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In vitro Fermentation, Methane Emission and Global Warming

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Abstract: *In vitro* fermentation of available 36 feeds was performed to assess the quality by investigating the methane (CH_4) production rate. For this purpose, a fermentation reactor was designed to capture the CH_4 gas emitted and to collect liquor from the reactor during *in vitro* fermentation. The result showed that the CH_4 production rate was greatly vary in different feed ingredients. The lowest CH_4 producing feeds were corn gluten feed, brewer's grain and alfalfa straw among all energy, protein and forage feeds, respectively. Significant differences were found in CH_4 emissions ($p<0.01$) in different feed ingredients during the 48 h of *in vitro* fermentation. Finally, an economically viable and eco-friendly dairy ration was suggested that would be produced a much less CH_4 than that of commercial dairy rations. Suggested dairy ration might be reduced CH_4 emission as well as global warming.

Key words: *In vitro* fermentation, CH_4 production, feed evaluation, eco-friendly ration, quality

INTRODUCTION

Ruminant animal depends on microorganisms to digest roughages (cell wall polysaccharides) and other feedstuffs to produce energy sources such as Volatile Fatty Acids (VFA) and other organic acids. Numerous microorganisms from different species (bacteria, protozoa and fungi) are involved in the ruminal digestion process to digest the fibrous constituents and other feed materials. A significant amount of methane (CH_4) is emitted during the fermentation of feedstuffs in the rumen (Wolin *et al*., 1997; Moss *et al*., 2000; Rymer *et al*., 2000; Getachew *et al*., 2004). Methane production in the rumen is an energetically wasteful process, representing a feed energy loss of about 6-8% depending on the level of feeding (Mould, 2003) which reduces the efficiency of utilization of feeds. Approximately 2-12% of dietary gross intake energy of feed is lost to the atmosphere as CH_4 gas (Yurtseven *et al*., 2009). Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Getachew *et al*., 1998; Blummel and Orskov, 1993).

The CH_4 emission rate largely depends on the types of feed and the ratio of forage to concentrate (F:C ratio) in the ration which have an influence on the Acetate:Propionate (A:P) ratio (Moss *et al*., 2000). It might be assumed that CH_4 production would be less when high concentrate diets are fed (Fahey and Berger, 1988). Only 2-3% gross energy loss is occurred when high grain concentrates (>90%) are offered at *ad libitum* intake levels (Johnson and Johnson, 1995). Chai *et al*. (2004) estimated the fermentation rate of six starch rich feed ingredients and eight maize silage samples at the *in vitro* fermentation technique and found a modest relationship ($r^2 = 0.80$) between measured *in vitro* starch degradation and gas production. Van Soest (1982) indicated that a high grain diet and the addition of soluble carbohydrates increases the passage rate and reduces the gas production but Moss *et al*. (1995) found a similar effect on gas production when grass silage was supplemented with barley.

Fermentation of fibrous materials or cellulose fraction is likely to produce a higher molar proportion of acetate and a lower proportion of propionate. On the other hand, feed with low fiber content would be expected to result in a reduction in the A:P ratio during rumen fermentation (Dougherty, 1984; Orskov and Ryle, 1990). Carbohydrate is the chief source of acetate and butyrate in the ruminal

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fermentation. The synthesis of acetate and butyrate in the rumen results in an increase Hydrogen (H_2) and the methanogenic bacteria in the rumen enhances CH_4 production by utilizing H_2 and CO_2 (Widiawati and Thalib, 2007). The stoechiometric reactions of CH_4 production in the rumen are stated (Rossi *et al.*, 2001):

- Glucose + $2H_2$ →2 acetate + $2CO_2$ + $4H_2$
- Glucose + $2H_2$ → 2 propionate + $2H_2O$
- Glucose → 1 butyrate + $2CO_2$ + $2H_2$
- $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$

It might be stated from the above stoechiometric reactions that higher production of acetate and CO_2 would lead to a higher CH_4 production which would represent a net loss of feed energy as well as inefficiency in feed utilization. Average global atmospheric concentration of CH_4 is 1720 parts per billion per volume (ppbv) (Bolle *et al.*, 1986) and this concentration is increasing at the rate of 10 ppbv per year (Steele *et al.*, 1992). The rising concentration of CH_4 is due to anthropogenic emission and enteric fermentation of ruminant animals (Moss *et al.*, 2000). It was estimated that CH_4 from domestic and non-domestic ruminants' accounts for about 15% of total global CH_4 production and about 75% of this produced from cattle (Hironaka *et al.*, 1996). This emitted CH_4 and other volatile organic compounds and gases from ruminants greatly affects on the environmental air quality. Methane contributes to climatic change by trapping outgoing terrestrial infrared radiation 20 times more effectively than CO_2 (Getachew *et al.*, 2005) in absorbing terrestrial thermal radiation in the troposphere that enhances global warming. Global warming increases the average temperature of Earth's near-surface air and oceans since the mid-20th century until now. Global surface temperature increased 0.74±0.18°C between the start and the end of the 20th century and this global surface temperature is likely to rise a further 1.1-6.4°C during the 21st century (IPCC, 2007). An increase in global temperature will cause the rise of sea water levels and will change the amount and pattern of rainfall, probably including expansion of subtropical deserts (Jian *et al.*, 2007). Not only these, rise in temperatures will also alter the delicate ecosystems, mountain flora and fauna, coral reefs and coastal regions, deserts and national parks. It will disrupt farming, fishing, forestry and many other industries that rely on the weather and natural ecosystems. There will be decreased crop yields and decreased arable land availability with subsequent starvation and malnutrition. Moreover, rise in temperature will alter the range of disease that threaten animals or human health such as malaria, sleeping sickness and other infectious disease that will affect the availability of human resources for the agricultural sector. Disease outbreaks will be rampant and the immune system of the animals will be lowered due to rise in temperature. It will also alter the endangered animal's habitat. Climate change will affect livestock productivity directly by influencing the balance between heat dissipation and heat production and indirectly through its effects on the availability of feeds and fodder (Gworgwor *et al.*, 2006). Ruminant animals are one of the important factors for all of this negative impact on environments.

Ruminant animals act as active component of global warming by emitting a huge amount of CH_4 to the atmosphere contributing 80 million tons of CH_4 from enteric fermentation every year. To keep this point into account, *in vitro* fermentation of available 36 feed ingredients were performed to identify less CH_4 producing feeds to prepare an eco-friendly ruminant ration which would be emitted less CH_4. For this reason, reduction of CH_4 and other gaseous productions from livestock animals is the common interest of worldwide scientist's and air regulatory agencies to save the environment from global warming. Knowledge about CH_4 production rate of the feed ingredients could help to minimize the CH_4 emission from livestock by formulating an eco-friendly ration for ruminants. Therefore, an eco-friendly ration for dairy cattle was suggested to minimize the CH_4 from enteric fermentation as well as global warming.

MATERIALS AND METHODS

Apparatus for *in vitro* fermentation: For the assessment of the livestock feeds, two types of fermentation reactors were designed (Fig. 1) to capture the CH_4 gas emitted during *in vitro* test and also to analyze the digestibility of feed during fermentation. One reactor was connected with tedlar bag to capture of gases and the other had 50 mL syringe and tube to collect liquor samples during the fermentation in addition to tedlar bag connection. The fermentation was performed in a shaking incubator (VS-8480 SR) to avoid settling of feed particles and to ensure proper physiological function of the microorganisms. *In vitro* fermentation of feeds was done according to the principles of Tilley and Terry (1963).

Preparation for *in vitro* fermentation (feed sample, buffer, inoculums and incubation): *In vitro* fermentation of available 35 feed ingredients (16 energy rich, 11 protein rich feed and 8 roughages) were carried out to investigate the production rate of CH_4 gas. All experimental feeds were arranged with 3 replications for capturing CH_4 gas and liquid sample collection. Detailed compositions of the

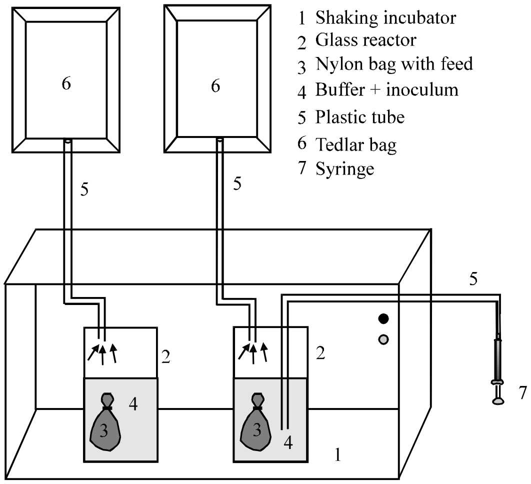

Fig. 1: Flow diagram of the *in vitro* fermentation

Table 1: Composition of buffer solution

Solution A		Solution B	
Reagent	g L^{-1}	Reagent	g L^{-1}
KH_2PO_4	10.0	Na_2CO_3	15.0
$MgSO_4.7H_2O$	0.5	$Na_2S.9H_2O$	1.0
NaCl	0.5		
$CaCl_2.2H_2O$	0.1		
Urea (reagent grade)	0.5		

experimental feed ingredients are shown in Table 1. Clean dry-nylon bags (mesh size: 30-50 μm; dimension: 5×10 cm) were rinsed in acetone for 3-4 min and completely air dried before sampling. After measuring the weight of nylon bag, 1.6 g basal feed (as correction factor) and 2.4 g ground experimental feeds were put into each bag. Basal feed was composed of 0.8 g ground rice straw and 0.8 g formula feed in every case. Nylon bags were sealed properly after adding 4 beads in each bag to ensure the complete immersion in the buffer solution. Prepared nylon bags with feed samples were placed in the marked fermentation reactor to make ready for the fermentation. When the fermentation reactors were warm up about 37°C, 80 mL diluted rumen inoculums were added in each reactor. Rumen fluid and contents were collected from non lactating fistulated Korean cattle (maintained at National Institute of Animal Science on a standard diet concentrate:roughage = 40:60) at approximately 30 min after feeding and put it into a pre-warmed insulated container. Then, anaerobic condition was maintained by injecting CO_2 gas and homogenized the liquor by blending at high speed for 30 sec. Homogenization needs to dislodge the microbes that were attached to the fibrous mat and assured an adequate microbial population for *in vitro* analysis. Homogenized digesta (liquor) was filtered through four layered cheesecloth continually purging with CO_2. After adding the liquor into the reactors with buffer solution and nylon bag with feed samples, tedlar bags were installed tightly with the reactors.

Fig. 2: Buffer, rumen liquor and feed sample containing reactors in the incubator

The buffer solution was prepared according to the principle as described by Menke and Steingass (1988). Composition of buffer solution is shown in Table 2. Solution A and B were prepared as the instruction given in the Table 1. Combined buffer solution was prepared by mixing in a proportion of 1:5 from solution A and B and adjusted the pH at 6.8. The 400 mL of the mixed buffer solution was added to each fermentation reactor (Fig. 2) and was allowed for warming for 20-30 min before starting the incubation. The reactors were then incubated at 39°C and stirred at 170 rpm for 48 h by placing the reactors on shaker incubator to avoid settling of feed particles and ensure proper physiological function of the microorganisms.

Sampling and analysis: Samples (liquor) were collected using the installed syringe at 0, 12, 24 and 48 h to analyze the pH, TOCs and VFA (acetic acid, propionic acid and butyric acid). The VFA was analyzed following the method described by Getachew *et al.* (2005). Approximately 6 mL of the liquid contents were taken into 10 mL plastic tubes and centrifuged at 11,000 RPM for 10 min. Subsequently, 3 mL of supernatants were removed and centrifuged again under the same conditions. Then, 0.1 mL aliquot of supernatant was pipetted into an auto-sampler vial containing 0.9 mL of internal standard (0.75 mM of 3 methylvaleric acid). VFA were analyzed using a Hewlett Packard 5890 Capillary Gas Chromatograph (GC). TOCs were analyzed by Total Organic Carbon Analyzer (Shimadzu, TOC-5000A). Volume of the collected gases in the Teadler bag was measured with gas flow meter and the CH_4 was analyzed

Table 2: Feed composition, digestibility and methane production characteristics of the feed ingredients (mean±standard error)

Feed ingredients	DM	Ash	EE	CP	CF	ADF	NDF	CH_4 (g kg^{-1} feed)	CH_4 (g kg^{-1} digested feed)
Energy rich feed									
Corn (USA)	89.86±0.17	1.63±0.08	3.67±0.16	8.29±0.32	0.49±0.28	2.22±0.26	15.10±1.72	1.161±0.005^{a}	2.095±0.021^{abc}
Corn (latin america)	88.00±0.00	1.43±0.02	4.98±0.09	8.45±0.21	0.37±0.14	2.99±0.77	44.12±5.64	0.850±0.084^{ab}	1.787±0.158^{bcd}
Corn cob	99.35±0.10	7.21±0.31	0.56±0.08	4.05±0.05	24.36±0.71	39.32±0.42	83.61±0.62	0.247±0.019^{c}	0.326±0.026^{de}
Corn gluten feed	92.67±0.02	9.51±0.26	2.70±0.07	19.06±0.02	6.67±0.10	10.10±0.13	44.25±0.40	0.129±0.030^{c}	0.146±0.037^{e}
Corn distillers grain	89.41±0.10	4.72±0.03	9.60±0.28	28.36±0.52	4.20±0.28	8.89±0.50	40.65±0.84	0.316±0.017^{bc}	0.453±0.041^{de}
Wheat	88.49±0.28	9.41±0.10	0.03±0.01	19.64±0.33	7.87±0.13	3.15±0.12	32.43±1.68	0.50±0.1220^{bc}	3.214±0.519^{ab}
Wheat bran	88.81±0.10	8.26±0.03	3.62±0.14	23.38±0.06	12.88±0.21	11.89±0.21	43.16±0.86	0.185±0.000^{c}	0.296±0.000^{de}
Rice bran	95.92±0.32	9.12±0.10	23.46±0.08	15.12±0.47	5.44±0.22	7.64±0.00	22.07±0.49	0.146±0.036^{c}	0.303±0.107^{de}
Beet pulp	90.61±0.01	4.18±0.06	1.73±0.08	10.94±0.08	23.39±0.32	29.25±0.08	57.59±0.43	0.230±0.006^{c}	0.318±0.012^{de}
Barley	89.00±0.00	15.03±0.19	3.38±0.13	10.98±0.27	21.71±0.73	36.33±1.16	67.40±3.45	0.271±0.131^{c}	0.320±0.148^{de}
Rye	96.90±0.07	4.58±0.02	0.78±0.03	3.48±0.48	39.19±0.35	47.72±0.58	76.78±1.47	0.560±0.007^{bc}	1.027±0.002^{cde}
Tapioca	97.78±0.21	6.90±1.15	1.78±0.03	2.81±0.01	12.44±0.17	21.68±0.10	33.29±1.94	1.295±0.356^{a}	3.569±1.222^{a}
Cottonseed hull	89.89±0.13	6.33±0.03	2.29±0.19	7.69±0.03	36.07±1.09	57.03±0.59	84.16±0.73	0.202±0.041^{c}	0.232±0.050^{de}
Lupine hull	90.41±0.24	3.02±0.02	2.11±0.24	13.23±0.41	42.77±0.22	48.90±1.45	61.16±1.82	0.521±0.072^{bc}	0.762±0.101^{cde}
Soybean hull	91.36±0.08	4.86±0.03	3.08±0.11	12.22±0.20	34.15±0.04	45.16±0.15	66.92±0.38	1.285±0.504^{a}	3.319±1.253^{a}
Apple pomace	90.00±0.00	4.02±0.07	9.17±2.41	7.19±0.29	23.88±0.75	41.12±1.88	55.93±0.49	0.375±0.012^{bc}	0.570±0.040^{cd}
Protein rich feed									
Corn gluten meal	93.64±0.01	4.06±0.28	1.11±0.17	65.91±0.07	0.15±0.00	3.19±1.08	15.10±1.63	0.714±0.346^{b}	1.641±0.796^{bc}
Brewers grain	97.76±0.07	4.28±0.02	6.97±0.43	23.33±0.10	13.70±0.13	18.89±0.62	59.29±0.88	0.050±0.00^{c}	0.055±0.000^{d}
Cottonseed meal	97.14±0.49	7.28±0.19	0.14±0.03	33.99±1.01	17.71±3.30	30.66±0.73	48.32±0.09	0.306±0.0002^{bc}	0.448±0.011^{cd}
Whole cottonseed	98.87±0.18	3.65±0.01	13.48±0.10	12.17±0.75	38.53±1.32	39.11±1.87	57.01±1.15	0.108±0.041^{c}	0.125±0.049^{d}
Soybean meal	96.62±0.06	8.58±0.17	1.85±0.00	52.49±0.14	2.96±0.05	5.03±0.20	12.94±0.10	0.541±0.225^{bc}	1.177±0.462^{bcd}
Soybean oil cake	96.11±0.20	8.34±0.05	2.20±0.13	51.66±0.03	4.11±0.09	7.08±0.35	16.94±0.16	10.33±0.216	4.220±0.382^{cd}
Rape seed meal	92.57±0.24	7.70±0.07	1.39±0.14	39.82±0.56	6.65±0.20	15.88±0.17	23.96±0.29	0.219±0.012^{bc}	0.384±0.008^{cd}
Coconut meal	90.30±0.00	5.22±0.02	3.12±0.07	12.01±0.18	38.91±0.65	31.04±0.43	62.53±0.33	0.272±0.190^{bc}	0.338±0.120^{cd}
Lupine	92.93±0.31	3.10±0.05	7.24±0.19	40.52±0.51	3.79±0.34	5.61±0.33	18.26±1.58	0.502±0.212^{bc}	2.241±1.021^{b}
Corn cake	98.28±0.17	2.31±0.04	5.20±0.35	22.09±0.19	9.64±1.04	12.84±0.59	61.82±0.33	0.161±0.024^{c}	0.247±0.035^{cd}
Palm cake	96.79±0.43	4.74±0.13	5.41±0.65	16.50±0.27	12.53±0.07	35.63±2.67	66.12±0.41	0.504±0.138^{bc}	1.519±0.526^{bcd}
Forages									
Alfalfa	90.16±0.58	7.25±0.08	0.11±0.06	14.81±0.42	38.28±0.30	42.01±0.01	53.97±0.17	0.186±0.008^{c}	0.264±0.009^{cd}
Oat	92.49±0.06	3.20±0.04	2.36±0.05	5.24±0.04	28.65±0.24	33.47±0.34	60.48±0.26	0.520±0.293^{bc}	0.733±0.414^{bc}
Rye grass	93.70±0.29	5.61±0.05	1.93±0.24	5.14±0.23	28.30±0.24	35.63±0.02	66.25±0.21	0.367±0.104^{bc}	0.507±0.147^{cd}
Perennial grass	93.33±0.31	3.86±0.47	1.57±0.04	8.56±0.23	24.62±0.80	40.00±0.48	73.43±0.35	0.353±0.044^{bc}	0.678±0.121^{bcd}
Orchard grass	98.67±0.53	5.74±0.02	1.23±0.08	3.11±0.26	38.65±0.29	46.91±0.75	77.58±1.22	0.259±0.024^{bc}	0.321±0.037^{cd}
Timothy grass	92.78±0.03	6.86±0.02	2.44±0.03	6.33±0.21	38.99±0.29	44.01±0.02	73.23±0.23	0.594±0.017^{b}	1.058±0.053^{b}
Talfescue grass	92.05±0.68	4.87±0.02	1.58±0.05	7.02±0.08	31.75±0.84	42.29±0.36	71.07±0.35	0.625±0.098^{b}	0.767±0.126^{bc}
Crain grass	97.11±0.14	8.43±0.56	1.39±0.03	11.02±0.31	20.65±0.05	35.48±0.35	72.94±0.82	1.578±0.099^{a}	6.065±0.063^{a}

by injecting 60 mL of gas into a GC (Varian, 450-GC) equipped with a thermal conductivity detector. Digestibility of feeds was measured by drying the nylon bags washed with cold tap water at 60°C for 4 days and by weighing of the residual feeds. All analyses were done according to the standard methods (AOAC, 2005).

Statistical analysis: The data was analyzed by the Statistical Package for the Social Sciences (SPSS, Version 12.0, 2003), computer statistical package program with one way Analysis of Variance (ANOVA). Differences among the treatment means were determined by the Duncan's Multiple Range Test (DMRT) value with the principles of Steet and Torrie (1980).

RESULTS AND DISCUSSION

CH_4 production rates of feeds: Experimental feeds were categorized according to DMRT value of CH_4 emitted during 48 h of *in vitro* fermentation (Table 2). In case of energy feed, there were 4 distinct categories of feed ingredients regarding CH_4 emission. Higher emission rate was found in tapioca, soybean hull and wheat with a range between 3.57-3.21 g CH_4 kg^{-1} digested feed in 48 h. Second category of CH_4 emission was found in the USA corn, Latin American corn, rye and lupine hull with a range between 2.09-0.76 g CH_4 kg^{-1} digested feed. The third category of feed such as apple pomace, corn distiller's grain, corn cob, barley, beet pulp, rice bran, wheat bran and cottonseed hull emitted a lower CH_4 during 48 h of *in vitro* fermentation (0.57-0.23 g CH_4 kg^{-1} digested feed). Corn gluten feed emitted the lowest CH_4 gas among all energy feeds. Differences in CH_4 emissions were significant among the different categories ($p<0.01$) mentioned in energy feeds.

In case of protein feeds, soybean oilcake emitted the highest CH_4 (4.22 g kg^{-1} digested feed) among all ingredients. Methane emissions were intermediate in lupine, corn gluten meal, palm cake and soybean meal with a range between 2.24-1.18 g kg^{-1} digested feed in 48 h and lower in cottonseed meal, rapeseed meal, coconut meal and corn cake (0.57-0.25 g CH_4 kg^{-1} digested feed). These intermediate and lower CH_4 emitted groups had no statistical differences but significant differences were found among the highest, lowest and intermediate groups ($p<0.01$). Brewers grain emitted the lowest CH_4 (0.05 g kg^{-1} digested feed) among all protein feeds.

Similarly, in the forages, crain grass was very prone to CH_4 production (6.07 g kg^{-1} digested feed). An intermediate emission rate of CH_4 was found in timothy grass, talfescue grass, oat and perennial grass (1.06-0.68 g kg^{-1} digested feed) and a comparatively lower emission was found in rye grass, orchard grass, alfalfa and rice straw (0.51-0.19 g kg^{-1} digested feed). Differences were also significant among the highest, lowest and intermediate CH_4 emitted from forages ($p<0.01$). It might be stated that corn gluten feed, brewer's grain and alfalfa would be the excellent feed ingredients from energy, protein and roughages, respectively due to low emission rate of CH_4.

Part of this experiment showed the pattern of CH_4 production rate and the Total Organic Carbon production (TOC) of feeds during *in vitro* fermentation (Kim *et al*., 2012). They found that lowest CH_4-producing feeds were corn gluten feed, brewer's grain and orchard grass among the energy, protein and forage feed groups, respectively. The result of the present study mostly supports the findings of Rossi *et al.* (2001) regarding CH_4 emission and feed quality. They found that corn silage produced the highest and rye grass produced the lowest CH_4 among all forages. In case of energy feeds, beet pulp and manioca produced the highest and rice bran produced the lowest CH_4. Whole soybean and soybean meal produced the highest and cotton meal produced the lowest CH_4 among all protein feed ingredients from *in vitro* fermentation.

CH_4 production rate and suggested eco-friendly ration to minimize CH_4 emission: Methane gas production rate is the most important criteria regarding the quality of feed ingredients. From this experiment, some quality feeds were identified that produced the lowest CH_4. In case of CH_4 production, researchers considered the amount of gas from per kg feed and per kg digested feed. Among the energy rich feeds, corn gluten feed showed the top ranking due to lower CH_4 production and higher digestibility. Accordingly, brewer's grain showed the highest performance among all protein rich feeds due to higher digestibility and lower gas production. In case of forages, alfalfa was ranked as 1. According to the results of this experiment, a ration could be suggested for ruminants that would be helpful to minimize CH_4 emission from ruminal fermentation. Ration should be prepared with a proper ratio of forage, energy and protein feeds according to the physiological state and nutrient requirements of the animals, cost effective and minimum CH_4 production characteristics. The forage to concentrate ratio of the ration has an impact on the rumen fermentation and hence the acetate:propionate ratio (declines with F:C ratio). Alfalfa and orchard grass would be the excellent forages for their digestibility and CH_4 production characteristics. Similarly, corn gluten feed, cottonseed hull and barley might be the best choice as energy rich feed. Brewer's grain whole cottonseed and corn cake are the top ranking protein feeds regarding CH_4 emission.

Finally, an eco-friendly ration would be suggested for ruminants (dairy cows) that emitted minimum CH_4 from the ruminal fermentation. Table 3 shows the inclusion levels of the feed ingredients of 3 commercial rations and suggested eco-friendly dairy ration and Table 4 shows the nutrient compositions of that rations.

Three different commercial rations and suggested ration were prepared with different formula of feed ingredients. Ration should be balanced to ensure proper physiological function and optimum production from the animals. Ration for a high producing dairy cow should contained 16-18% CP, 3-6% fat, 18-26% ADF and 20-26% forage NDF on the basis of DM and the DM intake is 3.13% of the body weight of a cow that produced 25 L milk/day. Also, Ca, P, Mg and other macro and micro nutrients including vitamins should be provided with ration for optimum production. A dairy cow of 600 kg body weight that produces 25 L of milk would require 19.72 kg mixed ration (as DM) daily with 5% allowances. Types of feed ingredients significantly influence on the CH_4 emission from the ruminal fermentation (Moss *et al*., 2000; Gworgwor *et al*., 2006). So, reduction of CH_4 emission from ruminal fermentation might be possible by selecting suitable feed ingredients that would be emitted less CH_4. Levels of organic matter, protein, fat, ADF, forage NDF and total

Table 3: Ingredients of commercial and eco-friendly dairy cattle rations

Feed ingredients (kg/day/cattle)	Commercial ration 1	2	3	Suggested eco-friendly ration
Oat	-	-	3.00	-
Alfalfa	-	2.00	-	3.00
Crain grass	3.73	-	-	-
Rye grass	3.00	-	-	-
Timothy grass	-	2.00	3.00	-
Talfescue grass	-	3.00	-	3.00
Corn (USA)	3.00	3.73	4.00	3.00
Corn (L.A.)	-	3.00	2.00	-
Wheat	3.00	-	-	-
Barley grain	-	-	-	2.00
Wheat bran	2.00	-	-	2.00
Rice bran	2.00	-	-	2.00
Corn gluten feed	1.00	-	-	2.00
Tapioca	1.00	2.00	2.50	-
Soybean hull	-	2.00	-	-
Soybean meal	2.00	3.00	-	-
Soybean oilcake	-	-	2.50	-
Lupine	-	2.00	2.00	-
Palm cake	2.00	-	3.00	0.73
Corn gluten meal	-	-	0.73	-
Cottonseed meal	-	-	-	2.00
Brewer's grain	-	-	-	3.00
Total	22.73	22.73	22.73	22.73

Table 4: Nutrient composition of commercial and eco-friendly dairy cattle rations

	Corn ration			
Parameters	1	2	3	Eco-friendly ration
DM intake (kg/day)	22.73	22.73	22.73	22.73
Organic matter (% DM)	86.50	87.61	88.86	86.87
Crude protein (% DM)	16.59	17.10	17.59	16.61
Fat (% DM)	4.20	3.01	3.55	4.73
CF (% DM)	12.25	15.93	12.86	17.14
ADF (% DM)	17.92	20.93	19.34	23.57
Total NDF (% DM)	43.10	41.01	40.53	45.63
CH_4 (g/day/cattle)*	16.15	16.58	19.29	9.30
CH_4 (g/day/cattle)**	50.37	39.91	47.04	14.89
Total CH_4 (kg/cattle/year)*	5.89	6.05	7.04	3.40
Total CH_4 (kg/cattle/year)**	18.39	14.57	17.17	5.44
Feed cost ($/kg mixed feed)***	0.284	0.313	0.286	0.284

*Amount of CH_4 g/kg feed; **Amount of CH_4 g/kg digested feed. ***Calculated on the basis of import price (2010) to animal feed industry

NDF of 3 commercial and suggested dairy ration were found in Table 4. Nutritional quality of suggested eco-friendly dairy ration also fitted well with the ideal characteristics of dairy ration. Suggested ration for dairy cows was also economically viable and no need to add more economic involvement to make it eco-friendly.

Table 4 also shows the amount of CH_4 produced from a cow/year that consumes 19.72 kg mixed ration (DM)/day. According to digested feed DM, the amounts of CH_4 produced were 22.13, 13.29, 14.90 and 3.28 kg/cow/year for commercial ration 1, 2, 3 and suggested ration, respectively. In that case, the amount of emitted CH_4 was >3 times higher than that of suggested eco-friendly ration. Methane is produced by the fermentation of feed within the Animal's Digestive System. Generally, the higher the feed intake, the higher the CH_4 emissions (Getachew *et al*., 2005). Feed intake and CH_4 production is positively related to animal's size, growth rate and production such as methane production head per year was 128, 117, 99, 68 and 46 kg for an American, Western European, Eastern European, Asian and African dairy cow respectively (IPCC, 2006). A far below production of CH_4 were found in Table 4 than that of IPCC estimation might be due to incomplete fermentation of feeds. Because, emitted amount of CH_4 in the Table 4 were estimated from 48 h of *in vitro* fermentation.

CONCLUSION

Agriculture contributes about 60% of the global CH_4 production. Among them a significant amount of CH_4 is produced from enteric fermentation of ruminant livestock every year. About 2-12% of intake feed energy is lost as CH_4 gas (Yurtseven *et al*., 2009), depending on the feed quality and animal status. Moss *et al.* (2000) stated that agricultural sector produced a total of 205-245 million tons of CH_4 every year in which enteric fermentation contributes 80 million tons and this atmospheric CH_4 is currently increasing at a rate of about 30-40 million tons per year. Preparation of an ideal eco-friendly ration with less CH_4 producing feed ingredients would be helpful to minimize this burning global warming issue. An ideal and balanced ration is important to maximize the efficiency of nutrient utilization, thereby reducing environmental pollution caused by excess nutrients leaving the animal as waste. Environmental pollution from ruminant animals can be caused by the emission of large amounts of CH_4 from the ruminal fermentation of feeds (Getachew *et al*., 2005). Suggested eco-friendly ideal ration could reduce the CH_4 emission at least 3 times than that of commercial rations without interrupting the milk production.

It might be stated that it would possible to reduce a huge amount of enteric CH_4 annually which might be an important event of reducing the global warming. A large amount of CH_4 might be minimized only by selecting less CH_4 producing feed ingredients. An eco-friendly ration is not only minimizes the global warming but also increase the animal production (milk or meat) from per unit of feed consumed which reduces CH_4 emissions of per unit product.

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REFERENCES

AOAC, 2005. Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemist, Washington, DC., USA.

Blummel, M. and E.R. Orskov, 1993. Comparison of gas production and nylon bag degradability of roughages in predicting feed intake in cattle. Anim. Feed Sci. Technol., 40: 109-119.

Bolle, H.J., W. Seiler and B. Bolin, 1986. Other Greenhouse Gases and Aerosols: Trace Gases in the Atmospheres. In: The Greenhouse Effect, Climatic Change and Ecosystems, Bolin, B., B.O.R. Doos, J. Jager and R.A. Warrick (Eds.). Wiley and Sons, Chichester, pp: 157-203.

Chai, W.Z., A.H. van Gelder and J.W. Cone, 2004. Relationship between gas production and starch degradation in feed samples. Anim. Feed Sci. Technol., 114: 195-204.

Dougherty, R.W., 1984. Physiology of the Ruminant's Digestive Tract. In: Duke's Physiology of Domestic Animals, Swenson, M. (Ed.). Cornell University Press, New York, USA., pp: 351-358.

Fahey, G.C. and L.L. Berger, 1988. Carbohydrate Nutrition in Ruminants. In: The Ruminant Animal: Digestive Physiology and Nutrition, Church, D.C. (Ed.). Prentice Hall, Englewood Cliffs, New Jersey, USA., pp: 269-297.

Getachew, G., E.J. de Peters and P.H. Robinson, 2004. *In vitro* gas production provides effective method for assessing ruminant feeds. California Agric., 58: 54-58.

Getachew, G., M. Blummel, H.P.S. Makkar and K. Becker, 1998. *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: A reveiw. Anim. Feed Sci. Technol., 72: 261-281.

Getachew, G., P.H. Robinson, E.J. DePeters, S.J. Taylor, D.D. Gisi, G.E. Higginbotham and T.J. Riordan, 2005. Methane production from commercial dairy rations estimated using an *in vitro* gas technique. Anim. Feed Sci. Technol., 123: 391-402.

Gworgwor, Z.A., T.F. Mbahi and B. Yakubu, 2006. Environmental implications of methane production by ruminants: A review. J. Sustainable Dev. Agric. Environ., 2: 1-14.

Hironaka, R., G.W. Mathison, B.K. Kerrigan and I. Vlach, 1996. The effect of pelleting of alfalfa hay on methane production and digestibility by steers. Sci. Total Environ., 180: 221-227.

IPCC, 2006. Emissions from Livestock and Manure Management. In: IPCC Guidelines for National Greenhouse Gas Inventories, Dong, H., J. Mangino, T.A. McAllister, J.L. Hatfield and D.E. Johnson *et al*., (Eds.). Cambridge University Press, Cambridge, UK.

IPCC, 2007. Climate Change 2007: The Physical Science Basis. In: Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Solomon, S., D. Qin, M. Manning, Z. Chen and M. Marquis *et al*., Cambridge University Press, Cambridge, UK.

Jian, L., G.A. Vecchi and T. Reichler, 2007. Expansion of the Hadley cell under global warming. Geophys. Res. Lett., Vol. 34 10.1029/2006GL028443.

Johnson, K.A. and D.E. Johnson, 1995. Methane emissions from cattle. J. Anim. Sci., 73: 2483-2492.

Kim, M.J., J.S. Lee, S. Kumar, M.M. Rahman, J.S. Shin and C.S. Ra, 2012. Indirect estimation of CH_4 from livestock feeds through TOCs evaluation. Asian Aust. J. Anim. Sci., 25: 496-501.

Menke, K.H. and H. Steingass, 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev., 28: 7-55.

Moss, A.R., D.I. Givens and P.C. Garnsworthy, 1995. The effect of supplementing grass silage with barley on digestibility, in sacco degradability, rumen fermentation and methane production in sheep at two levels of intake. Anim. Feed Sci. Technol., 55: 9-33.

Moss, A.R., J.P. Jouany and J. Newbold, 2000. Methane production by ruminants: Its contribution to global warming. Ann. Zootech., 49: 231-253.

Mould, F.L., 2003. Predicting feed quality-chemical analysis and *in vitro* evaluation. Field Crops Res., 84: 31-44.

Orskov, E.R. and M. Ryle, 1990. Energy Nutrition in Ruminants. Elsevier Science Publisher, London, UK, ISBN: 9781851664399, Pages: 149.

Rossi, F., P. Vecchia and F. Masoero, 2001. Estimate of methane production from rumen fermentation. Nutr. Cycling Agroecosyst., 60: 89-92.

Rymer, C., C.R. Stewart, T.F. Garstang and D.I. Givens, 2000. The use of mixtures of pure bacterial cultures as an alternative inoculum to rumen fluid in the *in vitro* gas production technique. Proceedings of the EAAP Satellite Symposium on Gas Production: Fermentation kinetics for Feed Evaluation and to Assess Microbial Activity, August 18-19, 2000, Netherlands, pp: 29-30.

Steele, L.P., E.J. Dlugokencky, P.M. Lang, P.P. Tans, R.C. Martin and K.A. Masarie, 1992. Slowing down of the global accumulation of atmospheric CH_4 during the 1980s. Nature, 358: 313-316.

Steet, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York, USA.

Tilley, J.M.A. and R.A. Terry, 1963. A two-stage technique for the *In vitro* digestion of forage crops. J. Br. Grassl. Soc., 18: 104-111.

Van Soest, P.J., 1982. Nutritional Ecology of the Ruminant. O and B Books, Oregon, USA., pp: 40-41.

Widiawati, Y. and A. Thalib, 2007. Comparison fermentation kinetics (*in vitro*) of grass and shrub legume leaves: The pattern of VFA concentration, estimated CH_4 and microbial biomass production. J. Anim. Vet. Sci., 12: 96-104.

Wolin, M.J., T.L. Miller and C.S. Stewart, 1997. Microbe-Microbe Interactions. In: The Rumen Microbial Ecosystem, Hobson, P.N. and C.S. Stewart (Eds.). Chapman and Hall, London, UK., ISBN: 97807-51403664, pp: 467-491.

Yurtseven, S., M. Cetin, I. Ozturk, A. Can, M. Boga, T. Sahin and H. Turkoglu, 2009. Effect of different feeding method on methane and carbon dioxide emissions milk yield and composition of lactating awassi sheep. Asian J. Anim. Vet. Adv., 4: 278-287.