Methane Production Potential of Feed Ingredients as Measured by *In Vitro* Gas Test

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ABSTRACT : This study was conducted to investigate *in vitro* methane production of feed ingredients and relationship between the content of crude nutrients and methane production. Feed ingredients (total 26) were grouped as grains (5 ingredients), brans and hulls (8), oil seed meals (9) roughages (3), and animal by-product (1) from their nutrient composition and their methane production protential were measured by *in vitro* gas test. Among the groups, the *in vitro* methane productions for both 6 and 24 h incubation were highest in grains, followed by brans and hulls, oil meals and roughages, animal byproducts. Within the group of grains, methane production from wheat flour was the highest, followed by wheat, corn, tapioca, and then oat. Within the brans and hulls, soybean hull showed the highest methane production and cotton seed hull, the lowest. Methane production from oil meals was lower compared with grains and brans and hulls, and in decreasing order production from canola meal was followed by soybean meal, coconut meal, and corn germ meal ($p<0.01$). Three ingredients were selected and the interactions among feed ingredients were evaluated for methane production. Correlation coefficient between measured and estimated values of the combinations were 0.91. Methane production from each feed ingredient was decreased with increasing amount of crude fiber (CF), protein (CP) and ether extract (EE), whereas positive relationship was noted with the concentrations of N-free extract (NFE). The multiple regression equation (n=134) for methane production and nutrient concentrations was as follows. Methane production (ml/0.2 g DM)=$(0.032\times CP)-(0.057\times EE)-(0.012\times CF)+(0.124\times NFE)$ ($p<0.01$; $R^2=0.929$). Positive relationship was noted for CP and NFE and negative relationship for CF and EE. It seems possible to predict methane production potential from nutritional composition of the ingredients for their effective application on formulating less methane emitting rations. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 8 : 1143-1150)***

Key Words : Methane Production, Feed Ingredients, *In vitro* Gas Test

INTRODUCTION

Ruminants utilize plant fiber by the help of rumen innates and over 200 kinds of microbes are also used as nutrients sources for the maintenance and production of their host. During the microbial fermentation in rumen, carbohydrates, protein and glycerol are fermented anaerobically to acetate, propinate, butyrate, carbon dioxide, ammonia and methane (Leng, 1991; McAllister et al., 1996). The methane, formed due to reduction of carbon dioxide by hydrogen via methanogenesis cannot be used as an energy substrate and is eructed in the air as greenhouse gas, resulting in environmental pollution. Methane is estimated to be responsible for 15% of global warming and is more efficient than carbon dioxide in absorbing infrared energy, which means that despite its very low concentration, its contribution to global warming is not negligible (Tyler, 1991).

It has been estimated that ruminants in the world produce 77 Tg (Tg=Teragram, 1 Tg=10^{12} g) of methane annually, which constitutes about 15% of total atmospheric methane emission (Crutzen et al., 1986, 1995). Methane produce 13.15 kcal/g of energy and therefore, 3-12% of gross energy intake is lost as methane depending on the nature of feeds. Since long, efforts have been made to reduce the energy loss as methane to increase efficiency of animal production.

Nutritional manipulation of methane production have been widely tried. Blaxter and Clapperton (1965) estimated methane production in sheep from feed intake and digestibility. Later, many results of methane production were reported about the effects of feed intakes, digestibility, species, physiological state, concentrate and roughage ratio, roughage quality, type of carbohydrates fed and feed processing (Moe and Tyrrell, 1979; Birkelo et al., 1986; Crutzen et al., 1986). Moreover, several feed additives, fat, antibiotics such as ionophore, halogen compounds have been investigated for their effects on methane production (Czerkawski, 1966; Haaland and Tyrrell, 1982; Whitelaw et al., 1984; O'Kelly and Spiers, 1992). But the effects were quite variable depending on the tested animals and their physiological state. Besides suppressing CH_4 production, some of the methane inhibitors reported also decreased the rumen fermentation influencing the animal performance. Despite much work, a lot has remained to be done for the development of methane reduction strategy.

This study was conducted to investigate *in vitro* methane production from feed ingredients and determine the possible application of these results to least methane-

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Table 1. Nutrient composition of feed ingredients

Feed ingredients	GE[1] (kcal/kg)	MO[2] %	(%) as-fed basis				
			Crude protein	Ether extract	Crude fiber	Ash	NFE[3]
Grains							
Corn	3,962	13.84	7.97	4.70	3.00	1.18	80.44
Oat	4,104	11.77	11.72	3.44	11.76	3.23	65.83
Wheat	3,832	13.21	11.35	1.53	2.35	1.55	82.90
Tapioca	3,620	12.42	2.36	0.73	5.27	5.94	83.67
Wheat flour	3,858	13.39	11.11	0.68	0.32	0.54	85.39
Brans and Hulls							
Wheat bran	4,061	11.42	14.28	2.99	8.77	4.07	64.94
Rice bran	4,851	11.64	14.04	16.91	8.53	7.85	46.25
Defatted rice bran	3,955	11.91	18.02	0.94	8.60	11.23	55.97
Lupin hull	3,922	11.19	16.81	2.60	34.34	2.31	36.88
Soybean hull	3,841	12.63	9.15	1.21	33.74	4.34	44.56
Corn gluten feed	4,037	15.52	18.42	0.96	13.18	6.13	54.20
Beet pulp	3,703	12.90	9.69	0.57	17.96	3.93	63.09
Cotton seed hull	4,205	10.70	4.96	2.00	35.93	3.47	48.09
Oil seed meals							
Corn germ meal	4,191	10.51	18.75	7.01	10.56	2.01	57.17
Sunflower meal	4,341	9.76	32.06	1.42	17.08	7.44	34.93
Palm kernel meal	4,879	6.42	49.31	8.72	8.93	9.78	18.00
Coconut oil seed meal	4,166	10.22	29.17	3.62	9.42	6.63	45.60
Cotton seed meal	4,556	11.98	36.83	1.88	7.88	6.21	48.09
Soybean meal	4,805	13.13	44.97	2.12	4.85	5.93	33.38
Canola meal	4,217	13.67	34.18	2.23	6.74	6.90	42.02
Rapeseed meal	4,241	6.59	35.73	1.05	6.69	8.58	44.28
Corn gluten meal	5,090	11.32	58.56	0.39	1.00	1.50	30.71
Forages							
Alfalfa hay	3,754	13.34	15.08	1.83	21.40	9.61	44.07
Rice straw	3,475	11.49	4.95	1.09	29.53	13.08	45.03
Orchardgrass hay	3,862	12.48	8.10	1.35	33.93	5.85	43.75
Animal byproduct							
Fish meal	4,541	10.07	53.92	11.53	0.91	17.75	6.47

[1] GE: gross energy. [2] MO: moisture. [3] NFE: nitrogen free extract, 100-(MO−Ash−CP−CF−EE)

producing formulation in ruminant diets.

MATERIALS AND METHODS

Animals

Ruminally cannulated, Korean native cattle, Hawoo steer (Body weight: 500 kg) were fed with diet containing 80% concentrate and 20% roughage (7.2 Kg of DM/d). Feed was provided twice a day and water and mineral block were offered *ad libitum*.

In vitro methane production

Preparation of feed ingredients : Total 26 feed ingredients namely 5 grains, 8 brans and hulls group, 9 oil seed meals, 3 roughages and 1 animal byproduct were tested. All feed ingredients were milled with Wiley Mill and screened with 1 mm mesh sieve. Moisture, crude protein, crude fiber and ether extract were analysed according to A.O.A.C. (1990) method and shown in Table 1.

Combination of feed ingredients : Equal amount of feed ingredients were combinated to give a 200 mg and methane production was measured to investigate the interaction of substrate utilization for methane production by rumen microbes among feed ingredients. Three feed ingredients were selected in each group, grain, bran and oil seed meal and combined to give 27 different combinations. Methane productions of combinated feed ingredients were compared with the expected.

Gas test : *In vitro* methane production was conducted by Menke's gas test (Menke, 1979). A total of 200 mg (DM: dry matter basis) of feed ingredients were loaded in glass syringe (volume: 100 ml) and treated with grease not to loose fluid and gas produced during incubation. Rumen fluid were obtained from Hanwoo just before feeding. The pH were determined immediately. Feed particles were removed by squeezing it through eight layers of cheesecloth. Feed ingredients were incubated triplicate with rumen fluid taken from two steers.

The composition of incubation medium was 400 ml distilled water, trace element solution, buffer solution, main element solution as was in Menke's method (Menke, 1979). The medium were saturated CO_2 gas and pH was adjusted

Table 2. Methane production of feed ingredients after 6 h and 24 h *in vitro* incubation

Feed Ingredients	CH_4 (ml/0.2 g DM)		Index[§]
	6 h	24 h	
Grains group			
Wheat flour	7.62[a]	11.60[a]	100
Wheat	5.80[b]	11.39[a]	98
Corn	4.03[c]	10.33[a]	89
Tapioca	5.45[b]	10.24[a]	88
Oat	4.34[c]	6.87[b]	59
SEM	0.83	1.19	-
Brans and Hulls			
Soybean hull	1.96[d]	11.12[a]	96
Beet pulp	2.81[c]	8.96[b]	77
Lupin hull	2.68[c]	8.66[b]	75
Wheat bran	4.80[a]	8.26[b]	71
Defatted rice bran	3.87[b]	7.00[c]	60
Corn gluten feed	3.67[b]	6.56[c]	56
Rice bran	3.40[b]	4.91[d]	42
Cotton seed hull	0.46[e]	0.86[e]	7
SEM	0.35	0.84	-
Oil seed meals			
Canola meal	4.44[a]	7.45[a]	64
Soybean meal	3.37[bc]	7.14[ab]	62
Coconut oil seed meal	2.86[c]	6.63[abc]	57
Corn germ meal	2.24[d]	6.07[bcd]	52
Rapeseed meal	3.60[b]	5.69[cd]	49
Sunflower meal	3.11[bc]	5.32[de]	46
Cotton seed meal	3.11[bc]	4.48[ef]	39
Palm kernel meal	1.72[e]	3.93[fg]	34
Corn gluten meal	1.44[e]	3.32[g]	29
SEM	0.45	1.05	-
Forages group			
Alfalfa Hay	2.71[a]	6.02[a]	57
Orchard Hay	1.25[b]	4.67[b]	40
Rice straw	0.96[b]	2.42[c]	21
SEM	0.39	1.20	-
Animal Byproduct			
Fish meal	1.57	1.63	14
SEM	0.21	0.30	-
Statistical difference among groups	NS	NS	*

a, b, c, d, e Values within the same column and group with different superscripts differ ($p<0.01$)

§ Index describes the relative amount of methane production to that of wheat flour for 24 h in vitro incubation.

* $p<0.01$, NS: Non-significnat

and mixed with rumen fluid at the ratio of 2:1.

Measurement of methane production : Glass syringe containing feed ingredients sample were incubated with 30ml of mixed rumen and buffer solution at 39°C incubator designed to circle. Gas was sampled at 6 h and 24 h. Head spaced gas (10 ml) from each syringe were removed and methane was measured by gas chromatograph (Varian 3800, USA) with column (Carbosieve S 8100 mesh column; Supelco Inc., USA). After 24 h incubation, pH was measured.

Statistieal analysis

Means values and standard errors of mean (SEM) were calculated. The differences in means between treatments were compared by Duncan (1955)'s multiple range test. Relation of nutritional constituent and methane production was deduced and estimated as regression equation and anlayzed by SAS (1995) program.

RESULTS AND DISCUSSION

Detailed nutrient composition of feed ingredients is given in Table 1. According to their compositional characteristics, they have been grouped under grains, brans and hulls, oilmeals, forges and animal byproduct. The results on *in vitro* methane production potential from there feeds are discussed below.

***In vitro* methane production of feed ingredients**

Methane production of feedstuffs after *in vitro* incubation with rumen fluid for 6, 24 h were shown in Table 2. The *in vitro* methane productions for both 6 and 24 h incubations were highest in grains, followed by brans and hulls, oil seed meals and roughages. When methane production of feed ingredients were ranked and expressed as relative value to that of highest production, wheat flour was the highest and followed by wheat, soybean hull and corn. Cotten seed was the lowest.

Grains : Methane production of grains for 24 h *in vitro* incubation were wheat flour, wheat, corn and tapioca in order and lowest in oat as 7.59 ml/0.2g DM ($p<0.01$). Comparative high methane production of grains among feed ingredients group might be attributed to high contents of easily fermentable starch, sugars, or hemicellulose as substrate to rumen microbes for gas production and nutritional composition of feed ingredients. Grains contain high amount of NFE which is readily fermented by microbes in rumen and provide the absolute large amount of substrates to microbes for methane production.

Corn produced less methane than wheat flour, which can be attributed to its low digestibility and in case of oat, tough fibrous outer membrane along with its high CF content (11.76% which is highest among grains studied) isn't easily digested by rumen microbes (low digestibility). On the contrary, wheat flour contains comparatively low ether extract and high nitrogen free extract that is fermented and produce high amount of gas resulting in highest methane production. Besides the high amount of easily fermentable substrates, Bonhomme (1990) reported that grains rich in soluble carbohydrates increase the population of cilliate protozoa and stimulate their hydrogen transfer to methanogens resulting in high methane production.

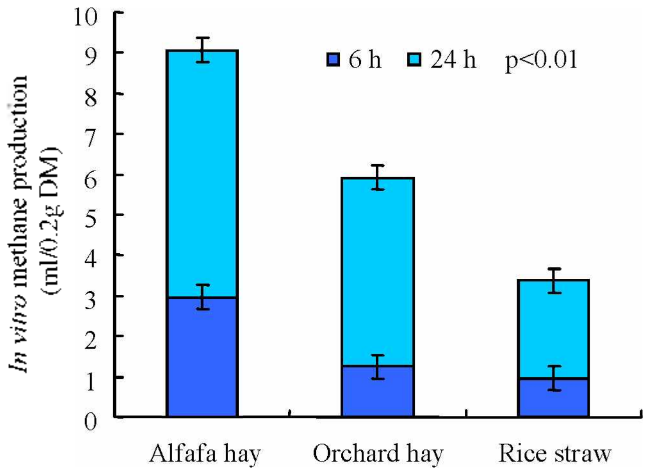

Figure 1. Methane production from forages after 6 h and 24 h *in vitro* incubation.

Blaxter and Clapperton (1965) showed that methane production rate in ruminants could be changed via the level of feeding. With three levels of feedings, maintenance, 2×maintenance, 3×maintenance, methane production increases with the increase of apparent digestibility of the diet at maintenance level and its rate decreases with the increase of apparent digestibility of the diet at 3×maintenance.

In gas test, sample amount of feed ingredients were far less than the maintenance level. It is obvians that feed ingredients which contains large amount of highly soluble and digestible substrates such as grains produce more methane than fibrous feed ingredients.

Brans and hulls : Methane production of brans and hulls for 24 h *in vitro* incubation were highest in soybean hull, as 11.12 ml ($p<0.01$) and lowered in the order of beetpulp, lupin hull, wheat bran, defatted rice bran, corn gluten feed, rice bran and cotton seed hull (Table 2). Soybean hull is the byproduct produced by defatting or casting off the soybean meal therefore contains high amount of cell wall component. Moe and Tyrell (1979) reported that cell wall components such as cellulose when fermented for long time and raises the acetate/propionate ratio which cause the increase in methane production. Soybean hull which contains high amount of cellulose as well as readily fermentable NFE produce highest quantity of methane.

Rice bran characteristically contains high amount of unsaturated fatty acid. Czerkawski et al. (1966) reported that unsaturated fatty acids are hydrogenated by rumen microbes resulting in low pressure of hydrogen which is pre-requisite for reduction in methane production. In addition, fat, itself, is considered to inhibit methane production by stimulating propionate production and inhibiting the protozoa activity as well as inhibitory effects on cellulolytic bacteria and feed digestion in rumen. In this experiment, rice bran produced 4.91 ml/0.2 g DM methane *in vitro* and it was statistically less methane (30%) compared with that of defatted rice bran (7.00 ml/0.2 g DM) which has same nutritional composition except for fat. Thus, high content of fat (unsaturated fatty acids) in rice bran suppressed the methane production.

Oil seed meals : Methane production of oil seed meals were comparatively lower than those of grains and brans and hulls. There were statistical differences in methane production among oil seed meals. Lupin showed highest methane production for 24 h *in vitro* incubation and followed by canola meal, soybean meal, coconut meal and corn germ meal ($p<0.01$) (Table 2). High contents of NFE and readily fermentable crude fiber in lupin is possibly used for the methane production by rumen methanogens.

When corn germ meal is compared with palm kernel meal for methane production in terms of nutritional composition, composition characteristics are similar except NFE. Thus, highly fermentable substrates for rumen microbes in corn germ meal seemed to cause higher methane and gas production. Low level of readily fermentable substrates in palm kernel meal and its appreciable fat content seemed to suppress microbial activity, leading to low digestibility and methane production. oil seed meals generally contain crude protein more than 20% and low amount of fiber. Protein is degraded to NH_4 in rumen and it combines to CO_2 resulting in $(NH_4)HCO_3$ (Getachew et al., 1998). Therefore NH_4 as the result of rumen incubation of high protein sources such as oil seed meals can be expected to combine with CO_2, the substrate for methane production, resulting in its lower production.

Roughages : Methane production was highest in alfalfa hay (6.02 ml) followed by orchardgrass hay and rice straw ($p<0.01$) (Figure 1). Rice straw contains large amount of lignin which is hardly utilized by rumen microbes resulting in lower methane production.

Effect of incubation time : During the first 6 h incubation, grain showed higher methane production, brans and hulls showed higher methane production after 6 h which might be because of its high contents of crude fiber which is degraded slowly in rumen compared. Methane production in oat was higher for first 6 h incubation but the production at 24 h incubation was less than any other grains. This corrobarates Herrer-Saldana et al. (1990) who showed that oat contains comparatively higher amount of soluble carbohydrates than corn and wheat and is quickly degraded within 2 h and later hardly degraded by its complex granule structure. Methane production of wheat bran during the first 6 h was significantly higher than any other brans and hulls ($p<0.01$) and this might be caused due to its high content of NFE (Table 1).

Interaction among feed ingredients for methane

Table 3. Methane production in combination of feed ingredients and their comparison with the estimates derived from individual value

Feed ingredients			CH_4 production		PRD***	B/A
Grains	Oil seed Meals*	Brans and Hulls**	6 h	24 h (A)	(B)	
Corn	SBM	WB	4.92	10.54	9.08	0.86
Corn	SBM	SBH	3.49	9.88	10.08	1.02
Corn	SBM	RB	4.00	7.86	7.87	1.00
Corn	RSM	WB	4.45	8.68	8.49	0.98
Corn	RSM	SBH	3.92	9.85	9.47	0.96
Corn	RSM	RB	3.94	7.02	7.32	1.04
Corn	CSM	WB	4.42	8.02	8.12	1.01
Corn	CSM	SBH	3.57	9.16	9.12	1.00
Corn	CSM	RB	3.86	6.65	6.92	1.04
Wheat	SBM	WB	4.76	9.23	9.46	1.02
Wheat	SBM	SBH	4.56	10.63	10.46	0.98
Wheat	SBM	RB	5.00	9.02	8.25	0.91
Wheat	RSM	WB	5.24	9.28	8.86	0.95
Wheat	RSM	SBH	4.11	9.21	9.84	1.07
Wheat	RSM	RB	4.61	7.97	7.69	0.96
Wheat	CSM	WB	5.31	9.11	8.50	0.93
Wheat	CSM	SBH	4.31	10.0	9.50	0.95
Wheat	CSM	RB	4.67	8.15	7.30	0.90
Oat	SBM	WB	4.71	8.32	7.85	0.94
Oat	SBM	SBH	3.74	9.90	8.85	0.89
Oat	SBM	RB	4.72	7.87	7.42	0.94
Oat	RSM	WB	4.85	8.32	7.29	0.88
Oat	RSM	SBH	4.09	8.98	8.28	0.92
Oat	RSM	RB	4.49	7.244	6.14	0.85
Oat	CSM	WB	4.59	7.47	6.90	0.92
Oat	CSM	SBH	3.54	8.21	7.91	0.96
Oat	CSM	RB	4.18	6.42	5.73	0.89
SEM			0.483	1.07	1.15	-

* SBM: Soybean meal, CSM: Cottonseed meal, RSM: Rapeseed meal,
** WB: Wheat bran, SBH: Soy bean hull, RB: Rice bran
*** PRD: Predicted value. Mathematical average of individual *in vitro* methane production for 24 h.

production : To investigate interactions among feed ingredients for methane production, three ingredients were selected from each group based on their level of methane which showed significant differences. Methane production from the combinations was measured and compared with their predicted value (PRD, mathematical average of three feed ingredients) estimated from individual production (Table 3).

There were no differences for methane production between actual and predicted values. The methane production values were higher in soybean meal, wheat, soybean hull combinations and lower in cotton seed meal, oat, rice which showed less methane production ($p<0.01$). Standard deviations between practical and predicted values were less than 5% and R^2 was 0.8099 (Figure 2). There seemed to be no substrate interaction for methane production among feed ingredients and the estimation of methane production from a feed ingredients would be possible without consideration of feed ingredient interaction.

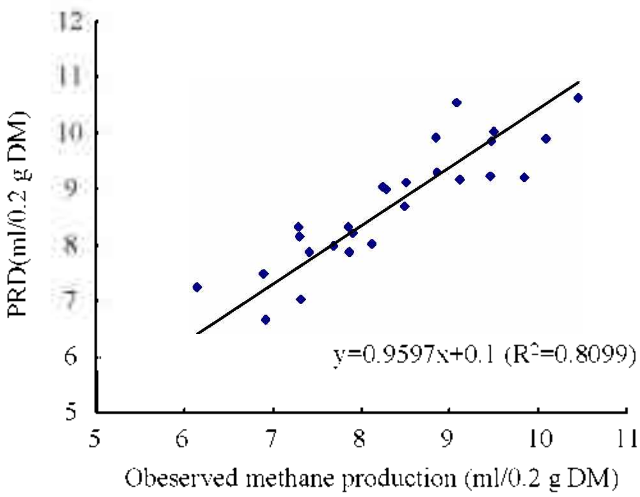

Figure 2. Relationship between the measured and the predicted value for 24 h *in vitro* methane production of feed ingredient combination.

Relation between methane production and nutritional constituents

Crude fiber : The relationship between the content of crude fiber and methane production in feed ingredients investigated are shown in Table 4. Methane productions decreased as the content of crude fiber increased. Several attempts have been made to predict methane production by determining the amount of crude nutrients in cattle and sheep (Holter and Young, 1992; Shibata et al., 1994) and it is known that crude fiber is an important component in methane production. Miller (1995) reported that feed ingredients rich in crude fiber stimulated some species of microorganism within the cellulolytic-methanogen consortium which serve to couple the degradation of carbohydrates with the use of H_2 for the reduction of CO_2 to methane.

Feed ingredients were grouped according to their compositional characteristics as grains, brans and hulls, oil seed meals and regression for methane production due to crude fiber for each group was developed. Thus, equations in Table 4 indicate that methane production decreases as crude fiber increases in grains. It was less affected by crude fiber in brans and hulls. Oil seed meals seemed to be less related to crude fiber than brans and hulls and and grain. *In vitro* methane production of feed ingredients with more crude fiber were negatively related with the amount of crude fiber.

Grains usually contain hard out layer, hull, which is cell wall component hardly degraded by rumen microbes and this might cause negative relationship with methane production.

Crude protein : The relationship of methane production

Table 4. Regression analysis of methane production by each crude constituent: total, grain, brans and hulls, oil seed meals

Feed ingredients		n[1]	Regression equation	R^2
Crude fiber	Total	134	$y=0.0011x^2-0.1123x+7.839$	0.076
	Grains	25	$y=-0.7014x^2-0.1638x+11.618$	0.694
	brans and hulls	33	$y=-0.0047x^2+0.3362x+3.6224$	0.471
	Oil seed meals	38	$y=0.0194x^2-0.5976x+9.6753$	0.145
Crude protein	Total	134	$y=-0.0021x^2+0.0716x+6.7667$	0.182
	Grains	25	$y=-0.0368x^2+0.5469x+8.9763$	0.035
	brans and hulls	33	$y=0.0463x^2-1.7193x+22.816$	0.364
	Oil seed meals	48	$y=-0.00072x^2+0.0276x+5.8952$	0.041
NFE	Total	134	$y=0.1098x+1.1048$	0.515
	Grains	25	$y=0.2317x-8.3677$	0.673
	brans and hulls	33	$y=0.0169x+6.8142$	0.008
	Oil seed meals	48	$y=0.0545x+3.6945$	0.210
Ether extract	Total	134	$y=-0.0006x^2-0.172x+7.2532$	0.071
	Grains	25	$y=-0.4259x+10.732$	0.158
	brans and hulls	33	$y=-1.4839x^2+5.871x+4.3041$	0.182
	Oil seed meals	48	$y=-0.1794x^2+1.743x+3.3747$	0.481

[1] n: number of samples, R^2: correlation coefficient.

Table 5. Estimation of methane production by crude constituent: total, grain, brans and hulls, oil seed meals

Feed ingredients	n[1]	Regression equation	R^2	Significacne of regression
Total	134	CH_4=0.032CP-0.057CFat-0.012CF+0.124NFE	0.929	**
Grains	25	CH_4=0.082CP-0.045CFat-0.017CF+0.129NFE	0.990	**
Brans and hulls	33	CH_4=-0.065CP-0.041CFat+0.150CF+0.115NFE	0.988	**
Oil seed meals	48	CH_4=0.062CP-0.067CFat-0.024CF+0.099NFE	0.962	**
Forage	15	CH_4=0.430CP+0.105CF-0.078NFE	0.986	**

[1] n: number of samples, R^2 : correlation coefficient, **: $p<0.01$.

and crude protein is shown in Table 4. The methane production was found to be decreased as the crude protein was increased (R^2: 0.182). Brans and hulls showed increase but grains and oil seed meals showed tendency to decrease the methane production with the increase in the amount of crude protein.

Getachew et al. (1998) reported that NH_4, which was released by protein degradation, combined with CO_2, methane substrate and resulted in less methane production. Kirchgessner et al. (1994) also reported that crude protein produce relatively little methane. Kurihara et al. (1997) found decreased methane in cattle fed more than maintenance and also when the crude protein of diet was increased.

Nitrogen free extract : The relationship of methane production and NFE is shown in Table 4. NFE containing high amount of easily fermentable carbohydrates such as starch, sugars, some cellulose which are highly soluble in rumen shift the fermentation pattern toward process which are linked to the consumption of H_2 for propionate consumption.

Shibata et al. (1992) from the multiple regression analyses relating CH_4 production to various nutrient intakes suggested that NFE had positive correlation with methane production. Our findings (Table 4) agrees with Shibata et al. (1992). Especially high correlation was estimated in grains.

Ether extracts : Fat and other compounds included in the ether extract fraction are mostly not fermented by rumen microbes, and especially unsaturated fatty acids are known to inhibit the methanogenic microbial system (Czerkowski et al. 1966; Demeyer and Van Nevel, 1975). Hydrogenation of unsaturated fatty acid increases propionate synthesis, inhibits protozoa and cellulolytic bacterial activity and thereby affect the methane production (Czerkawski et al., 1966). Also, Roger et al. (1992) reported that glycerol, released from fat hydrolysis suppress the cellulolytic bacteria activity. Those trends were not clearly shown in this results but as a whole, ether extract tends to reduce the methane production.

Generally the relationship between nutrient composition and methane production among feed ingredients was not clear. Correlation was high with crude fiber, NFE and low with crude protein, ether extract in grains, showing that crude fiber and NFE are important factors. In oil seed meals which is high in crude protein, correlation was high with ether extracts. It seems that methane production of feed ingredients are affected by nutritional composition and their interactions not by only single nutrient or a factor.

Estimation of methane production by nutritional constituents : The results of multiple regression analyses relating methane production to various nutrient composition are summarized in Table 5.

Methane production in brans and hulls was positively co-related to NFE and CF and negatively co-related to ether extracts and CP contents. In roughages, NFE had negative impact on methane production. It was shown that NFE generally has positive effects on methane production except roughages and the contribution of individual nutrient to methane production were different depending on the group of feed ingredients. Moe and Tyrell (1979) investigated methane production relating to the type of carbohydrates in beef cattle and reported that methane production from cellulose (1 g) was 3 times greater than soluble residue showing that methane productions were different depending on the type of carbohydrates and among them, cell wall component was most affecting.

Our results showed that NFE was the most important factor in methane production different from other results above mentioned. This might be caused from low digestibility of crude fiber *in vitro* condition and limited time of incubation and relatively compared with the NFE which is digested and produce large amount of gas in a short time. Further *in vivo* experiment are needed for application because the extent and rate of digestibility of feed ingredients are quite related to methane production. As most of feed ingredients constituting the concentrates are digested within 24 h in rumen condition, quite similar results to *in vitro* studies, as described here, can be expected.

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