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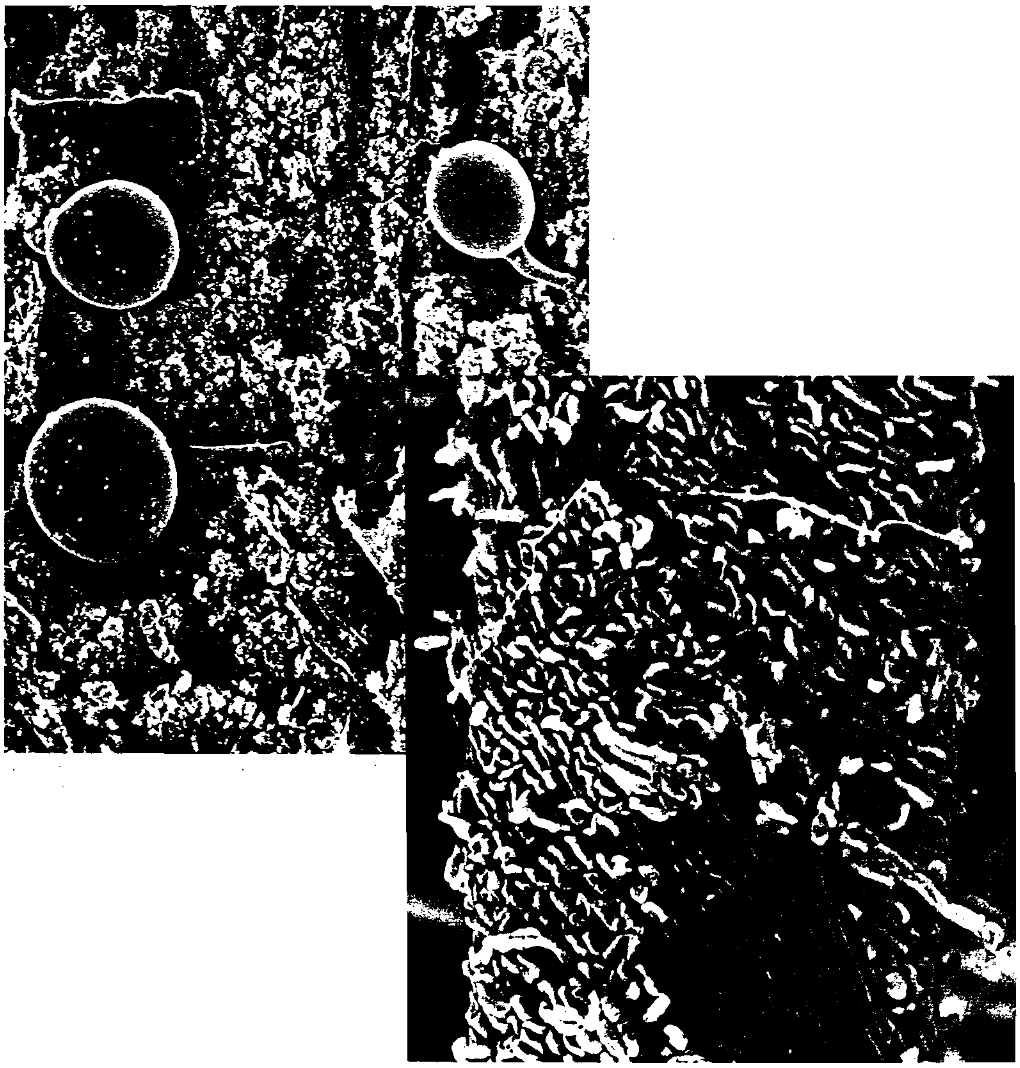

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Methane Production and Its Dietary Manipulation in Ruminants

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Methane (CH_4) production in ruminants has been investigated with a view to reducing energy loss of diets during the fermentation process in the rumen (*1*). However, at the same time, CH_4 produced by methanogenic bacteria plays an important role in eliminating hydrogen from the rumen because hydrogen is toxic to microbial growth.

It was recently shown that CH_4 was one of the major greenhouse gases (*6*). The most important sources of CH_4 are natural wetlands (21%), fossil fuel related to natural gas, coal mines, and coal industry (16%), enteric fermentation (16%), rice paddies (11%), biomass burning (7%), landfill (7%), and animal waste (5%) (*6*). Current CH_4 emission from enteric fermentation in domestic and wild animals is estimated to be about 85 (65 to 100) teragrams ($g \cdot 10^{12}$)/year (*6*). Among animals, ruminants are thought to be major anthropogenic emitters of this gas. Estimates of potential CH_4 reductions from ruminant animals are approximately 30% (*7*). However, the estimated value of emission rates is not certain yet because a variety of factors have strong influence on the amount of CH_4 produced in ruminants.

The objective of this review is 1) to clarify the effect of dietary factors and level of milk production on CH_4 production, 2) to evaluate the change in total CH_4 emission from lactating cows in Japan, and 3) to identify possible methods for reducing CH_4 production in ruminants.

I. METHANE PRODUCTION IN RUMINANTS

In adult ruminants the rumen represents about 85% of the total stomach capacity. The main function of the rumen is to digest plant materials which cannot be digested by the host enzymes. The materials are fermented into volatile fatty acids, carbon dioxide, and CH_4 by microorganisms such as bacteria, fungi, and protozoa. Rumen methanogenic bacteria utilizes either hydrogen and carbon dioxide or formate, acetate, methylamine, and methanol to produce CH_4. The primary mechanism is the conversion of hydrogen and carbon dioxide, or formate.

1. Effect of Feed Intake

Daily CH_4 production (liter/day) was significantly different among ruminant species fed to meet approximately 1.5 times their maintenance requirements (*15*). Heifers produced about 7 times and 9 times the CH_4 produced by sheep and goats, respectively. These results are not far from the multipliers for "Livestock units": cattle=0.8, sheep=0.1, and goats=0.1 reported by Gibbs *et al.* (*4*). However, there was no significant difference among ruminants in CH_4 production (liter/kg) for various nutrient intakes of dry matter (DM), crude fiber (CF), and nitrogen free extracts (NFE) (*15*). This confirms that CH_4 production is influenced by the level of nutrient intake, and not by size of the ruminants.

Shibata *et al.* (*16*) investigated the relationship between DM intake and CH_4 production using the data from 190 total energy balance trials with dairy cattle, beef cattle, sheep, and goats. Dairy cattle included lactating, pregnant, and dry Holstein cows. Beef cattle included steers, pregnant cows and dry cows of Japanese Black species, and Holstein steers. Lactating cows showed the highest DM intake and the lowest CH_4 production per unit DM intake. This production per unit DM intake decreased with increased level of feeding although overall daily CH_4 production increased. Coppock *et al.* (*2*) found a similar relationship and the reason could be various factors such as changes in the rumen fermentation pattern or the mean retention time of feed in the rumen (*17*). The relationship between CH_4 production (Y1, liter/day) and DM intake (DMI, kg/day) can be expressed as follows:

$$Y1 = -17.766 + 42.793DMI - 0.849DMI^2 \ (r=0.97^{**}) \qquad \text{(i)}$$

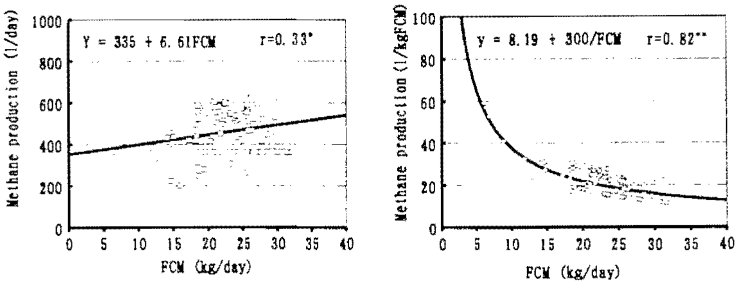

Fig. 1. Relationship between 4% FCM and methane production.

Using this equation, CH_4 production by ruminants can be estimated adequately from DM intake alone, although the equation may not be applicable to ruminants given poor quality diets such as agricultural by-products.

2. *Effect of Milk Production*

CH_4 production resulting from maintenance metabolism has to be regarded as non-productive since this is gas released without any benefit to milk production (*9*). The ratio of non-productive to productive CH_4 decreases with higher milk yield (*9*). In an investigation using 115 total energy balance trials with lactating cows, as daily 4% fat-corrected milk yield (FCM, kg/day) increased, daily CH_4 production also increased, but CH_4 production per unit FCM (Y2, liter/kg FCM) hyperbolically decreased as shown in Fig. 1 (*11*). A regression equation was derived as follows:

$$Y2 = 8.19 + 300/FCM \ (r = 0.82^{**}) \qquad \text{(ii)}$$

This result indicates that if FCM increases from 5 to 10, 20, and 30 kg/day, CH_4 production per unit FCM decreases from 68 to 38, 23, and 18 liter/kg FCM, respectively.

3. *Changes in Methane Emission from Lactating Cows in Japan*

Figure 2 shows the changes in FCM and CH_4 emission from lactating cows in Japan (*11*). Total CH_4 emission per year was derived from lactating cow populations and CH_4 production per head was estimated by using the above equation (ii), average milk yield and average milk fat percentage each year. Until 1980, the increase in total CH_4 emission per year paralleled the increase in total FCM per year; after 1980 total FCM per year increased by 30%, although total annual CH_4 emission was approximately constant

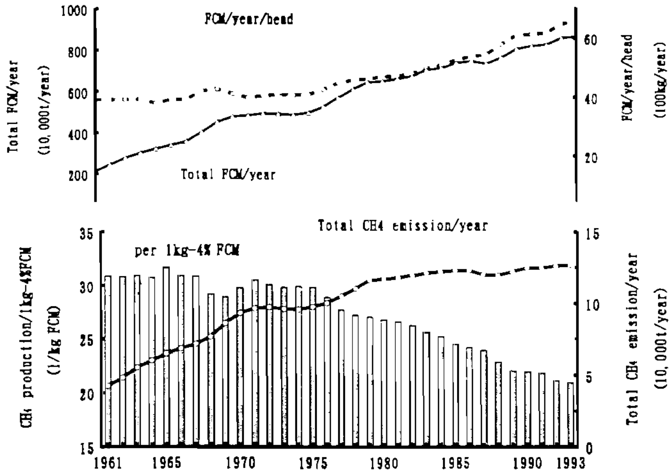

Fig. 2. Changes in 4% FCM and CH_4 emission from lactating cows in Japan.

because CH_4 production per unit FCM decreased with the increase in FCM per head. These results suggest that it is possible to achieve both an increase in total milk production and to control total CH_4 emission by increasing the level of milk production.

II. DIETARY MANIPULATION OF METHANE PRODUCTION IN RUMINANTS

CH_4 production is believed to be influenced by the type and level of diet. Moe and Tyrrell (*13*) showed the relationship between CH_4 production and type of carbohydrate digested. Holter and Young (*5*) reported the relationship between CH_4 production and several dietary factors such as chemical composition of diet, nutrient intake, and digestibility. Also, Shibata *et al.* (*15*) conducted regression analyses of CH_4 production on nutrient intake 1.5 times below maintenance needs of the animals. A multiple regression analysis showed that CH_4 production had a positive or negative correlation with carbohydrate intake, such as the amounts of CF, NFE, neutral detergent fiber, acid detergent fiber, and starch intake, or nitrogen intake, respectively (*14, 15*). However, CH_4 production was not influenced as much by type of carbohydrate at low intake as at higher intake (*13, 15*).

TABLE I
Effect of Roughage-concentrate Ratios on Methane (CH_4) Production

	Goats				
	H90	H75	H60	H45	H30
DM intake, kg/day	0.660*	0.589**	0.574**,***	0.545***	0.513****
DM digestibility, %	62.9*	63.5*,**	71.6**,***	74.6***	76.9***
CH_4 l/kg DM	26.1*	28.7*,**	31.7*,**	34.6**	33.9**
% ME intake	11.2	12.6	11.8	12.2	11.4

	Lactating cows		
	R70	R50	R30
DM intake, kg/day	15.9*	18.2**	18.1**
DM digestibility, %	62.8*	66.2**	66.5**
CH_4 l/kg DM	33.3	31.1	25.8
% ME intake	14.3	12.9	10.5

Goats were fed to meet their maintenance energy requirement. Roughage-concentrate ratio: H90, hay 90%; H75, hay 75%; H60, hay 60%; H45, hay 45%; H30, hay 30%; R70, roughage 70%; R50, roughage 50%; R30, roughage 30%. *,**,***,****Means in the same row within animal with different superscripts differ ($p<0.05$) by Student's t test. Data from Kurihara *et al.* (*10, 12*).

1. Effect of Hay-concentrate Ratio

Table I indicates the effect of hay to concentrate ratios at the same crude protein content (12%) of diet on CH_4 production in goats (*12*). The five levels of hay to concentrate ratios were 90 to 10 (H90), 75 to 25 (H75), 60 to 40 (H60), 45 to 55 (H45), and 30 to 70 (H30). These approximately met maintenance energy requirements. CH_4 production per unit DM intake increased with rise in the ratio of concentrate intake to hay intake until H45. However, the ratios of CH_4 energy loss to metabolizable energy (ME) intake were similar. These results imply that if ME intake of goats is at a maintenance energy level, daily CH_4 production is not influenced by the hay to concentrate ratio.

On the other hand, lactating cows were fed one of the three levels of roughage to concentrate ratios: 70 to 30 (R70), 50 to 50 (R50), or 30 to 70 (R30). High concentrate diet reduced both CH_4 production per unit DM intake and the ratio of CH_4 energy loss to ME intake compared with low concentrate diet. Therefore, in lactating cows it is important to increase the ratio of concentrate to total diet in order to reduce daily CH_4 production.

2. Effects of CP Content of Diets

Table II shows the effect of crude protein (CP) content on CH_4 production in goats fed a maintenance energy level diet (*12*). The four levels

TABLE II
Effect of Dietary CP Content on Methane Production in Goats

	CP4	CP7	CP9	CP12
DM intake, g/day	618	630	632	637
DM digestibility, %	55.0	57.7	58.5	57.0
CH_4 l/kg DM	29.3*	32.2*,**	36.3***	34.6**,***
% ME intake	14.9	15.7	17.7	17.0

Goats were fed to meet their maintenance energy requirement. Data from Kurihara *et al.* (*10, 12*).

TABLE III
Effect of CP and Zn Concentration of Diets on Methane Production in Dry Cows

	CP6.5	CP8.5	CP8.5 Zn[a]
DM intake, kg/day	6.1*	7.8**	6.4*
Zn content, ppm	12*	12*	1,310**
DMdigest.,[b] %	49	51	47
DMdisap.,[c] %	56*	66**	38***
CH_4 l/kg DM	19*(90)	21*(100)	8**(38)
% ME intake	10.0*	11.2*	4.4**

[a]Dry cows were fed diets supplemented with zinc sulfate (35 g/head/day). [b]DMdigest.: dry matter digestibility. [c]DMdisap.: dry matter disappearance in rumen after 48 hr incubation. Data from Kurihara *et al.* (*12*).

of CP content were 4% (CP4), 7% (CP7), 9% (CP9), and 12% (CP12). CH_4 production per unit DM intake and the ratio of CH_4 energy loss to ME intake increased with the rise in CP content of diets from CP4 to CP9. However, the difference between CP9 diet and CP12 diet was not clear. These tendencies also agreed with the results in dry cows (Table III). When ruminants were fed sufficient CP above the maintenance energy level, however, daily CH_4 production was negatively related to daily CP intake (*14, 15*).

3. *Effects of Zinc Sulfate Supplementation to Diet*

Since protozoa generates a relatively large amount of hydrogen, methanogenic bacteria attach to the surface of protozoa. Zinc sulfate supplementation of ruminant diets at levels greater than 1,000 ppm was demonstrated to defaunate the rumen (*3*). Therefore, defaunation caused by zinc sulfate supplementation may result in decreasing CH_4 production. Tables III and IV show the effect of zinc sulfate supplementation (35 g/head/day) on CH_4 production and rumen fermentation in dry cows (*12*). The ruminal protozoa numbers and cellulolytic bacteria numbers tended to decrease.

TABLE IV
Effect of CP and Zn Concentration of Diets on Rumen Fermentation in Dry Cows

	CP6.5	CP8.5	CP8.5 Zn
pH	7.1	7.0	6.9
Zn (ppm)		0.175*	2.455**
NH_3-N (mg/100 ml)	2.0	13.7	10.1
Total VFAs (mmol/l)	57.1	65.0	53.1
Protozoa (log no./ml)	4.8	4.8	2.2
Bacteria (log MPN/ml)			
Cellulolytic	8.1	8.4	6.8
Methanogenic	7.7	7.9	7.2

VFAs: volatile fatty acids. MPN: most probable number. *,**Same as in Table I.

CH_4 production of dry cows fed zinc supplemented diet significantly decreased by 60% compared with that of cows fed CP8.5 diet. However, in a high zinc diet, DM intake and DM disappearance rate of hay in the rumen significantly decreased. DM digestibility, ammonia nitrogen, and total volatile fatty acid concentration in the rumen also tended to decrease. Thus, the reduction in CH_4 production in this experiment is thought to be related to the repression of rumen fermentation. Itabashi *et al.* (*8*) also reported a decrease in CH_4 production and fiber digestibility by defaunation. Effective regulation of rumen microorganisms without lowered digestion of diet should therefore be implemented to reduce CH_4 production.

4. Effect of Calcium Salts of Fatty Acid (CaFA)

Methanogenic bacteria requires hydrogen to form CH_4, and some microorganisms in the rumen use hydrogen to hydrogenate the double bonds of unsaturated fatty acids. Therefore, the addition of unsaturated fatty acids to the diet may result in an inhibition of CH_4 production. Table V shows the effects of feeding CaFA on CH_4 production and rumen fermentation in goats (*12*). Goats were fed diets consisting of 60% hay and 40% concentrate. CH_4 production decreased with the feeding of CaFA and the extent of decrease became greater as the number of double bonds of fatty acids increased. CH_4 production from goats fed linoleic acid decreased by approximately 15% compared with control. In experiment 1, DM intake, DM digestibility, and rumen fermentation were not affected by feeding CaFA. However, in experiment 2, DM digestibility was slightly depressed in stearic acid feeding treatment, and DM intake and rumen fermentation were slightly depressed when linoleic acid was fed. Ruminal protozoa and bacteria numbers were not affected. In an experiment where lactating cows were fed 40% roughage and 60% concentrate, CH_4 production, DM intake,

TABLE V
Effect of Calcium Soap of CaFA on Methane Production and Rumen Fermentation in Goats

Item	Experiment 1			Experiment 2		
	Control	C18 : 0	C18 : 1	Control	C18 : 0	C18 : 2
DM intake, g/day	714	745	744	652	685	630
Supplemented CaFA, g DM		31[a]	31[b]		31[c]	31[d]
DM digestibility, %	64.2	64.7	65.3	70.1	64.8*	71.2**
CH_4 prod., l/kg DM	32.4	31.2	28.8	31.9	29.1	27.9
	(100)	(96)	(89)	(100)	(91)	(87)
% ME intake	13.4*	11.9**	10.9**	12.3	11.7	9.3
Ruminal parameter						
pH	6.83	6.66	6.68	6.60	6.61	6.90
NH_3-N, mg/100 ml				15.8	18.2	13.1
Total VFAs, mmol/l	76.3	77.0	77.4	61.8*	68.7*	53.8**
Protozoa, log no./ml				5.87	5.55	5.79
Cellulolytic bacteria, log MPN/ml				7.83	7.65	8.41
Methanogenic, log MPN/ml				8.47	>8.11	>9.11

[a]Stearic acid (C18 : 0) 97.8%. [b]Oleic acid (C18 : 1) 90.5%. [c]Stearic acid (C18 : 0) 93.6%. [d]Linoleic acid (C18 : 2) 80.0%. *,**Same as in Table I. Data from Kurihara *et al.* (*12*).

and DM digestibility were not affected by feeding CaFA. CaFA was a mixture of saturated fatty acid and monounsaturated fatty acids (*10*). These results imply that the effects of feeding CaFA on CH_4 production and rumen fermentation vary with the nature of the fatty acids, the level of feeding, and the hay to concentrate ratio. Therefore, it is necessary to optimize the combination of diet and CaFA for practical feeding conditions.

SUMMARY

CH_4 production (Y1, liter/day) in ruminants was influenced by dietary factors and production level. A simple and applicable estimation equation was $Y1 = -17.766 + 42.793DMI - 0.849DMI^2$. The relationship between 4% FCM (kg/day) and CH_4 production per unit FCM (Y2, liter/kg FCM) in lactating cows could be expressed by the equation, $Y2 = 8.19 + 300/FCM$. From this equation, lactating cow populations and their FCM, it is evident that total CH_4 emission per year from lactating cows has remained approximately constant in Japan during the last ten years although total annual FCM increased. Furthermore, at maintenance energy level, increasing the ratio of concentrate to total diet containing optimum CP was not always

useful for the reduction in CH_4 production. In lactating cows, it was important to increase the ratio of concentrate to total diet with optimum CP. Defaunation by zinc sulfate supplementation remarkably decreased CH_4 production by 60%, however, DM disappearance of hay in the rumen was also reduced. By feeding CaFA, CH_4 production decreased by 0 to 15%, although the degree of this reduction was influenced by the nature of CaFA, feeding level, and hay to concentrate ratio.

Considering the importance of ruminant livestock which have the unique ability to convert low quality plant materials into useful products, the establishment of an economically feasible way to improve their productivity as well as reduce their CH_4 emission is sorely needed.

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