#### **B-2.4.1** Methane Production in Ruminants

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#### **Abstract**

The relationship among diet composition, intake and methane (CH4) production should be investigated to clarify the factors affecting CH4 production in the rumen and to estimate the CH4 emissions from ruminant in Japan and all over the world. To achieve the above purpose, we investigated (1) the relationship of CH4 production and volatile fatty acids (VFA), which is the main energy source of ruminants, in rumen, (2) the effects of quality and quantity of diet on CH4 production in ruminant, and (3) the simple estimation equation of CH4 production in ruminants. The results were as follows; (1) The VFA in the rumen was found no relation to CH4 production. (2) Although the daily CH4 production was goats < sheep < cattle, there were no significant differences in CH4 production per dry matter intake among ruminants. (3) The CH4 production was influenced by the hay-concentrate ratio of diets. (4) The CH4 production by ruminants fed good quality diets can be estimated adequately from DM intake (X, g/day) alone by the equation  $CH4(1/day) = -17.766 + 42.793X - 0.849X^2$ , (r=0.950). (5) The CH4 production of the cows given low digestibility and low nitrogen content roughage was 0 to 10% higher compared with the estimated value obtained from the estimation equation. (6) Annual CH4 emission from all livestock in Japan was estimated to be 0.345 teragrams (Tg, g\*10<sup>12</sup>)/year. (7) Annual CH4 emission from cattle in the world was estimated to be 67-71 Tg/year.

Key Words Ruminant, Methane, estimation, Dry matter intake, diets

#### 1. Introduction

The report by IPCC<sup>6)</sup> shows that current methane (CH4) emission from domestic and wild animals is estimated to be about 80 teragrams (Tg, g\*10<sup>12</sup>)/year, representing 15-25 % of the total CH4 released to the atmosphere from all sources. Among animals, the ruminants are the major emitters of CH4. Their rumen, a large "fore-stomach," is a continuous fermentation system. Microorganisms such as bacteria, fungi and protozoa in the rumen ferment carbohydrates and other plant materials to produce mainly volatile fatty acids. During the fermentation process in the rumen, methanogenic bacteria produce a considerable quantity of CH4. The conversion of hydrogen or formate and carbon dioxide (CO2) is the primary mechanism<sup>3)</sup>. However, the amount of CH4 production in ruminants is strongly influenced by the level and type of diet. Therefore, in this research, dietary factors affecting CH4 production and a simple method for estimating CH4 production in ruminants to predict total CH4

emission from livestock in Japan and all over the world are discussed.

# 2. Research objective

The relationship among diet composition, intake and CH4 production should be investigated to clarify the factors affecting CH4 production in the rumen and to estimate the CH4 emissions from ruminants in Japan and all over the world. To achieve the above purposes, we investigated (1) the relationship of CH4 production and volatile fatty acids (VFA), which is the main energy source of ruminants, in rumen, (2) the effects of quality and quantity of diet on CH4 production in ruminants, and (3) the simple estimation equation of CH4 production in ruminants.

#### 3. Research method

# (1) Experiment 1

Two ruminally cannulated cows were used for 2 trials. They were fed hay at the 1st trial, and fed high concentrate diet (hay-concentrate ratio=3:7) at the 2nd trial as a basal diet (BD). Three VFA mixtures containing acetic, propionic and butyric acid in the following molar proportions (%) 20, 70 and 10 (A2P7); 45, 45 and 10 (A4.5); 70, 20 and 10 (A7P2) were infused into the rumen to supply 50% of metabolizable energy (ME) requirement for maintenance. The treatments for both trials were (1)BD50; BD equivalent to 50% of ME required for maintenance was fed, (2)A2P7; BD50 plus A2P7 solution infused; (3)A4.5; BD50 plus A4.5 solution infused; (4)A7P2; BD50 plus A7P2 solution infused; (5) BD100; BD equivalent to 100% of ME required for maintenance was fed.

## (2) Experiment 2

Six Holstein heifers, 10 Corriedale wethers and 11 castrated male goats of Japanese native breed were used to clarify the effects of hay-concentrate ratios on CH4 production in ruminants fed at 1.5 times maintenance. The three levels of hay-concentrate ratios were 100:0 (H100), 70:30 (H70), and 30:70 (H30).

## (3) Experiment 3

The relationship between dry matter intake (DMI) and methane (CH4) production was investigated using results obtained from 190 energy balance trials with dairy cattle, beef cattle, sheep and goats. Dairy cattle include lactating, pregnant and dry Holstein cows. Beef cattle include steers, pregnant cows and dry cows of Japanese Black, and Holstein steers. All the animals were given roughage and concentrate. The animals were grouped in consideration of species or breed, physiological status and DM intake for the least square analysis. The six groups were Holstein lactating cows, Holstein pregnant cows and dry cows, Holstein fattening steers, Japanese Black pregnant cows and dry cows, Japanese Black fattening steers, and sheep and goats.

## (4) Experiment 4

The influence of ruminal infusion of urea on CH4 production were determined with 4 Holstein cows given low digestibility and low protein diet as a basal diet (BD) which was mixture of steamed wood and italian ryegrass hay wafer. The amount of BD was equivalent to 50% of ME and to less than 10% crude protein required for maintenance. Three levels of urea infusion were no urea (N0), 50% (N50) and 100% (N100) of crude protein requirements for maintenance. Urea was infused into the rumen as a solution in VFA. The amount of VFA

infused was calculated to meet 50% of ME required for maintenance.

# (5) Experiment 5

The influence of ruminal infusion of urea on CH4 production were determined with 3 Holstein cows given high digestibility and low protein diet as BD which was mixture of steamed wood and italian ryegrass hay wafer. The amount of BD was equivalent to 50% of ME and to 60% crude protein required for maintenance. Two levels of urea infusion were no urea (N60) and 40% (N100) of crude protein requirements for maintenance. Urea was infused into the rumen as a solution in VFA. The amount of VFA infused was calculated to meet 50% of ME required for maintenance.

#### 4. Result and discussion

# (1) Relationship of CH4 production and VFA in the rumen

Table 1 shows the effects of various VFA infusions on CH4 production. Almost no CH4 was produced from fasting cow. Also no increase in CH4 production was observed by VFA infusion treatment. Although it is considered that methanogens can convert acetate to CH4, rates of acetate conversion to CH4 are low in the rumen. Results shown in Table 1 confirm that the contribution of acetic acid to CH4 production is insignificant. The CH4 production per dry matter (DM) intake was higher in cows with low intake level than in cows with high intake level. This effect was more marked when cows were fed high concentrate diet. Digestibility of high concentrate diet was higher than hay. So, it is considered that higher feed intake leads to the fall in CH4 production per unit feed intake in the rumen and the fall in CH4 production is more marked when ruminants consume highly digestible feed.

9	Item		50%BD +A4.5		

Item	50% BD	50%BD +A2P7	50%BD +A4.5	50%BD +A7P2	100% BD	Fast	
<u> </u>		BD: Hay					
CH4 production (l)	150	140	136	136	266	11	
(l/kgDM)	34	32	31	31	31		
		BD: High	concentrate	diet			
CH4 production (l)	157	136	145	144	264	11	
(l/kgDM)	46	41	43	43	39		

Table 1. Methane (CH4) production in cows during VFA infusion.

50% BD: Basal diet (BD) equivalent to 50% of energy required for maintenance was fed. 100% BD: Basal diet (BD) equivalent to 100% of energy required for maintenance was fed.

VFA solutions: Acetic:Propionic:Butyric=2:7:1 (A2P7), 4.5:4.5:1 (A4.5), 7:2:1 (A7P2).

### (2) Effects of the hay-concentrate ratios of diets on CH4 production

Table 2 shows the effects of hay to concentrate ratios on CH4 production in heifers, sheep and goats. The daily CH4 production was significantly different among animal species. Heifers produced about 7 times and 9 times as much as sheep and goats, respectively. The daily CH4 production was also significantly different among treatments. CH4 production in H30 treatment was significantly lower than that in H70 treatment. It has been shown that the amount of digested cellulose contributed to CH4 production more than that of other carbohydrate components. Since, moreover, the conditions that promote the synthesis of microbial cells and propionate production lead to reduced CH4 production, it is considered that the diets which have the characteristics of lower cellulose content, lower digestibility of fiber fractions and promoting propionate type fermentation, such as high concentrate diet, lead to lower CH4 production. There were no significant differences in CH4 production per various nutrient intakes among animals though treatment effect of them was significant. It confirms that CH4 production is a function of the nutrient intake and not of the size of the animal.

Table 2. Least square means for daily methane (CH4) production in heifers, sheep and goats consuming various diets.

	•	Animal				Treatment			Effect	
		Heifer	Sheep	Goats	H100	H70	H30	Animal	Treatment	
CH4	(1)	230.9ª	34.3 <sup>b</sup>	25.2 <sup>b</sup>	93.0ª,b	115.0°	82.5 <sup>b</sup>	**	*	
CH4/DM							25.4 <sup>b</sup>	NS	*	
CH4/CF						135.2 <sup>b</sup>	$202.8^{b}$	NS	**	
CH4/NFE						59.1 <sup>b</sup>	41.1 <sup>b</sup>	NS	**	

Hay-concentrate ratio:H100; hay 100%, H70; hay 70%, H30; hay 30%.

DM:Dry matter, CF:Crude fiber, NFE:Nitrogen free extracts.

Level of significance: NS Not significant; \*\* P<0.01; \* P<0.05.

a,b:Means in the same row within animal and within treatment with different superscripts differ (P<0.05) by Student's t test.

# (3) Estimation of CH4 production from ruminants

Table 3 shows the mean of DM intake and CH4 production in ruminants used to investigate the estimation equation of CH4 production. There were significant differences among animal groups in all the variables. Although the daily CH4 production was increased with increasing the intake of dry matter, the CH4 production per unit DM intake was conversely decreased.

Table 3. Mean daily intakes of dry matter (DM) and methane (CH4) production.

		Difference						
Item		HL-L	HL-PD	HL-S	JB-PD	JB-S	SG	
DM intake								**
CH4 production	l/day	464.04°	268.43 <sup>b</sup>	259.32°	211.65 <sup>d</sup>	205.63 <sup>d</sup>	28.55°	**
CH4/DM intake	l/kg	27.17²	33.84 <sup>b</sup>	33.85 <sup>b</sup>	33.40 <sup>b</sup>	36.49 <sup>b</sup>	26.70°	**

Level of significance: \*\* P<0.01. a, b, c, d, e: Means in the same row with different superscripts differ (P<0.05) by Student's t test.

HL-L: Holstein lactating cows, HL-PD: Holstein pregnant cows and dry cows,

HL-S: Holstein fattening steers, JB-PD: Japanese Black pregnant cows and dry cows,

JB-S: Japanese Black fattening steers, SG: Sheep and goats.

Table 4 summarizes the results of regression analyses. The effect of feeding level,

expressed as the amount of digestible energy consumed divided by the amount required at maintenance, on the relationship between DM intake and CH4 production was studied by deriving separate regressions within three classes of feeding level. The regression coefficient was significantly decreasing with increasing level of feeding. It means that CH4 production per unit feed intake is decreasing with feeding level although the absolute amount of CH4 produced increases.

Since CH4 production per unit feed intake was decreasing with increasing level of feeding as mentioned above, the relationship between CH4 production (Y, l/day) and DM intake (X, kg/day) could be expressed as a quadratic form as shown in Fig. 1.

Table 4. Regressions of methane production (Y, l/day) on dry matter intake (X, kg/day) for different levels of digestible energy (DE) intake.

DE intake (x maintena	N ance)	Regression equation	_	nificance regression
<1.5	108	Y=- 0.127+34.360X	0.899	**
1.5-2.5	68	Y= 29.973+27.047X	0.928	**
>2.5	14	Y= 347.992+ 6.741X	0.016	NS
All data	190	Y= 54.663+24.133X	0.889	**
		$Y=-17.766+42.793X-0.849X^2$	0.934	**

Level of significance: NS Not significant; \*\* P<0.01.

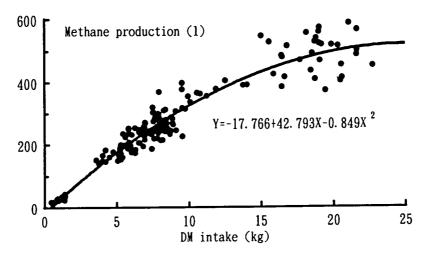


Fig. 1. Relationship between dry matter (DM) intake (kg/day) and methane production (l/day)

From the above results, it can be concluded that CH4 production by ruminants can be estimated adequately from DM intake alone. However, the above equation may not be applicable to the animals given poor quality roughages such as agricultural by-products.

# (4) Methane production of cattle given low quality diets

Table 5 shows the influences of ruminal infusion of urea on CH4 production of cattle given low or high digestibility and low protein content basal diets. CH4 production of the cows given low digestibility and low protein content diet in experiment 4 was 0 to 10% higher compared with the estimated value obtained from the estimation equation reported in this report. On the other hand, CH4 production of the cows given high digestibility and low

Table 5. Effect of urea infusion on methane production of cattle given low (experiment 4) or high (experiment 5) digestibility and low nitrogen content diets

		Exper	iment 4		Experiment 5			
Item	N0	N50	N100	Mean	N60	N100	Mean	
DM <sup>1)</sup> digestibility (%	) 27.5	29.3	33.8	29.5	64.4	66.2	65.3	
CH4 production (l/day	(r) 44.5	50.1	46.3	46.8	98.2	107.3	102.8	
CH4/DM intake (1/kg	() 26.7	22.1	31.5	26.2	29.1	31.9	30.5	
CH4 energy/GE <sup>2)</sup> (%	•	5.1	7.2	6.0	4.5	4.8	4.7	
CH4/estimated CH4 <sup>3)</sup> (%	) 115.3	81.4	131.7	107.7	84.0	92.3	87.4	

<sup>1)</sup> DM: dry matter, 2) GE: gross energy,

protein content diet in experiment 5 was 13% lower compared with the estimated value. From the above result, CH4 production of the cows given low digestibility and low protein content diet was 0 to 10% higher compared with the estimated value obtained from the estimation equation. More data should be accumulated to estimate accurately CH4 production of ruminants in the developing countries.

(5) Estimation of methane emission from livestock in Japan and all over the world

Table 6. Methane emission from livestock in Japan.

Animal	DMI (kg) (l/d	CH4 prod /head) ani	Number of mals <sup>1)</sup> (Tg	Total emission /year)
Dairy cows			2,068,400	0.182
Lactating cows	15.8	446.5	1,082,000	0.126
Dry cows <sup>2)</sup>	7.5	255.4	332,300	0.022
Heifers <2 yr	7.9	267.3	$654,100^{5}$	0.034
Beef cattle			2,804,700	0.150
Breeding cows	5.8	201.9	$713,700^{6}$	0.037
Fattening				
Beef breed <sup>3)</sup> >1 yr	r 7.3	249.4	565,000	0.037
<1 yr	5.2	181.8	$453,000^{6}$	0.011
Dairy breed4)	9.5	312.2	$1,073,000^{5}$	0.066
Sheep and Goats	0.8	15.9	66,800	< 0.001
Pigs		4.2	11,335,000	0.012
Horses		69.0	24,300	< 0.001
Total			16,299,200	0.345

<sup>1)</sup> Investigated on February, 1991. 2) Including heifers older than 2 years. 3) Mainly Japanese Black steers and females. 4) Mainly Holstein steers and females. 5) Twenty five percent of the animal population younger than 2 years old were excluded for the calculation of CH4 production. 6) Fifty percent of the animal population younger than 1 year old were excluded for the calculation of CH4 production.

<sup>3)</sup> Estimated CH4=-17.766+42.793\*DM-0.8486\*DM<sup>2</sup>

From the above equation, using average DM intake in various stages of cattle, sheep and goats estimated from Japanese Feeding Standard<sup>1,2)</sup>, NRC<sup>9)</sup> and Itoh et al.<sup>7)</sup>, and the number of livestock<sup>8)</sup>, total CH4 emission from ruminant livestock in Japan was estimated as shown in Table 6. Annual CH4 production was estimated to be 0.182 Tg/year from dairy cattle and 0.150 Tg/year from beef cattle. Total CH4 emission by ruminant livestock in Japan was estimated to be 0.332 Tg/year and total CH4 emission by all livestock including ruminants, pigs and horses was 0.345 Tg/year in Japan. It only accounts for around 0.5 % of the total CH4 emissions from animals in the world.

Table 7. Methane emission (CH4) from cattle in the world.

Region _	LC1)	ntake Others //head	LC	emission Others ay/head	LC	or of animals Others 000head	Total CH4 emission Tg/year
Africa	4.95	3.73	173.3	129.9	37,505	150,018	$6.78(7.46)^{4)}$
North America	16.20	5.79	452.7	201.5	11,256	101,305	6.65
Mid America	8.00	6.65	270.2	229.4	6,533	43,722	3.08(3.39)
South America	8.00	6.65	270.2	229.4	36,075	241,428	16.98(18.68)
Japan, Korea	15.80	4.39	446.4	153.7	1,659	5,883	0.43
Central Asia	7.70	5.69	261.4	198.4	2,771	89,598	4.82(5.30)
South East Asia	6.33	2.98	218.9	102.4	41,196	233,447	8.58(9.44)
Middle East Asia	4.95	3.73	173.3	129.9	4,636	18,545	0.84(0.92)
West Europe	13.75	6.20	410.1	214.9	27,301	60,767	6.32
East Eur., USSR <sup>2)</sup>	11.00	7.24	350.2	247.6	41,366	96,519	10.01
Oceania	9.50	7.33	312.2	250.2	2,285	30,362	2.17
Dev.ped all <sup>3)</sup>	12.49	6.46	384.3	223.4	83,867	294,836	25.58
Dev.ping all <sup>3)</sup>	6.42	4.74	221.9	166.1	128,717	776,757	41.08(45.18)
All <sup>3)</sup>	8.55	5.20	286.0	181.8	212,584	1,071,593	66.65(70.76)

<sup>1)</sup> LC: lactating cows, 2) USSR: former USSR

From the above equation, using average DM intake in various stages of cattle estimated from EAP<sup>4)</sup> and the number of livestock from FAO<sup>5)</sup>, total CH4 emission by cattle in the world was estimated as shown in Table 7, to be 67-71 Tg/year. Total CH4 emission by cattle in the developing countries accounts for around 62 % of the total CH4 emissions from cattle in the world.

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<sup>3)</sup> DM intake and CH4 emission are mean values of every region. Population and total CH4 emission are sum of every region.

<sup>4)</sup> Figures in parenthesis were CH4 emissions from developing countries which were assumed to be 10% more than that from developed countries.

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