

## Estimation of Methane Production by Lactating and Dry Crossbred Holstein Cows in Thailand

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### Abstract

The methane (CH<sub>4</sub>) produced by lactating and dry crossbred Holstein cows, together with factors affecting CH<sub>4</sub> production, were investigated using data obtained from balance trials of cows in Thailand (48 observations). The CH<sub>4</sub> production per dry matter intake (DMI) of lactating cows tended to be lower than that for dry cows (25.7 L/kg vs. 28.7 L/kg DMI;  $P < 0.10$ ), which was due to the higher energy intake of lactating cows. A positive correlation was observed between CH<sub>4</sub> production and DMI ( $r = 0.70$ ;  $P < 0.001$ ). The CH<sub>4</sub> production was also founded to be related to crude fiber (CF) and nitrogen free extract (NFE) intake from a multiple regression equation ( $r = 0.77$ ,  $P < 0.001$ ), and the coefficient of CF was higher than that of NFE, indicating higher methanogenesis of CF than of NFE. The decreasing CH<sub>4</sub> production per milk production with the increase in milk production ( $r = -0.62$ ;  $P = 0.003$ ) shows that improvements in the individual milk yield suppress the CH<sub>4</sub> production per milk production. From the findings of the present study and available statistical information in Thailand, it was estimated that CH<sub>4</sub> production by cows increased as the cow population increased over a 5-year period from 1999. Because the CH<sub>4</sub> production per milk yield showed little change from 1999 to 2004, in terms of milk productivity and suppression of CH<sub>4</sub> production, improving individual milk yield will be important in Thailand.

**Discipline:** Animal industry

**Additional key words:** carbon source, dry matter intake, milk production

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The results reported in this paper were obtained through collaborative research between JIRCAS and the Department of Livestock Development, Ministry of Agriculture and cooperatives, Thailand, through studies entitled “Comprehensive Studies on Sustainable Agricultural Systems in Northeast Thailand, 1995–2000” and “Increasing Economic Options in Rainfed Agriculture in Indochina through Efficient Use of Water Resources, 2001–2005.” This study was also partly supported by a grant from the “Global Environment Research Fund S-2-3a(2)” of the Ministry of Environment, Japan.

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Received 13 August 2009; accepted 12 January 2010.

## Introduction

The global warming potential of methane ( $\text{CH}_4$ ) is 21 times greater than that of carbon dioxide ( $\text{CO}_2$ ), and enteric fermentation in ruminants accounts for 16% of anthropogenic  $\text{CH}_4$  emissions<sup>12</sup>. The Intergovernmental Panel on Climate Change (IPCC)<sup>13</sup> has proposed two methods (Tier 2 and Tier 3) for estimating anthropogenic  $\text{CH}_4$  emissions in countries with large livestock populations. However, according to Kebreab et al.<sup>16</sup>, the prediction accuracy of the Tier 2 method is insufficient due to the use of a fixed  $\text{CH}_4$  conversion factor (MCF; expressed as the percentage of  $\text{CH}_4$  production per gross energy intake). For the Tier 3 method, more detailed data derived from direct experimental measurement is required<sup>13</sup>. In Southeast Asia, the demand for meat and milk is projected to increase<sup>8</sup>. However, compared to Western countries, the direct measurement of enteric  $\text{CH}_4$  production by ruminants is limited.

Integrated dairy farming in Thailand resulted from a collaboration project between the Thai and Danish governments in the 1960s. In 2004, the population of dairy cattle and lactating dairy cows reached 408,000 and 165,000 heads, respectively<sup>11</sup>. The enteric  $\text{CH}_4$  production in Thailand has also been increasing and is projected to have grown: it was 13.0 and 10.4 million ton (Mt)  $\text{CO}_2$  eq in 1990 and 2000 and is projected to be 13.3 and 16.1 Mt  $\text{CO}_2$  eq in 2010 and 2020, respectively<sup>7</sup>. Dairy cows in Thailand are mainly crossbreeds of Holstein and native Thai cattle: compared to purebred Holstein, they have a smaller body size, lower milk yield and greater resistance to heat stress and parasitic infections. Dairy farms are mainly distributed in the central, northern and northeastern regions of Thailand. Tropical grasses such as ruzi grass (*Brachiaria ruziziensis*) and guinea grass (*Panicum maximum*) are mainly used as roughage in dairy farms, except in some parts of the central region where corn silage is predominantly used. Additionally, rice straw is an important source of roughage in regions with extremely low rainfall in the dry season, such as the northeastern region.

JIRCAS and the Department of Livestock Development developed a respiration calorimeter with a ventilated face mask for large ruminants<sup>15</sup> at the Khon Kaen Animal Nutrition Research and Development Center (KKANRDC). The objective of this study was to evaluate the  $\text{CH}_4$  produced by lactating and dry cows in Thailand, and to consider the factors affecting  $\text{CH}_4$  production using data obtained from the balance trials at KKANRDC.

## Materials and methods

Data were collected from 20 observations of multiparous lactating cows and 28 observations of dry cows (Holstein  $\times$  native Thai cattle) from 4 experiments that involved 11 treatments: The details of experiments 2 and 4 can be obtained from the study by Odai et al.<sup>21,22</sup>, and those for experiments 1 and 3 were not published (Table 1). In addition to the feed listed in Table 1, 100 g of mineral and vitamin premix was fed per day to the cows in experiments 1 and 2, with 70 g per day of the premix fed to the cows in experiments 3 and 4. The mineral and vitamin premix contained 150,000 IU vitamin A; 30,000 IU vitamin  $\text{D}_3$ ; 100 IU vitamin E; 11.81 g Na; 18.22 g Cl; 330.00 g Ca; 171.32 g P; 1.03 g S; 1.20 g Zn; 499.3 mg Fe; 6.03 g Mg; 15.10 mg Co; 205.30 mg Cu; 15.30 mg I; 499.50 mg Mn; 7.00 mg Se; 5.00 mg Mo; and 4.70 mg K in 1 kg of feed as fed. All the roughage used was produced in northeast Thailand, and the concentrate was purchased from the dairy cooperative in Khon Kaen Province. Cows were fed *ad libitum*, except in experiment 4, when they were given feed equivalent to 1.7% of their body weight on a dry matter (DM) basis. The feed used for the experiments, except that used in experiment 4, was formulated according to the Japanese feeding standard<sup>1</sup> to fulfill total digestible nutrients and crude protein (CP) requirements. Cows were fed in individual pens and had free access to water. Experiments 1, 2 and 3 consisted of a 15-d adaptation period and a 5-d data collection period, whereas experiment 4 consisted of a 7-d adaptation period and a 5-d data collection period. During the 5-d data collection period, the oxygen expenditure, and the  $\text{CO}_2$  and  $\text{CH}_4$  produced by the experimental cows were measured 7 times per day for a duration of 6 min each time, by using the face-mask method<sup>15</sup>, and the orts, feces and urine were also collected daily. Lactating cows were milked twice daily in their individual pens. Milk samples were collected during each milking session and were refrigerated at 4°C until analysis. All animals were cared for according to the guide for the care and use of experimental animals by Curtis and Nimz<sup>5</sup>.

The  $\text{CH}_4$  volume, measured in liters, was converted to kilojoule and gram by multiplying it with the factors 39.54 and 0.716, respectively<sup>3</sup>. The collected orts, urine and feces were pooled separately for each cow during each trial period. Samples of the feed and the collected ort and feces were dried (60°C, 48 h) for chemical analysis. The DM, ash, ether extract, and crude fiber (CF) contents of the feed, ort and feces samples were determined according to the methods described by the Association of Official Analytical Chemists (AOAC)<sup>2</sup>. The nitrogen

content of the feed, ort, feces, and urine samples was also determined according to AOAC methods<sup>2</sup>. The energy content of the feed, ort, feces, urine, and milk samples was determined using an adiabatic calorimeter (CA-4PJ; Shimadzu, Kyoto, Japan), and the fat, protein and lactose contents of the milk samples were measured using the Milko-Scan (133B; Foss, Hillerød, Denmark).

Simple and multiple regression analyses were performed using the PROC REG of SAS<sup>23</sup>. Analysis of variance was performed to analyze the data on CH<sub>4</sub> production, with cow status as a factor, using the PROC GLM.

## Results and discussion

The daily CH<sub>4</sub> production of lactating cows was significantly higher than that of dry cows (262.7 L/d vs. 153.0 L/d;  $P < 0.01$ ) since the lactating cows consumed around twice the amount of DM as compared to the dry cows (10.2 kg vs. 5.3 kg; Table 2). However, when the CH<sub>4</sub> production was corrected for DM intake (DMI), it tended to be lower for lactating cows than for dry cows (25.7 L/kg vs. 28.7 L/kg DMI;  $P < 0.10$ ; Table 2). Moe and Tyrell<sup>20</sup> have reported that the fermentation of structural carbohydrate produces more CH<sub>4</sub> than that of non-structural carbohydrate. In addition, Kurihara et al.<sup>17</sup> found that CH<sub>4</sub> production increased as the forage-to-concentrate ratio increased. Therefore, lower CH<sub>4</sub> production per DMI by lactating cows in the present study would result from a lower CF content (13.7% of DM vs. 31.1% of DM) and a lower forage- to-concentrate ratio in the ration, as compared to dry cows (Tables 1 and 2). Shi-

bata et al.<sup>24</sup> obtained similar results for lactating and dry cows (27.2 L/kg and 33.8 L/kg of DMI, respectively).

The daily CH<sub>4</sub> production by lactating cows observed in the present study at 263 L/d was lower than the reported values in Japan<sup>24</sup> and North America<sup>20</sup> (464 L/d and 346 L/d, respectively). The CH<sub>4</sub> production by dry cows in this study was also lower than that observed in Japan<sup>24</sup> (153 L/d vs. 268 L/d). One of the reasons for this difference may be explained by the measurement method. The data in the present study were obtained by using the face-mask method, while the data in the literature cited were obtained by using a whole-body open-circuit respiration chamber. Liang et al.<sup>18</sup> showed that the heat production measured using the face-mask method was 9% lower than that measured using the respiration chamber, and Johnson & Johnson<sup>14</sup> also mentioned this underestimation. Thus, CH<sub>4</sub> production measured by the face-mask method would also be lower than that measured using the respiration chamber. Even if this 9% underestimation is accounted for in CH<sub>4</sub> production, the levels of daily CH<sub>4</sub> production observed in the present study will be lower than those in the cited literature. Another one of the reasons is cattle breed. The main dairy breed used in Japan and North America is Holstein (Holstein-Friesian), but crossbreeds of Holstein and native cattle were used in this study. This crossbreed is most commonly used in Thailand, which has a smaller body size and lower DMI and milk production compared to purebred Holstein cows. Therefore, the difference in the measurement method and in the dairy breed probably resulted in the lower CH<sub>4</sub> production observed in the pres-

**Table 1. Number of cows and feed formula used in the present study**

Exp. no.	Cow status	Treatment	n	Feed formula <sup>1,2</sup>
1	Lactating	1	5	21% CVH, 40% RS, 12% SBM, 10% DC, 10% RB, and 7% CCM <sup>3</sup>
		2	5	44% CVH, 5% SBM, 11% DC, 23% CSC, 11% RB, and 7% CCM <sup>3</sup>
2	Lactating	1	5	21% CVH, 40% RS, 12% SBM, 10% DC, 10% RB, and 7% CCM <sup>3</sup>
		2	5	40% CS, 15% SBM, 10% DC, 15% CSC, 11% RB, and 9% CCM <sup>3</sup>
3	Dry	1	4	92% CSS and 8% SBM <sup>4</sup>
		2	4	82% CSS and 18% SBM <sup>4</sup>
		3	4	59% CSS, 5% SBM and 36% DBG <sup>4</sup>
4	Dry	1	4	100% RGH <sup>4</sup>
		2	4	93% RGH and 7% SBM <sup>4</sup>
		3	4	86% RGH and 14% SBM <sup>4</sup>
		4	4	78% RGH and 22% SBM <sup>4</sup>

<sup>1</sup>: Percentages of feed formula are given on a dry matter basis.

<sup>2</sup>: RGH: Ruzi grass hay, CVH: Cavalcade hay, RS: Rice straw, CS: Corn silage, CSS: Corn stover silage, SBM: Soy bean meal, DC: Dried corn, CSC: Cassava chip, RB: Rice bran, CCM: Coconut meal, DBG: Dried brewer's grain.

<sup>3</sup>: 100 g/head/d of vitamin and mineral premix was included in the feed.

<sup>4</sup>: 70 g/head/d of vitamin and mineral premix was included in the feed.

ent study.

The CH<sub>4</sub> production, corrected for the DMI, measured in the present study was similar in lactating cows (25.7 L/kg DMI vs. 27.2 L/kg DMI) but was lower in dry cows (28.7 L/kg DMI vs. 33.8 L/kg DMI) when compared to the results obtained in a Japanese study<sup>24</sup>. Hindrichsen et al.<sup>9</sup> compared the *in vitro* CH<sub>4</sub> production during the fermentation of lignified fiber with that of non-lignified fiber, and found that lower fiber digestibility resulted in lower CH<sub>4</sub> production. The dry cows in the present study were fed tropical grass, rice straw or corn stover silage, whereas the dry cows in the study conducted by Shibata et al.<sup>24</sup> were mainly fed temperate grasses. Fiber digestibility has been reported to be generally lower for tropical grasses with a C4 photosynthetic pathway than for temperate grasses with a C3 photosynthetic pathway<sup>4</sup>. Therefore, it was considered that in the present study, the CH<sub>4</sub> production, corrected for the DMI, of dry cows was lower than that reported by Shibata et al.<sup>24</sup> due to lower fiber digestibility.

In order to estimate enteric CH<sub>4</sub> production using

the Tier 2 method, the IPCC<sup>13</sup> has recommended an MCF of  $6.5 \pm 1.0\%$  for dairy cows and their young in case country-specific data on MCF is unavailable. The MCF of lactating and dry cows in the present study was within the range recommended by the IPCC<sup>13</sup> (Table 2). Additionally, when the 9% difference in the results of the present and other studies was taken into consideration, the MCF of lactating and dry cows was 6.3% and 7.3%, respectively, both within the range recommended by the IPCC<sup>13</sup>.

CH<sub>4</sub> production was significantly influenced by CP, CF and nitrogen-free extract (NFE) intake (Equations 1 and 2; Table 3). The coefficient of CF intake was higher than that of NFE intake in equations 1 and 2, indicating that fiber carbohydrate has a higher methane-producing capacity than non-fiber carbohydrate. Moe and Tyrell<sup>20</sup> also found CH<sub>4</sub> production to be significantly related to the non-fiber carbohydrate, hemicellulose and cellulose by multiple regression analysis, and they also observed a higher coefficient for the fiber than for the nonfiber component in the regression equation. Regression analysis

**Table 2. Mean and range of feed chemical contents, body weight, intake, milk production and composition, and methane production**

		Lactating cows				Dry cows			
		Mean	SD	Min	Max	Mean	SD	Min	Max
Feed									
OM <sup>†</sup>	%DM	93.2	1.6	90.4	95.0	92.5	2.5	85.6	96.8
CP	%DM	13.9	2.0	11.2	18.0	10.4	4.2	3.2	15.7
EE	%DM	3.1	0.8	1.9	4.0	1.8	1.1	1.1	4.2
CF	%DM	13.7	2.8	9.8	17.8	31.1	6.3	22.2	39.7
NFE	%DM	62.5	3.5	56.2	69.0	49.4	1.7	46.7	52.4
ME	MJ/kgDM	10.7	0.8	9.0	12.1	8.2	1.1	5.6	10.1
Body weight	kg	424.5	54.6	375.5	532.5	373.9	44.6	283.0	461.0
Intake									
DM	kg/d	10.2	1.2	6.8	12.2	5.3	0.7	4.1	6.6
GE	MJ/d	183.4	20.9	125.2	215.5	92.5	13.0	66.5	117.7
ME	MJ/d	107.8	11.5	72.5	122.2	43.9	8.6	24.7	61.0
Milk production	kg/d	13.6	2.3	9.7	17.1	–	–	–	–
Milk composition									
Fat	%	3.8	0.8	1.8	4.7	–	–	–	–
Protein	%	3.1	0.3	2.7	3.7	–	–	–	–
Lactose	%	4.6	0.3	4.1	5.1	–	–	–	–
CH <sub>4</sub> production	L/d	262.7**	80.5	125.5	390.2	153.0	28.5	84.3	203.0
	L/kgDMI/d	25.7	6.6	12.5	35.2	28.7 <sup>+</sup>	4.8	18.5	38.3
	MJ/100MJGE/d	5.6	1.5	2.7	7.8	6.6*	1.2	4.2	9.0

<sup>†</sup>OM: Organic matter, CP: Crude protein, EE: Ethel extract, CF: Crude fiber, NFE: Nitrogen free extract, ME: Metabolizable energy, DM: Dry matter, GE: Gross energy, DMI: DM intake.

<sup>+</sup>: P < 0.10, \*: P < 0.05, \*\*: P < 0.01.

showed a significant positive relationship between CH<sub>4</sub> production and DMI or the metabolizable energy intake (MEI) (Equations 3 and 4). However, the determination coefficient of the equation using only DMI was lower than for the other equations (Equations 1, 2 and 4). This positive regression between CH<sub>4</sub> production and DMI has also been reported by Shibata et al.<sup>24</sup>, Mills et al.<sup>19</sup> and Ellis et al.<sup>6</sup>; moreover, Mills et al.<sup>19</sup> and Ellis et al.<sup>6</sup> have also reported positive regression between CH<sub>4</sub> production and MEI. Kurihara et al.<sup>17</sup> found that CH<sub>4</sub> production per kilogram of milk yield was inversely proportional to milk production. In the present study, a negative linear regression was found between CH<sub>4</sub> production per kilogram of milk yield and milk production (Equation 5). This equation indicated that the CH<sub>4</sub> production per kilogram of milk yield was lower for cows with a high milk yield than

for cows with a low milk yield. Therefore, the CH<sub>4</sub> production from a dairy farm will be reduced by increasing the individual milk yield and reducing the number of cows, without any reduction in the milk production on the farm.

The estimated regional production of CH<sub>4</sub> in 1999 and 2004 is shown in Table 4. These estimates were calculated using CH<sub>4</sub> production per head (Table 2) or Equation 5 in Table 3, and the statistical data on the cow population and milk production, both sources obtained from the Information Technology Center<sup>10,11</sup>. Regional milk production for all regions increased over the five years from 1999 to 2004. This was due to an increase in the number of lactating cows, rather than to an improvement in the milk production of individual cows. Consequently, the estimated CH<sub>4</sub> production in 2004 was 1.4 times high-

**Table 3. Regression of methane production on nutrients intake and milk production**

Equation no.	Regression equation <sup>†</sup>	Database	n	R <sup>2</sup>	Significance of regression
1	CH <sub>4</sub> (L/d) = 0.083 (±0.036) ×CPI (g/d) +0.058 (±0.016) ×CFI (g/d) +0.013 (±0.008) ×NFEI (g/d) -24.673	Lactating & dry cows	48	0.63	<0.001
2	CH <sub>4</sub> (L/d) = 0.042 (±0.015) ×CFI (g/d) +0.027 (±0.005) ×NFEI (g/d) +9.370	Lactating & dry cows	48	0.60	<0.001
3	CH <sub>4</sub> (L/d) = 24.19 (±2.72) ×DMI (kg/d) +20.94	Lactating & dry cows	48	0.49	<0.001
4	CH <sub>4</sub> (L/d) = 1.63 (±0.25) ×MEI (MJ/d) +83.70	Lactating & dry cows	48	0.63	<0.001
5	CH <sub>4</sub> (L/kgMilk/d) = -2.22 (±0.66) ×Milk (kg/d) +50.40	Lactating cows	20	0.39	0.003

<sup>†</sup>CH<sub>4</sub>: Methane production, CPI: Crude protein intake, CFI: Crude fiber intake, NFEI: Nitrogen free extract intake, DMI: Dry matter intake, MEI: Metabolizable energy intake, Milk: Milk production.

**Table 4. Population of cows, milk yield and estimated methane production from lactating and dry cows in each region of Thailand in 1999 and 2004**

		Central		Northeast		North		South		Thailand	
		1999	2004	1999	2004	1999	2004	1999	2004	1999	2004
Lactating cows											
No. of cows	×10 <sup>3</sup> head <sup>1</sup>	80	113	25	35	9	13	2	3	115	165
Milk yield	kg/head/d <sup>1</sup>	9.0	9.2	11.0	10.7	9.8	11.8	10.0	9.3	9.6	9.8
CH <sub>4</sub> production	t/d <sup>2</sup>	15.0	21.3	4.6	6.6	1.8	2.5	0.3	0.6	21.7	30.9
	g/kgMilk/head/d <sup>3</sup>	21.8	21.4	18.6	19.1	20.6	17.4	20.3	21.4	20.9	19.6
Dry cows											
No. of cows	×10 <sup>3</sup> head <sup>1</sup>	24	28	8	9	3	8	1	1	36	46
CH <sub>4</sub> production	t/d <sup>2</sup>	2.6	3.0	0.9	1.0	0.3	0.9	0.1	0.1	3.9	5.0
Lactating + dry cows											
CH <sub>4</sub> production	t/d	17.7	24.4	5.5	7.6	2.1	3.4	0.4	0.7	25.6	36.0

<sup>1</sup>: Data source from Information Technology Center (1999 and 2004).

<sup>2</sup>: Calculated from no. of cows × CH<sub>4</sub> production per head in Table 2.

<sup>3</sup>: Calculated from individual milk yield and equation 5 in Table 3.

er than that in 1999. On the other hand, CH<sub>4</sub> production per kilogram of milk yield in 2004 was similar to that in 1999 because the amount of milk produced by individual cows was similar in both years. In 2004, the milk production by individual cows was highest in the north region, followed by the northeast and then the south and central regions. The CH<sub>4</sub> production per kilogram of milk yield in the northern region was estimated to be 27% lower than that in the central region. This regional difference could be due to the ambient temperature, feed and feeding management. Therefore, there is a possibility that the CH<sub>4</sub> production per kilogram of milk yield in the central region could be made to advance to the levels observed in the northern region by improving feed quality or feeding management. In order to meet the increase in milk demand in the future and the reduction in CH<sub>4</sub> production by dairy cows, an improvement in milk production by individual cows will be important in Thailand.

## Conclusion

In this study, CH<sub>4</sub> production by lactating cows and dry cows, and the factors affecting CH<sub>4</sub> production were evaluated using a data set from cows in Thailand. The CH<sub>4</sub> production with DMI correction of lactating cows was lower than that of dry cows, which was due to a difference in the energy content of the feed. In addition, a multiple regression analysis indicated that the carbohydrate type affected CH<sub>4</sub> production. Because higher individual milk production resulted in lower CH<sub>4</sub> production per milk production, improvement in feed quality or feeding management could provide reduction of CH<sub>4</sub> production while maintaining milk productivity.

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