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In vitro Rumen Fermentation Patterns of Environment Friendly Whole Crop Barley, Italian Ryegrass and Rice Straw Silages*

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친환경 청보리, 이탈리안 라이그라스, 볏짚사일리지의 *In vitro* 반추위 발효성상 비교연구

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Rumen fermentative characteristic is useful indicators of the quality of ruminant feed stuffs and diets. An *in vitro* rumen fermentation experiment was therefore carried out to compare fermentation patterns among three forage sources. These were whole crop barley (WCBS), Italian ryegrass silage (IRGS) and rice straw silages (RSS). Rice straw (RS) was the control, making the treatments 4 in total. Forages were randomly allocated to serum bottles. The incubation times were arranged 0, 3, 6, 9, 12, 24, 48 and 72h at 39℃, respectively. Each forage source was replicated 3 times per incubation time. At each sampling time, total gas and pH were measured, whilst individual volatile fatty acids (VFAs), total volatile fatty acids (TVFAs) and ammonia nitrogen (NH_3-N) were determined later after storing samples at -20℃. Acetate: Propionate ratio (A/P) was then calculated. Forage source had a significant effect ($P<0.001$) on pH and NH_3-N. RSS maintained higher pH values than the rest of the forage sources. A decreasing pH trend with increased time of incubation, in agreement with literature, was observed for all forage sources. WCBS recorded NH_3-N values higher than all the other treatments. Total gas, individual and total VFA and A/P ratio were not affected by forage

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source. However, there was a significant difference in all parameters ($p<0.05$) among forage sources at sampling periods at 3 to 72h. Therefore, the present results indicating that WCBS, IRGS, RS and RSS maintained *in vitro* rumen pH above the critical value. Also, WCBS produced the highest NH3-N and on this merit could be of better nutritive value, in vivo, in the ruminant.

Key words : *in vitro fermentation, rumen, forage*

Ⅰ. Introduction

Forage quality is the basis of the formulation of productively efficient ruminant diets that supply sufficient nutrients to rumen microbes and the host (Van Saun, 2007). Good quality forage sources for ruminants, among other things, provide highly digestible energy and protein even when concentrate is provided for at low levels. Such forage sources have the potential of reducing feed costs, which generally account for up to 65% of production costs in beef enterprises. In an effort to improve beef production, farmers in South Korea have made it a priority to formulate high quality fattening diets. This has resulted in the introduction of other forage sources that include barley and Italian ryegrass, in an effort to replace the commonly used rice straw. Rice straw is of relatively low nutritive value. Low nitrogen content, high level of lignin and the slow and limited degradation of its carbohydrates have been pointed out as the major limitations (Sarnklong *et al.*, 2010). South Korean weather patterns are favourable for the production of barley and Italian ryegrass.

Whole crop barley and Italian ryegrass are widely used in beef diets in temperate climates. They can be fed alone or in total mixed rations (TMR). Fibrous components of ruminant diets can be fed as fresh, hay, haylage or silage. Ensiling is a common method of preserving the nutritional quality of forages. It also improves digestibility, palatability and nutritive value of straws (Gao *et al.*, 2008). This ensures a guaranteed supply of high quality forage throughout the year. An evaluation of forage sources is necessary before incorporating them into animal diets. Rumen fermentation parameters are good indicators of the quality and the potential of the feed component to supply nutrients to rumen microbes and the host. *In vitro* fermentations are generally considered sufficient approximators of in vivo fermentations (Mansfield *et al.*, 1995; Mould *et al.*, 2005). Gizzi *et al.* (1998) noted that *in vitro* fermentation is a useful tool in evaluating individual feedstuffs.

The objective of this study was to compare *in vitro* rumen fermentation parameters among rice straw silage (RSS), whole crop barley silage (WCBS) and Italian ryegrass silage (IRGS).

Ⅱ. Materials and methods

Forage silages and rice straw were collected from Chonbuk Hanwoo Association and frozen dried. The forages were then ground through a 1mm sieve on the day they were used in the *in vitro* fermentation experiment. Rumen contents were collected from a rumen fistulated Hanwoo steer weighing 350Kg. Rumen content was collected just before morning feeding. The steer was receiving a roughage:concentrate ration of (50:50). These contents were squeezed through four layers of cheese cloth into an Erlenmeyer flask with an O_2-free CO_2 headspace. On reaching the lab, the liquor was further strained through four layers of cheese cloth again. The particle-free liquor was transferred into a medium, McDougal buffer (pH 6.5), prepared according to Troelsen and Donna (1966). It contained 9.8g $NaHCO_3$, 4.62g $Na_2HPO_4.2H_2O$, 0.57g KCl, 0.47g NaCl, 0.12g $MgSO_4.7H_2O$ and 4(5.3)g $CaCl_2$($CaCl_2.2H_2O$) per 100ml H_2O. Fifty mL of the inoculum were anaerobically transferred into 96 serum bottles, each containing about 50g of ground (1mm sieve) forage source. The *in vitro* incubation was carried out according to method outlined by Tilley and Terry (1963).

Each forage source was replicated 3 times per sampling period (0, 3, 6, 9, 12, 24, 48 and 72h). All serum bottles, except for 0h sampling time bottles, were immediately incubated into an incubator (VS-1203P3N) at 39℃. At each sampling period, total gas was measured first by reading the displacement of a syringe plunger. Serum bottles were then uncapped and pH measured immediately using a calibrated pH meter (Mettler Toledo, seven easy). The inoculum in each bottle was emptied into a conical tube and centrifuged (10,000rpm for 15min) and cell-free supernatant stored at -20℃ for further analyses.

Ammonia was measured using calorimetry as specified by Chaney and Marbach (1962). Rumen inoculum was centrifuged (3,000rpm × 15min). 20μL of cell-free supernatant was supernatant was collected from each conical tube and transferred to test tube. 1ml of each of the 2 reagents, Phenol colour reagent and Alkali-hypochlorite reagent were added into the test tube and thoroughly mixed using a voltex mixer. The test tube contents were then incubated for 7 minutes at 50℃ in a water bath. Readings were then obtained from a spectrophotometer (spectrophotometer, optizen 2120UV, Mecasys Co., LTD).

VFAs were determined using gas liquid chromatography according to Erwin *et al.* (1961). A Varian GC-3400 (Walnut Creek, California, 94598, USA) gas chromatograph (column temperature = 120℃ to 170℃ at 10℃/min, injector temperature = 170℃, detector temperature = 190℃) equipped with an auto sampler and Stabilwax-DA (30m × 0.5mm I.D. × 0.5film) column (Resteck).

An ANOVA was carried out using the R- statistical passage (Version 2.15.0) to determine

effect of the forage sources on fermentation parameters.

Ⅲ. Results

Forage source had a significant effect (P<0.001) on *in vitro* ruminal pH values. RSS with an overall mean of 6.96, was significantly higher than the other forage sources. IRGS and WCBS tended to have lower values than RS, although the difference among these three was not significant (p>0.05). Figure 1 shows a decreasing trend in pH that was observed among all forage sources with increased incubation time.

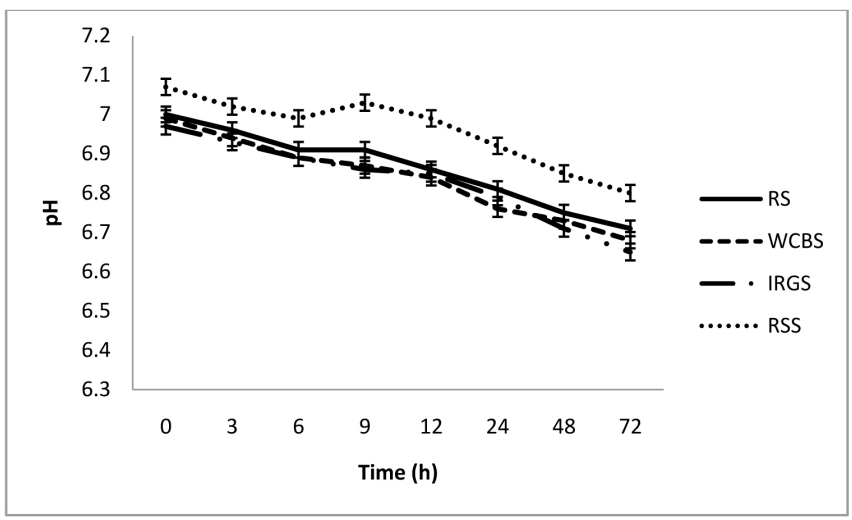

Figure 1. Effect of forage source on pH values over time. RS = Rice straw, WCBS = Whole crop barley silage, IRGS = Italian ryegrass silage, RSS = Rice straw silage.

Forage source had no effect (p>0.05) on TVFAs, the molar proportions of VFAs and also the A/P ratio. However, there were significant differences (p<0.05) between treatments in the same parameters in fermentation time periods from 3 to 72h (Figure 2. and Table 1). The amount of individual VFAs and subsequently TVFAs increased as sampling time increased, whilst the A/P ratio showed a decreasing trend in that same period. The amount of individual VFAs and TVFAs differed significantly (p<0.05) in the different sampling periods except for 0h.

WCBS had significantly higher (p<0.001) NH_3-N production than other forage sources. Significant interactions (p<0.01) between incubation time and forage source were noted. As

incubation time increased (from 12 - 72h) the rate of NH_3-N production varied among forage sources also (Figure 3).

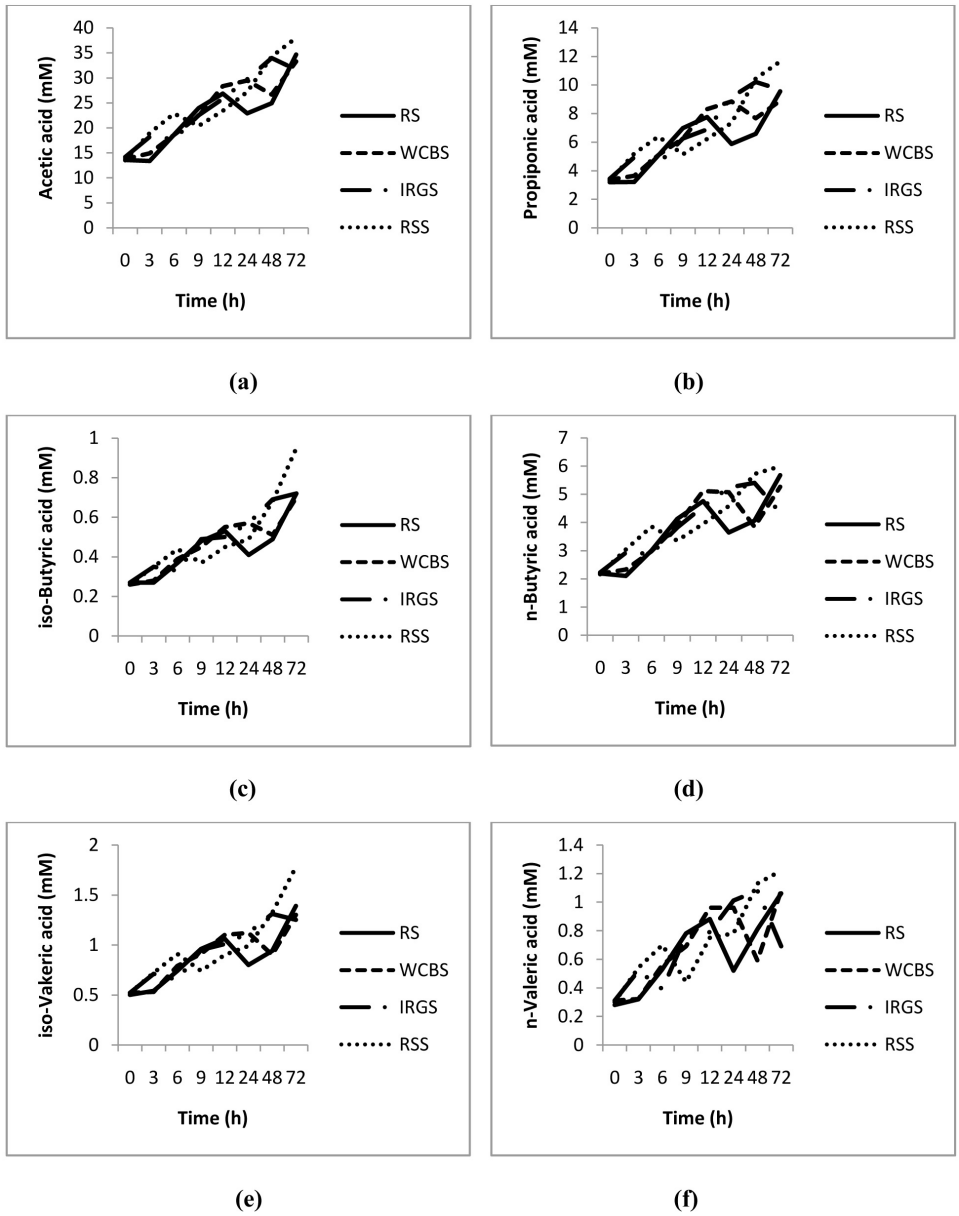

Figure 2. Changes in individual VFA production (a) acetic acid, (b) propionic acid, (c) iso-butyric acid, (d) n-butyric acid, (e) iso-valeric acid (f) n-valeric acid in different forage sources over time. RS = Rice straw, WCBS = Whole crop barley silage, IRGS = Italian ryegrass silage, RSS = Rice straw silage.

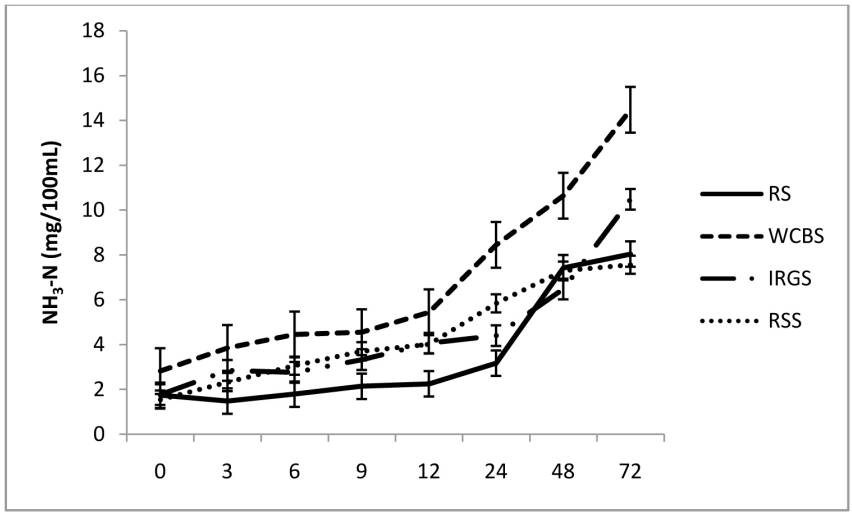

Figure 3. Changes in NH_3-N production in different forage sources over time. RS = Rice straw, WCBS = Whole crop barley silage, IRGS = Italian ryegrass silage, RSS = Rice straw silage.

Table 1. Effect of forage source on TVFA (mM) and Acetate:Propionate ratio (A/P ratio)

Time (h)	Forage source				SEM	Level of significance
	RS	WCBS	IRGS	RSS		
TVFAs (mM)						
0	20.40	20.40	20.89	20.09	0.17	NS
3	19.80^{a}	21.97^{b}	27.62^{c}	28.80^{c}	1.15	*
6	28.38^{a}	28.39^{a}	26.48^{a}	35.15^{b}	1.06	*
9	37.27^{b}	34.54^{b}	34.76^{b}	30.47	0.85	*
12	41.38^{bc}	44.36^{c}	39.52^{ab}	35.69^{a}	1.12	*
24	34.13^{a}	45.99^{c}	46.85^{c}	41.39^{b}	1.63	*
48	37.78^{a}	40.14	52.60^{b}	53.60^{b}	2.24	*
72	53.06^{c}	50.50^{b}	48.24^{a}	59.60^{d}	1.31	*
A/P ratio						
0	4.24	4.10	4.12	4.11	0.02	NS
3	4.17^{b}	4.10^{b}	3.68^{a}	3.64^{a}	0.07	*
6	3.67^{b}	3.65^{b}	3.97^{c}	3.57^{a}	0.05	*
9	3.43^{a}	3.58^{b}	3.60^{b}	3.96^{c}	0.06	*

Time (h)	Forage source				SEM	Level of significance
	RS	WCBS	IRGS	RSS		
12	3.47[a]	3.40[b]	3.75[b]	3.76[a]	0.05	*
24	3.90[b]	3.33[a]	3.26[a]	3.71[b]	0.09	*
48	3.79[c]	3.47[b]	3.33[a]	3.28[a]	0.06	*
72	3.63[b]	3.73[c]	3.27[a]	3.26[a]	0.06	*

[abcd] Means in a row with different superscripts are significantly different ($p<0.05$). RS = Rice straw, WCBS = Whole crop barley silage, IRGS = Italian ryegrass silage, RSS = Rice straw silage.

There were significant differences ($p<0.05$) in total gas produced in the sampling periods 24, 48 and 72h. RSS recorded the lowest at every sampling period whilst IRGS and WCBS recorded the highest output. Forage source however did not have an overall significant effect ($p>0.05$) on total gas produced. The total gas recorded for each of the forage sources followed an increasing trend as incubation time increased as is shown in Figure 4.

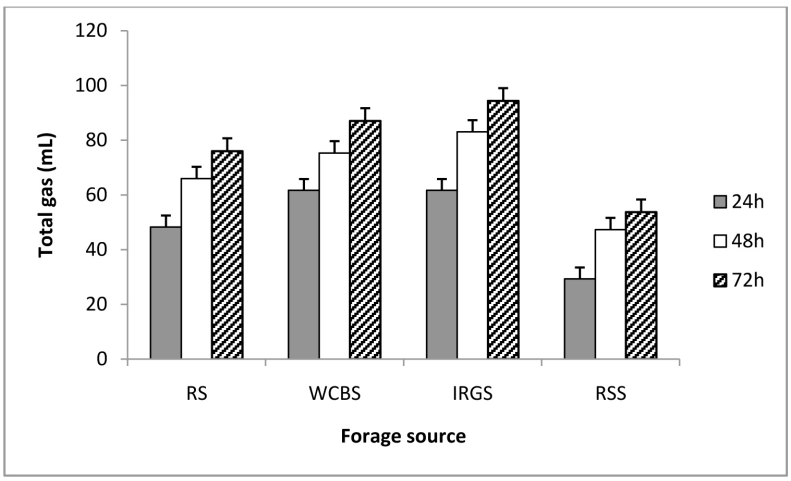

Figure 4. Total gas production in different sampling periods from different forage sources. RS = Rice straw, WCBS = Whole crop barley silage, IRGS = Italian ryegrass silage, RSS = Rice straw silage.

Ⅳ. Discussion

The observed decreasing trend in pH values is the normal and expected pattern. This is

because with increasing time, more VFAs are produced and these lower the pH. According to Owens and Goetsch (1993) roughage diets are expected to maintain pH ranges of between 6.2 and 7. The observed values are in agreement with this expectation. Actually, all the forage sources maintained values above the critical value of 6.3, below which cellulolytic bacteria function is reduced (Stewart, 1977; Hiltner and Dehority, 1983).

Depending on the rumen digestibility of the carbohydrate source, VFAs are produced in varying proportions. Forages are lower in energy density and also they contain high amounts of hemicellulose and cellulose than grains. Such a nutritive content supports a rumen fermentation pattern that favours more acetate than propionate (Beever and Mould, 2000). This is the pattern observed in all the forages used in the experiment. Hence higher A/P ratios were realised. VFAs can meet up to 80% of the animal's energy requirements (France and Siddons, 1993).

The trend observed suggests that WCBS has higher content of nitrogen or soluble nitrogen compared to the other forage sources. NH_3-N is recognised as the most important source of N for rumen microbes. Microbial protein can meet up to 60% of the animal's protein requirements. For maximum nutrient utilisation, Perdok and Leng(1989) stated that NH3-N concentrations should be within a range of 15-20mg/100ml rumen fluid. The NH3-N production for all forages in all sampling periods was not within this optimal range. This proves the inadequacy of forages fed alone as a complete ration in meeting animal nutritive requirements.

Gas production *in vitro*, is closely related to *in vivo* rumen digestibility of the feed or feedstuff (Menke *et al.*, 1979). The trend observed, where gas production increased over time is as expected also with increasing retention time in vivo. All the forages would be expected to be of almost the same rumen digestibility according to the *in vitro* gas out-put which did not significantly differ among the treatments.

Ⅴ. Conclusion

WCBS, IRGS, RSS and RS maintained a desirable rumen pH, although RSS silage had significantly higher pH values. Since forage source did not have significant differences in TVFAs, individual VFAs, A/P and gas production, WCBS was concluded to be the better forage source on the basis that it produced significantly high NH_3-N.

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