Analysis of Rumen Fermentation According to Various Association of Forage and Concentrate Feed

Seul Lee, Banji Kim, Chaehwa Ryu, Jinyoung Jeong, Byeonghyeon Kim, Youlchang Baek
Animal Nutrition Physiology Team, National Institute of Animal Science, Wanju 55365, Republic of Korea
e-mail:tabababy@korea..kr

조사료 및 농후사료 조합에 따른 반추위 발효 성상 분석

이슬, 김반지, 류채화, 정진영, 김병현, 백열창
국립축산과학원 영양생리팀

Abstract

The objective of this study is to analyze ruminal fermentation and methane emission according to different feed association of forage and concentrate. Alfalfa hay, oat hay, and concentrate feed were used for in vitro fermentation experiment, with the different feed ratio of 9:1, 5:5, 1:9 (forage:concentrate feed ratio). After 24 hours of incubation, rumen fermentation was evaluated. In overall, low forage treatments had higher total gas, CH_4, NH_3-N, TDMD, and total VFA to other treatments, which are used as a parameter of rumen fermentation ($P < 0.05$). The feed ratio had an effect on the copy number of total archaea, and genus *methanobrevibacter* ($P = 0.015$, $P = 0.010$). The trend of PCR result is similar with that of CH_4 per DDM. However, the copynumber of methanogen does not reflect the total emission of CH_4. This result corresponds with the earlier study, the composition of methanogen affects the level of CH_4, not the entire copynumber of methanogens. The results of this study can be applied to predict the rumen fermentation and methane emission of the cattle which fed a variety of associated feedstuffs. To implement more accurate applications, it is necessary to conduct in vivo studies using the cattle with same methods.

1. Introduction

Methane generation by ruminants is linked not only to global environmental problems, but also to the economic loss. To mitigate methane emission from ruminants, there have been many studies to reduce partial ruminal fermentation which producing methane. For the basis of these research, the methane-producing microbiome were investigated using many experimental methods, as the analysis technology evolves. In the current, real-time PCR is known as a highly sensitive method which is applicable for the detection and quantification of microbial populations without cultivating them in anaerobic environment. There has been studies for analyzing the rumen methanogen in cattle, but little study has been done with the Korean native cattle, Hanwoo. The objective of this study is to investigate the effect of analyze methane emission and rumen methanogen according to various feed association. The objective of this study is to analyze ruminal fermentation, methane emission, and microbial change according to different feed association of forage and concentrate.

2. Materials and Methods

2.1 Preparation of Experimental Diets

Alfalfa hay, oat hay, and concentrate feed were used for in vitro fermentation experiment. To measure the ruminal fermentation characteristics, the feedstuffs were incubated in various combination as follows: 90% alfalfa and 10% concentrate feed (HA), 50% alfalfa and 50% concentrate feed (MA), 10% alfalfa and 90% concentrate feed (LA), 90% oat hay and 10% concentrate feed (HO), 50% oat hay and 50% concentrate feed (MO), 10% oat hay and 90% concentrate feed (LO). The feed samples were oven-dried at 60°C for 72 h, then grounded through a cyclone mill (Foss, HillerØd, Denmark) with a 1 mm and 2 mm screen, for the chemical analysis and in vitro trial, respectively.

2.2 *In vitro* Ruminal Fermentation Experiment

All experimental procedures were approved and performed under the guidelines of the National Institute of Animal Science Institutional Animal Use and Care Committee in Korea. Three cannulated Hanwoo steers were individually housed in a pen and fed a diet composed of 80% concentrate feed and 20% of mixed

hay (45% tall fescue, 45% orchard grass, and 10% Kentucky bluegrass). Animals were fed twice a day with 4 kg of concentrate and 2.5 kg of roughage in total. Water and mineral blocks were freely accessible. Rumen liquid was collected one hour before morning feeding, squeezed through four layers of cheesecloth before pH was measured. The collected rumen fluid was filtered with 4 layers of cheese cloth, and then bubbles with O_2-free CO_2 gas to maintain anaerobic condition. Rumen fluid from three donors was pooled and combined with McDougall's buffer in a 4:1 ratio under strict anaerobic conditions [M1], and 50 mL of inoculum was then added (n = 3/treatment). The control setup was three blank flasks containing only inoculum. Each treatment and control flask contained total 0.5 g of fermentation substrates: the association of alfalfa, oat hay, and concentrate feed according to each treatment. 125 mL of flasks were sealed with butyl rubber stoppers and aluminum caps and then incubated for 24 h at 39°C. Subsequently, rumen-fluid pH and total gas production were measured; the latter with a glass syringe (Truth, Pvt. Ltd. 100 mL, India). Rumen fluid was centrifuged at 6,000 × g and 20°C for 15 min to remove remaining feedstuff. Dry matter degradability was calculated by filtering the solid fraction of rumen fluid after centrifuge and washing the residue in the flask with distilled water. Samples were filtered by filter paper (Whatman, No. 541) and dried in the oven for 48 hours.

2.3 Statistical Analysis

All data were analyzed using the MIXED procedure in SAS. Between-treatment differences were also compared with the Tukey's range test to determine the significance of the overall treatment effect. Significance was set at $P < 0.05$, while $0.05 \leq P < 0.1$ was considered a trend.

3. Results and Discussion

The in vitro fermentation characteristics after 24 h incubation are presented in table 2. There was a significant difference in pH, total gas, CH_4, CH_4 per digested dry matter(DDM), NH3-N, true dry matter degradability (TDMD), total volatile fatty acids(VFA), acetate, propionate, acetate:propionate ratio(A:P ratio), and valerate among all of the treatments ($P < 0.05$). The pH of rumen fluid showed a significant difference according to the forage source and feed ratio ($P = 0.003$, $P < 0.001$), and the pH was significantly lower in low forage. Total gas and CH_4 showed a significant difference based on the feed ratio ($P < 0.01$), and the values were significantly increased in low forage. On the other hand, CH_4 per DDM significantly decreased in low forage ($P < 0.01$). TDMD showed a significant difference according to the forage source and feed ratio ($P < 0.01$, $P < 0.01$), and the TDMD value increased as the forage ratio decreased. Total VFA value showed a significant difference according to the feed ratio ($P = 0.03$), and the value increased as the forage ratio decreased.

In overall, LA and LO had higher total gas, CH4, NH3-N, TDMD, and total VFA to other treatments, which are used as a parameter of rumen fermentation ($P < 0.05$). This result is thought to be arise from the relatively high ratio of concentrate feed accounting for 90% in the total feed of LA and LO. These general outcomes of the experiment are corresponding with the widely known idea about the rumen fermentation. It is known that the generation of acetate is related to the forage and the amount of propionate is related to the concentrated feed [D2, D3]. The A:P ratio of LA and LO was lower than that of other treatments. Also, the concentrate feed has higher NFC and lower NDF than roughages, and tends to be easily digested, consequently [D4]. Consequently, the overall ruminal fermentation results support the earlier studies about the difference according to the composition ratio of feed.

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[Table 1] Chemical composition of feeds used in *in vitro* experiment (% DM)

Item	Alfalfa	Oat hay	Concentrate
Dry matter	91.4	92.7	91.5
Crude protein	20.1	6.2	19.0
Ether extract	1.5	1.3	3.3
Non-fiber carbohydrate	17.0	27.4	43.8
Neutral detergent fiber[1)]	53.1	61.5	25.9
Acid determent fiber	41.9	35.6	9.1
Ash	8.3	3.6	8.0
GE(Kcal/g)	4.2	4.2	4.3

[1)]Neutral detergent fiber analyzed using a heat stable amylase and expressed inclusive of residual ash

[Table 2] Effect of different forage sources and levels on in vitro rumen fermentation at 24h

Item[1)]	Treatments[2)]						SEM[3)]	Significance[4)]		
	HA	MA	LA	HO	MO	LO		F	R	F x R
pH	6.67 ^{a}	6.59 ^{b}	6.51 ^{d}	6.62 ^{b}	6.54 ^{c}	6.51 ^{d}	0.010	0.003	<0.001	0.050
Total gas, mL	68.3 ^{e}	80.5 ^{c}	85.3 ^{b}	62.3 ^{f}	75.0 ^{d}	89.0 ^{a}	1.14	0.02	<0.01	<0.01
CH_4,mL	3.9 ^{c}	4.7 ^{b}	5.0 ^{b}	3.3 ^{d}	4.6 ^{b}	5.5 ^{a}	0.14	0.73	<0.01	0.02
CH_4,mL/gDDM	21.8 ^{b}	19.5 ^{bc}	17.5 ^{c}	25.0 ^{a}	21.5 ^{b}	19.6 ^{bc}	0.67	<0.01	<0.01	0.72
NH_3-N,mg/dL	10.4 ^{a}	10.6 ^{a}	9.4 ^{ab}	3.1 ^{d}	6.1 ^{c}	8.3 ^{b}	0.63	<0.01	0.02	<0.01
TDMD, %	44.0 ^{d}	57.3 ^{b}	66.0 ^{a}	37.4 ^{e}	51.5 ^{c}	64.9 ^{a}	1.15	<0.01	<0.01	0.07
Total VFA, mM	66.9 ^{bc}	77.8 ^{ab}	79.1 ^{a}	65.9 ^{c}	66.8 ^{bc}	75.0 ^{abc}	3.53	0.09	0.03	0.38
Acetate, mM	46.4 ^{ab}	52.0 ^{a}	50.5 ^{ab}	44.4 ^{ab}	43.6 ^{b}	47.3 ^{ab}	2.33	0.04	0.33	0.37
Propionate, mM	13.6 ^{c}	17.0 ^{ab}	19.4 ^{a}	14.3 ^{c}	15.4 ^{bc}	18.9 ^{a}	0.78	0.50	<0.01	0.37
A:P ratio	3.4 ^{a}	3.1 ^{b}	2.6 ^{d}	3.1 ^{b}	2.8 ^{c}	2.5 ^{e}	0.02	<0.01	<0.01	<0.01
Butyrate, mM	5.0	6.5	7.0	5.8	15.3	6.8	3.87	0.35	0.37	0.47
Valerate, mM	1.61 ^{ab}	1.70 ^{a}	1.69 ^{a}	1.49 ^{b}	1.51 ^{b}	1.62 ^{ab}	0.04	<0.01	0.09	0.39

[1)]DDM: digested dry matter, DDM: digested dry matter, TDMD: true dry matter digestibility, VFA: total volatile fatty acids, A:P ratio: acetate/propionate ratio

[2)]HA: 90% alfalfa and 10% concentrate feed, MA: 50% alfalfa and 50% concentrate feed, LA: 10% alfalfa and 90% concentrate, HO: 90% oat hay and 10% concentrate, MO: 50% oat hay and 50% concentrate, LO: 10% oat hay and 90% concentrate

[3)]SEM: standard error of the mean

[4)]F: effect of forage sources, R: effect of forage to concentrate ratio, F x R: effect of interaction between feed sources and forage to concentrate ratio