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Indirect Estimation of CH_4 from Livestock Feeds through TOCs Evaluation

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ABSTRACT : Thirty-five available feeds were fermented *in vitro* in order to investigate their soluble total organic carbon (TOCs) and methane (CH_4) production rate. A fermentation reactor was designed to capture the CH_4 gas emitted and to collect liquor from the reactor during *in vitro* fermentation. The results showed that CH_4 production rate greatly varied among feeds with different ingredients. The lowest CH_4-producing feeds were corn gluten feed, brewer's grain, and orchard grass among the energy, protein, and forage feed groups, respectively. Significant differences ($p<0.05$) were found in digestibility, soluble total organic carbon (TOCs), and CH_4 emissions among feeds, during 48 h of *in vitro* fermentation. Digestibility and TOCs was not found to be related due to different fermentation pattern of each but TOCs production was directly proportional to CH_4 production ($y = 0.0076x$, $r^2 = 0.83$). From this *in vitro* study, TOCs production could be used as an indirect index for estimation of CH_4 emission from feed ingredients. (**Key Words :** Feed, Fermentation, Methane, Total Organic Carbons)

INTRODUCTION

Ruminants depend on plant source feed that is digested anaerobically in their rumen through microbial enzymes. Volatile fatty acid (VFA) and other organic acids are the primary energy sources in rumen fermentation. Microbial fermentation in the rumen also produces waste products such as CH_4 and carbon dioxide (CO_2). CH_4 production in the rumen is an energetically wasteful process that reduces the efficiency of feed utilization. Approximately 2 to 12% of the dietary gross intake energy of feed is lost to the atmosphere as CH_4 (Moss et al., 2000; Kumar et al., 2009; Yurtseven et al., 2009). Knowledge regarding ruminant feed digestion kinetics is necessary for a better understanding of the pattern of degradation and CH_4 emission. *In vitro* digestion technique is a rather simple method of feed evaluation, and a large number of feeds can be simultaneously incubated and analyzed. Therefore, it is the most appropriate and widely utilized technique to measure gas emission from livestock feed ingredients. When feedstuff is incubated with buffered rumen fluid *in vitro*, carbohydrates are fermented by microbial cells to short-chain fatty acids and gases. Gas production is essentially the result of fermentation of carbohydrate to acetate, propionate, and butyrate (Blummel and Orskov, 1993; Getachew et al., 1998). The amount of gas produced due to protein fermentation is smaller than that produced due to carbohydrate fermentation. Carbohydrate is the chiefsource of acetate and butyrate in rumen fermentation. The synthesis of acetate and butyrate in the rumen increases hydrogen production (Widiawati and Thalib, 2007), and the methanogenic bacteria in the rumen enhance CH_4 production by utilizing hydrogen and CO_2.

Increased production of acetate and CO_2 leads to increased CH_4 production, which represents a net loss of feed energy as well as inefficient feed utilization. In contrast, increased propionate production reduces CH_4 emission in the rumen. CO_2 production from carbohydrate fermentation contributes to approximately 40% of total gas production in the rumen (Mcdonald et al., 1995); therefore, feeds containing large amounts of carbohydrate should produce more gas than feeds containing less amounts of carbohydrate. Amount of feed intake, feed composition, and organic matter degradability are also related to CH_4 and CO_2 production (Monteny et al., 2006): higher feed intake reduces digestibility and increases CH_4 and CO_2 production.

Since, volatile fatty acids and other organic carbon sources represents the soluble total organic carbons, there could be a possibility of correlation between CH_4 production rates from feed ingredients and total organic carbons. Therefore, the rates of CH_4 emission and other

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fermentation parameters of 35 available feed ingredients were assessed by *in vitro* fermentation in the present study and the interrelationship between CH_4 emission and total organic carbons (TOCs) levels was studied to determine the correlation for the indirect estimation of CH_4 from livestock feeds.

MATERIALS AND METHODS

Apparatus for *in vitro* fermentation

Two types of fermentation reactors were designed to collect liquid samples, to capture CH_4 gas emitted during the *in vitro* test, and to analyze TOCs production during fermentation. One reactor (500 ml) was connected to a tedlar bag to capture gases, and the other reactor was equipped with a 50-ml syringe and tube to collect liquor samples during fermentation A set of 210 reactors, containing one bag in one reactor, were placed in 6 shaking incubators and three replicates of each were tested at the same time. 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 the feeds was carried out according to the principles of Tilley and Terry (1963).

Preparation for *in vitro* fermentation (feed sample, inoculums, and incubation)

In vitro fermentation of the 35 available feed ingredients (16 energy-rich and 11 protein-rich feeds and 8 roughages) was performed to assess CH_4 production rate. The detailed composition of the experimental feed ingredients is shown

Table 1. Feed composition, digestibility, TOCs and methane production characteristics of the feed ingredients

Feed ingredients	DM	Ash	EE	CP	CF	ADF	NDF	Digestibility (%)	TOCs (g/kg digested feed)	CH_4 (g/kg digested feed)	Ranking
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	67.76±4.95^{ab} (2)	264.75±32.18^{fg}	2.54±0.021^{ed} (7)	7
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	58.56±0.46^{abcd} (3)	394.93±57.39^{e}	2.28±0.158^{e} (6)	6
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	22.30±6.96^{ghij} (9)	33.73±15.31^{klm}	0.09±0.002^{hi} (2)	10
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	50.98±6.78^{abcdef} (4)	27.02±14.80^{lm}	0.08±0.003^{hi} (2)	2
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	22.84±1.28^{ghij} (9)	16.74±02.69 ^{lm}	0.14±0.041^{hi} (2)	10
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	59.80±0.25^{abc} (3)	1,807.66±166.48^{b}	8.92±0.519^{a} (10)	13
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	51.96±2.18^{abcde} (4)	100.26±34.62^{jklm}	0.26±0.00^{hi} (2)	2
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	47.34±1.31^{bcdefg} (5)	104.17±17.41^{jkl}	0.27±0.010^{hi} (2)	3
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	43.35±5.17^{bcdefgh} (6)	21.27±14.31^{lm}	0.19±0.012^{hi} (2)	4
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	31.52±3.07^{efghij} (8)	42.90±14.97^{klm}	0.12±0.014^{hi} (2)	8
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	28.89±1.84^{efghij} (8)	159.57±28.77^{hij}	0.54±0.002^{hi} (2)	8
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	53.33±2.31^{abcde} (4)	684.48±148.97^{d}	4.59±1.222^{c} (8)	11
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	10.20±0.76^{j} (11)	0.73±0.76^{m}	0.02±0.005^{i} (1)	16
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	42.07±8.18^{bcdefgh} (6)	36.06±08.17^{klm}	0.46±0.010^{hi} (2)	4
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	36.47±0.57^{edefghi} (7)	399.70±71.41^{e}	3.18±1.253^{d} (7)	14
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	29.58±6.99^{efghi} (8)	31.11±12.28^{klm}	0.24±0.040^{hi} (2)	8
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	20.11±0.21^{hij} (10)	105.04±16.52^{jkl}	0.76±0.079^{gh} (3)	10
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	20.87±1.40^{hij} (10)	5.21±0.96^{lm}	0.01±0.00^{i} (1)	10
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	34.20±2.19^{cdefghij} (7)	13.81±09.98^{lm}	0.21±0.011^{hi} (2)	8
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	16.67±1.02^{ij}(11)	2.32±0.80^{lm}	0.02±0.000^{i} (1)	16
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	51.14±2.18^{abcdef} (4)	230.71±85.68^{ghi}	1.84±0.462^{gf} (5)	5
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	53.54±2.58^{abcde} (4)	816.15±56.47^{c}	7.33±0.382^{b} (9)	13
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	53.56±2.84^{abcde} (4)	231.65±29.09^{gh}	0.38±0.008^{hi} (2)	2
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	53.37±0.84^{abcde} (4)	60.85±4.17^{jklm}	0.25±0.120^{hi} (2)	2
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	75.37±3.17^{a} (1)	2,295.14±409.29^{a}	7.01±1.021^{b} (9)	12
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	47.48±2.52^{bcdef} (5)	44.94±10.52^{klm}	0.18±0.035^{hi} (2)	3
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	38.21±1.65^{cdefghi} (7)	340.04±90.57^{ef}	1.51±0.526^{f} (4)	9
Forages											
Alfalfa	90.16±0.58	7.25±0.08	0.11±0.06	14.8±0.42	38.28±0.30	42.01±0.01	53.97±0.17	38.93±1.09^{cdefghi} (7)	27.43±6.96^{klm}	0.14±0.009^{hi} (2)	8
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	65.82±4.21^{ab} (2)	155.72±17.35^{hij}	0.67±0.414^{hi} (2)	1
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	26.03±2.82^{fghij} (9)	40.67±13.14^{klm}	0.18±0.147^{hi} (2)	10
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	18.98±3.11^{hgi} (10)	51.18±10.59^{klm}	0.23±0.121^{hi} (2)	16
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	15.20±3.85^{ji} (11)	11.56±03.01^{lm}	0.06±0.037^{i} (1)	16
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	29.82±2.56^{efghij} (8)	129.44±42.74^{ji}	0.57±0.053^{hi} (2)	10
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	14.42±0.18^{ji} (11)	24.26±03.73^{lm}	0.13±0.010^{hi} (2)	16
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	25.75±3.96^{fghij} (9)	276.37±104.95^{fg}	5.04±0.063^{c} (8)	15

$^{a-m}$ Different superscripts in same column differs significantly ($p<0.001$); ±: SEM (n = 3).

DM = Dry matter; EE = Endogenous energy; CP = Crude protein; CF = Crude fibre.

ADF = Acid detergent fibre; NDF = Neutral detergent fibre; TOCs = Soluble total organic carbons.

in Table 1. Clean, dry, nylon bags (mesh size: 30-50 μm; dimension: 5×10 cm) were rinsed in acetone for 3 to 4 min and completely air-dried before sampling. After measuring the weight of the nylon bag, 1.6 g of basal feed (as the correction factor and for optimizing the best ecosystem conditions for microbial growth and rapid colonization) and 2.4 g of dried and ground experimental feed (1.0-2.0 mm) were put into each bag. The basal feed comprised 0.8 g ground rice straw and 0.8 g formula feed. The nylon bags were sealed after adding 4 beads to each bag to ensure complete immersion in the Menke buffer solution. Prepared nylon bags (2 each) with feed samples were placed in the marked fermentation reactor for fermentation. Buffer solution was prepared according to the method described by Menke and Steingass (1988), and the pH was adjusted to 6.8. The buffer solution (320 ml) was added to each fermentation reactor and warmed to 39°C for 20 to 30 min; thereafter, rumen inoculum (80 ml) was added to each reactor. All the 35 ingredients were tested in the same batch, with same media and inocula and in triplicates. Rumen fluid and contents were collected from fistulated non lactating Korean cattle (maintained at National Institute of Animal Science on a standard diet concentrate:roughage = 40:60) approximately 30 min after feeding and placed in a O_2- free CO_2 flushed, pre-warmed insulated container. Anaerobic conditions were maintained by injecting CO_2 gas, and the liquor was homogenized by blending at high speed for 30 s. Homogenization was required to dislodge microbes attached to the fibrous mat and ensure an adequate microbial population for *in vitro* analysis. Homogenized digesta (liquor) was filtered through four-layered cheesecloth and continually purged with CO_2. After addition of the liquor to the reactors with buffer solution and nylon bag with feed samples, the tedlar bags were tightly installed in the reactors. The reactors were then incubated at 39°C and stirred at 170 rpm for 48 h by placing the reactor on shaker incubator to avoid settling of feed particles and ensure proper physiological function of the microorganisms.

Sampling and analysis

Liquor samples were collected using the installed syringe at 0, 12, 24, and 48 h to analyze the TOCs levels. TOCs production was analyzed by a Total Organic Carbon Analyzer (Shimadzu, TOC-5000A). Methane was analyzed from the total volume of the gases collected in the tedlar bag 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 d and by weighing the residual feeds. All feed analyses (ash, EE, CP, CF, ADF and NDF) were done according to standard methods (AOAC, 2005).

The methane values were converted in g/kg feed as:

CH_4 (g) = ((percent methane/100)
×(specific gravity of methane/1,000)
×volume of gas)

CH_4 (g/kg feed) = CH_4 (g)×feed weight×250

Where, 250 is used as a factor to convert the 4 g feed into Kg.

CH_4 (g/kg digested feed)
= ((CH_4 (g)×digested feed weight)×(conversion factor))

Where, conversion factor is 1,000/digested feed weight.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 12.0, 2003), a computer statistical package program with one-way analysis of variance (ANOVA). Differences among treatment mean values were determined by the Tukey Multiple Range Test (MRT) according to the principles of Steel and Torrie (1980).

RESULTS AND DISCUSSION

CH_4 production rate of feeds

Experimental feeds were categorized according to the Tukey MRT value of CH_4 emitted during 48 h of *in vitro* fermentation (Table 2). In the case of energy feed, there were 6 distinct categories of feed ingredients regarding CH_4 emission. The CH_4 emission rates were higher in the case of wheat (8.29 g/kg digested feed) and thus placed in the sixth category. The fifth-category feed included tapioca (4.59 g/kg digested feed) and fourth and third categories include soybean hull, USA corn and Latin American corn emitting CH_4 in range of 2.28-3.18 g/kg digested feed. Second category feed (rye, lupine hull, apple pomace, corn distiller's grain, corn cob, barley, beet pulp, rice bran, wheat bran, and corn gluten feed) emitted lower CH_4 levels during 48 h of *in vitro* fermentation (CH_4 emission rate, 0.08-0.54 g/kg digested feed). Cottonseed hull emitted the lowest amount of CH_4 gas (0.02 g/kg digested feed) among all energy feeds and was ranked first. Differences in CH_4 emissions were significant among the categories ($p<0.05$) of the energy feeds.

In the case of protein feeds, soybean oilcake and lupine produced the highest CH_4 levels (7.09-7.33 g/kg digested feed) followed by soybean meal and palm cake. Methane emissions from corn gluten meal, rape seed meal, coconut meal, cottonseed meal and corn cake were in range of 0.18 to 0.76 g/kg digested feed, and were lower. Although there

Table 2. Methane production by feed ingredients

Treat	N	CH_4 production (g/kg digested feed)						
Energy feed								
Cottonseed hull	3	0.02						1st
Corn gluten feed	3	0.08	0.08					
Corn cob	3	0.09	0.09					
Barley	3	0.12	0.12					
Corn distillers grain	3	0.14	0.14					
Beet pulp	3	0.09	0.09					
Apple pomace	3	0.24	0.24					2nd
Wheat bran	3	0.26	0.26					
Rice bran	3	0.27	0.27					
Lupine hull	3	0.46	0.46					
Rye	3	0.54	0.54					
Corn (Latin America)	3			2.28				3rd
Corn (USA)	3			2.54	2.54			4th
Soyabean hull	3				3.18			
Tspioca	3					4.59		5th
Wheat	3						8.92	6th
Sig.		0.02	0.01	0.05	0.04	0.04	0.12	
Protein feed								
Brewers grain	3	0.01						1st
Whole cottonseed	3	0.02						
Corn cake	3	0.18	0.18					2nd
Cottonseed meal	3	0.21	0.21					
Coconut meal	3	0.25	0.25					
Rape seed meal	3	0.38	0.38					
Corn gluten meal	3		0.76	0.76				3rd
Palm cake	3			1.51				
Soybean meal	3			1.84				
Lupine	3					7.09		4th
Soybean oil cake	3					7.33		
Sig.		0.04	0.03	0.11		0.07		
Forages								
Orchard grass	3	0.06						1st
Talfescue grass	3	0.13		0.13				2nd
Alfalfa	3	0.14		0.14				
Rye grass	3	0.18		0.18				
Perennial grass	3	0.23		0.23				
Timothy grass	3	0.57		0.57				
Oat	3	0.67		0.67				
Crain grass	3					5.04		3rd
Sig.		0.07		0.06		0.15		

Means for groups in homogenous subsets are displayed, uses harmonic mean sample size = 3.000, N is number of replicates.

was no statistical difference between the second category and lower CH_4-emission groups, significant differences were found between the highest group and the lowest and intermediate groups ($p<0.05$). Brewer's grain produced the lowest CH_4 levels (0.01 g/kg digested feed) and was ranked first among the protein feeds.

Amongst the forages, crain grass produced the highest level of CH_4 (5.04 g/kg digested feed). Lowest CH_4 emission rate was observed in the case of timothy, tallfescue, oat, perennial grass, rye grass, orchard grass, and alfalfa (0.06-0.67 g/kg digested feed). Differences were significant between the forage feeds with the highest and lowest CH_4

levels ($p<0.05$). The results of the present study mostly supported by the findings of Rossi et al. (2001) regarding CH_4 emission and feed quality, wherein corn silage was found to produce the highest CH_4 levels, and rye grass produced the lowest CH_4 levels among all the forage feeds. Furthermore, their data indicated that for energy feeds, beet pulp and manioca produced the highest and rice bran produced the lowest CH_4 levels. Whole soybean and soybean meal produced the highest and cotton meal produced the lowest CH_4 levels among all protein feed ingredients during *in vitro* fermentation.

Relationship between CH_4 emission and TOCs

Since different ingredients in the categories of energy, protein and forage feed, have different fermentation pattern (Table 1) hence no relationship between digestibility and TOCs production could be established. Fermentation pattern of proteins/protein rich diets results in both amino acids and short chain peptides which can end up either in microbial biomass or in fermentation end products such as VFA, CO_2, or ammonia, which is quite different with that of carbohydrate based diet (Cone and Van Geldar, 1999). Thus results obtained are in accordance with the previous study but fails to establish any relationship between TOCs and digestibility.

During *in vitro* fermentation, metabolism of TOCs occurs in the microbial body to produce new cells. Short-chain fatty acids, gases, and new microbial cells are produced from carbohydrate sources during *in vitro* fermentation (Getachew et al., 1998 and 2006). TOCs are the backbone of simple sugars, VFAs, and amino acids. Low TOCs production from highly digestible feeds might be due to the frequent utilization of TOCs for microbial assimilation and synthesis of new cells whereas, diets having high TOCs and better digestibility leads to more CH_4 production. Feed having high TOCs may get digested with the help of the complete rumen consortia as rumen fungi takes more time to colonize and degrade the feed ingredients with their array of hydrolytic enzymes and thus contributes to slow and efficient digestion. This leads to slow release of hydrogen and more interspecies hydrogen transfer and thus CH_4 production (Kamra, 2005).

TOCs levels during *in vitro* fermentation were directly proportional to CH_4 production (Table 2 and Figure 1); thus, the amount of CH_4 could be estimated from the amount of TOCs produced during *in vitro* fermentation. Furthermore, TOCs levels could be used as an indirect index to measure CH_4 in the ruminal fermentation process ($y = 0.0076x$, $r^2 = 0.83$).

CONCLUSION

CH_4 emission of feed ingredients during *in vitro* fermentation and interrelationships among digestibility, TOCs, and CH_4 emission were examined in this study. The following conclusions were made on the basis of our results:

i) CH_4 emission during *in vitro* fermentation varied among feed ingredients. Tapioca, soybean hull, wheat, soybean oilcake, lupine, and crain grass resulted in the highest CH_4 production levels among the 35 tested feed ingredients. Cottonseed hull, corn gluten feed, brewer's grain, whole cottonseed, and orchard grass resulted in the lowest CH_4 production levels.

ii) *In vitro* fermentation of the feeds showed that there was no relation between digestibility, TOCs levels and CH_4 production. TOCs levels could be used as an indirect index to measure CH_4 levels in ruminal fermentation because TOCs levels during *in vitro* fermentation were directly proportional to CH_4 emission.

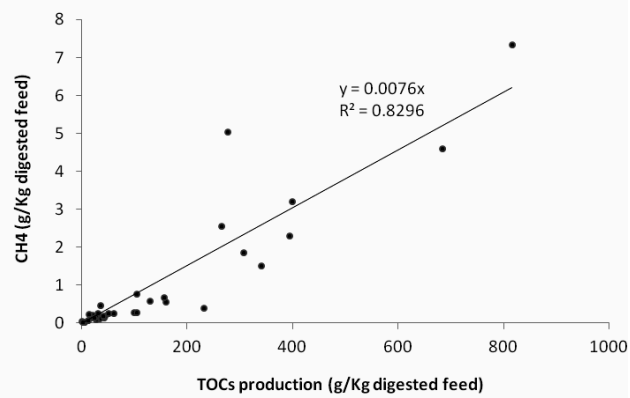

Figure 1. Correlation between TOCs and CH_4 production.

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