rice straw and the annual ryegrass straw. There was no significant difference ($p > 0.05$) in the ADF disappearance rate between the rice straw and annual ryegrass straw. The ADF disappearance rate occurring at zero hour was the disappearance from the pores of the nylon bag.

A large amount of ADF disappeared quickly from Klein grass hay compared with rice straw and annual ryegrass straw, both straws did not show any difference in ADF disappearance rate. A particular thing observed was that the disappeared ADF of annual ryegrass straw increased from 12 hours of the fermentation in the rumen, and after 24 hours the ADF disappearance tended to be slightly higher in annual ryegrass straw than in rice straw.

The disappearance rates of DM, CP, NDF, and ADF overall showed the highest values in the Klein grass hay during all the observation times. Meanwhile, although the disappearance rates of the above components in the annual ryegrass were not significantly higher ($p > 0.05$) than those in the rice straw, decreasing trends of DM, CP, NDF, and ADF were observed. It was reported that the digestion rate of pasture plants was higher than that of the straws (Ndlovu Buchanan-Smith, 1985; Poore *et al.*, 1991). The results of this study, in which the disappearance rates of DM, CP, NDF, and ADF of the good quality pasture plants Klein grass hay were significantly higher ($p < 0.01$) than those of rice straw and annual ryegrass straw, are in conformity with the preceding researches.

CH₄ production by animals fed rice straw, annual ryegrass straw, or Klein grass hay

CH₄ production

The average CH₄ productions per day by feeding rice straw, annual ryegrass, or Klein grass hay are presented in Table 7.

Table 7. Effects of experimental diets on CH₄ emission in Hanwoo cows

Item	Treatment		
	Rice straw	Annual ryegrass straw	Klein grass hay
CH ₄ (mg/hr/day)	139.92±18.89 ^{1)ab}	169.89±6.94 ^a	128.20±4.91 ^b

¹⁾ Values are mean ± standard deviation.

^{a,b} Means with different letters in the same row are significantly different ($p < 0.01$).

Average CH₄ production per hour was 139.92±18.89 mg in rice straw, 169.89±6.94 mg in annual ryegrass straw, and 128.20±4.91 mg in Klein grass hay, respectively. The CH₄ production was significantly higher ($p < 0.01$) in annual ryegrass straw than in Klein grass hay. The least CH₄ production was found in Klein grass hay with the highest contents of CP. Getachew *et al.* (1998) reported the relationship between CP content in the feed and CH₄ production, where NH₄⁺ produced from CP degradation was bonded with CO₂ (a substrate for CH₄ production) and the reduced production of NH₄⁺ resulted in the reduced CH₄ production. Therefore, we need additional researches about the CH₄ production in Klein grass.

CH₄ production pattern

The CH₄ production during 24 hours by feeding rice straw, annual ryegrass straw, or Klein grass hay is shown in Figure 6. Though the CH₄ production was greatly different according to the types of the forage ingested, the CH₄ production patterns were generally similar in rice straw, annual ryegrass straw, and Klein grass hay.

The CH₄ production showed rapid increases in one to two hours after feeding rice straw, annual ryegrass straw, or Klein grass hay. This agrees with the result by Song (2008) who reported that the highest CH₄ level was due to the responsive burp by the animal, fitted with the high

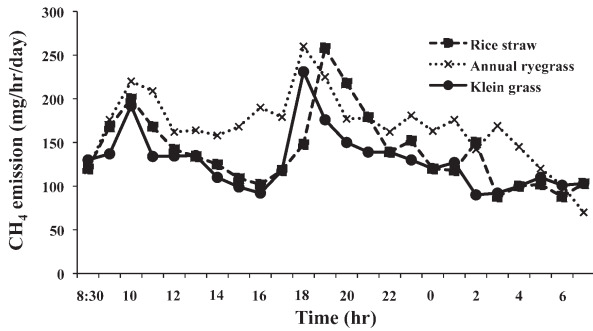


Fig. 6. Effects of experimental diets on CH₄ emission in Hanwoo cows.

level of gas pressure inside the rumen, that actively occurred in one to two hours after feeding. When day and night were divided at hour 20, the CH₄ production was generally higher during day than during night. This result matches with the report by Jeon (1988) that cows ruminate more during day than during night.

Correlation between the nutrient disappearance rate in the rumen and CH₄ production by feeding rice straw, annual ryegrass straw, or Klein grass hay

Figure 7 shows the correlation between the nutrient disappearance rate in the rumen and CH₄ production by

feeding rice straw, annual ryegrass straw, or Klein grass hay to the animals. The correlation between the disappearance rates of DM, CP, NDF, and ADF and CH₄ production investigated using *in situ* method tends to display a negative (-) relationship for all the feeds types. Klein grass hay with a significantly higher ($p < 0.01$) disappearance rate in the rumen showed the significantly lower ($p < 0.01$) CH₄ production. This result was consistent with the report by Johnson and Johnson (1995) that when the feed was given to the livestock at an energy maintaining level, the CH₄ production decreases per feed intake as the digestion rate increases.

CONCLUSIONS

As a conclusion, it would be beneficial to use pasture plants having higher CP and TDN, instead of straws like rice straws being used most in Korea that produce a high amount of CH₄. Imported forage annual ryegrass straws might have lower CP and TDN contents compared with those in Italian ryegrass and crop barley that are produced in Korea. Further, it would give an adverse effect on the ruminal disappearances and CH₄ production. Therefore, we would need additional researches about the effects of forage feeding on the nutrient disappearance rates in the rumen and CH₄ production among Hanwoo species in the future.

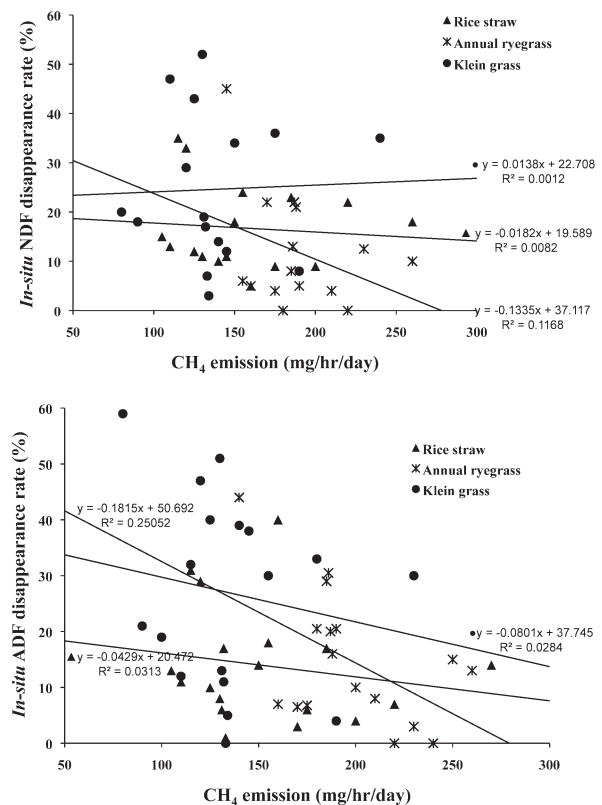
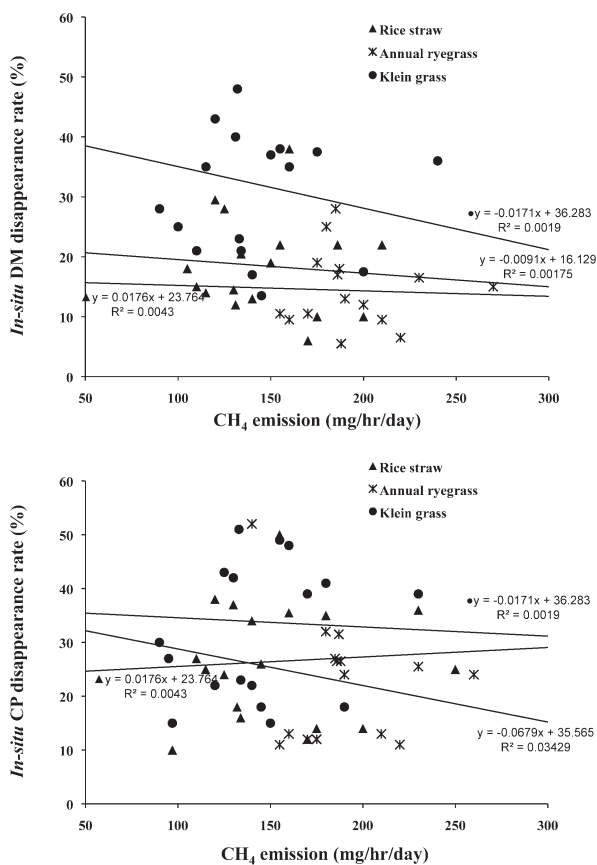


Fig. 7. Relation between *in situ* disappearance rates of nutrients and CH₄ emission of experimental diets.

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