Back to Amornsak content

Evaluation of the effects of local plants by *in vitro* rumen fermentation and their effects on fermentation end-products

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Abstract

Feed resources could be divided into three groups: high, medium and low depending on CT concentrations, which were from 11.4 to 16.8%, 2.1 to 4.6% and 0.7 to 1.7%, respectively. Mangosteen peel had the highest and pak kayaeng had lowest in terms of CT value. However, CT could not be detected in fresh banana fruit, Indian mulberry fruit, banana flower and rice straw. There was a significant difference ($P< 0.001$) in gas production after 48 h incubation. Gas production was lower in the group which had the highest level of CT (from 32.2 to 181.9 ml g^{-1} DM). There were significant differences ($p<0.05$) in total VFAs (from 48 to 88 m*M* L^{-1}), individual VFA production and acetate to propionate ratio (from 1.6 to 4.6), but the values were variable. However, propionate production was slightly higher in the group treatment which contained CT than those without CT. The correlation coefficients (*r*) were relatively low between gas production after 48 h incubation with total VFAs (0.39), acetate (0.16) and butyrate (0.05) production. Moreover, negative correlations were obtained between gas and propionate production (-0.20).

Key words: *In vitro* gas technique; Condensed tannins; Saponins; Local feed resources.

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1. Introduction

Feed resources for ruminant production in the tropics are becoming increasingly important because of rising costs and limited supplies. It is, therefore, imperative to use fully the available feed resources and crop residues, including non-conventional supplies, to meet the rising demands of the rapidly increasing animal production. This is especially critical during the time of feed shortage in the dry season and also during the hectic season of cultivation where labour is limited and paddy fields are being harvested cropped (Wanapat, 1986;

Wanapat and Devendra, 1992). Furthermore, crop residues, shrubs, and tree fodders are available locally in large amounts and are potentially important in small farms to alleviate shortages of feed and increase the efficiency of the production systems in the tropics (Leng, 1993) including the northeast of Thailand (Wanapat, 1999).

Numerous studies have demonstrated that local feed resources such as cassava root/hay/silage, corn stovers, kapok meal, baby corn, cow-pea, cotton seed meal, leuceana leaves, sweet potatoes, sugarcane, sesban seed/leaves, mulberry leaves, moringa seed, sapindus fruit etc, have a potential as ruminant feeds to improve and increase the efficiency of the production system (Abdullah and Rajion, 1997; Liu *et al*, 2001; Preston, 2001; Hossain and Becker, 2002; Hess *et al*., 2003; Lam, 2003; Promkot, 2003; Sokerya, 2003; Vongsamphanh, 2003; Wanapat, 2003; Anhwange *et al*., 2004; Hristove *et al*., 2004; Khampa *et al*., 2004). However, some of these feeds contain secondary plant compounds such as condensed tannins, saponins, gossypol, mimosine, and trypsin inhibitor, which may diminish the effects of these feedstuffs with respect to feed quality and animal production. Comprehensive reviews on the effects of secondary compounds and detoxification methods on animal nutrition and feeding in the tropics have been reported by Reed (1995), Abdullah and Rajion (1997) and Markar and Becker (1999).

In vitro gas methods have several advantages over the *in sacco* or other *in vitro* methods which are based on gravimetric determination of residues to study the action of anti-nutritive factors. The latter techniques based on gravimetric determination of residues, lead to the solubilization of anti-nutritional factors, thus, making no contribution to energy production in the system but being measured as dry matter digestibility. This could lead to misleading conclusions, whereas in the *in vitro* gas method the effects of anti-nutritional factors on rumen fermentation are reflected in the gas production. Furthermore, the *in vitro* gas method is also expected to be better than chemical methods for quantification of anti-nutritional factors. Generally, chemical methods measure anti-nutritional factors related to one or another standard. The nature of the standard and, hence, their biological effects could be different from the anti-nutritional factors present in feeds. This is particularly true for heterogeneous classes of anti-nutritional compounds such as tannins, saponins, alkaloids, etc. In addition, chemical assays do not indicate the possible interaction of different anti-nutritional factors that take place during fermentation (Siaw *et al*., 1993; Khazaal *et al*., 1994; Nsahlai *et al*., 1994; Bonsi *et al*., 1995). Nevertheless, detailed studies on the relationship of gas produced and fermentation end-products of tropical feed resources have been rather limited.

Therefore, the objective of this study is to investigate condensed tannins (CT) and/or crude saponins (CS) concentrations in local plants and their relationship with the fermentation and end-produts by using the *in vitro* gas technique.

2. Materials and methods

2.1 Location and duration

This experiment was conducted on station during June to November 2004, at the Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand.

2.2 Experimental design

A Completely randomized design (CRD) with seventeen treatments and three replicates per treatment was used.

2.3 Feed samples

Seventeen kinds of local plants were used as substrates in this experiment. Most of them were collected from areas located around Khon Kaen University and some bought from local markets. Samples were dried in a 60 ^{o}C forced air oven then ground to pass a 2 mm sieve and used for chemical analysis and *in vitro* gas production. Local plant materials investigated were as follows:

1. Fresh banana *(Musa sapientum)*, Fruit
2. Indian mulberry *(Morinda citrifolia*), Fruit
3. Bitter cucumber *(Mormormdica charantia)*, Fruit
4. Siam neem tree *(Azadirachta indica)*, Leaf
5. Sugar apple *(Annona squamosa)*, Leaf
6. Guava *(Psidium guajava)*, Leaf
7. Mangosteen *(Garcinia mangostana)*, Peel of fruit
8. Sesbania *(Sesbania grandoflora*), Leaf
9. Cassava hay *(Manihot esculenta* Crantz)
10. Banana *(Musa sapientum)*, Flower
11. Mulbery *(Morus indica)*, Leaf
12. Pak Kayaeng;Paddy rice weed (*Limnophila aromatica*), Leaf
13. Bai Yanang, Leaf (*Tiliacora triandra*)
14. Coral, Leaf (*Erythrina variegate)*
15. Star gooseberry, Leaf (*Phyllanthus acidus)*
16. Banana *(Musa sapientum)*,Leaf
17. Rice straw (*Oryza saliva*)

2.4 In vitro gas production

The method used for *in vitro* fermentation was based on the technique described by Menke *et al.* (1979), and slightly modified for use in our laboratory (Wanapat and Ngamsaeng, 2004). Two hundred milligrams of feed samples were weighed into 60 ml plastic syringes with pistons lubricated with vaseline.

Buffered mineral solution was prepared and placed on a magnetic stirrer at 39 ºC under continuous flushing with CO_2. Rumen fluid was collected before the morning feeding from two ruminally fistulated steers fed on rice straw as roughage. Rumen fluid was taken from the middle part of the rumen by using a 60-ml hand syringe and transferred into two pre-warmed thermos flasks, combined, filtered through three layers of cheesecloth and flushed with CO_2. About 30 ml of buffered rumen fluid was taken into syringes containing the feeds. After closing the three way clips, the syringes were gently swirled and tubes opened to remove gas by pushing the piston upwards to achieve complete removal. The clip was closed, this initial volume recorded, and syringes placed in an incubator at 39 ºC. Gas production rates were recorded at 2, 4, 6, 12, 18, 24 and 48 h of incubation and each syringe was gently swirled after reading. At 2, 4, 6 and 48 h of incubation, the fluid samples were drawn into plastic bottles where 3 ml of H_2SO_4 solution (1M) were added to 30 ml of sample. The mixture was centrifuged at 16,000 x *g* for 15 minutes and the supernatant stored at –20 ^{o}C prior to VFA analysis using High Performance Liquid Chromatography (HPLC; Model Water 600; UV detector, Millipore Crop.) according to the method of Samuel *et al.* (1997). Rate and extent of gas production was determined for each substrate by fitting gas production data to the non-linear equation $Y = b(1\text{-}e^{-ct})$(Orskov and McDonald, 1979), where Y is the volume of gas production at time *t*, *b* the potential gas production(mlg^{-1}DM), and *c* the fraction rate of gas production (h^{-1}).

2.5 Chemical analysis

Substrates were analyzed for DM, Ash, CP, P, K, Ca, Mg and S using the procedure of AOAC (1990), and NDF and ADF according to Goering and Van Soest (1970). Condensed tannins (CT) were estimated by the Vanillin-HCL method (Burns, 1971 modified by Wanapat and Poungchompu, 2001) and saponins were measured by using methanol extraction following the method of Kwon *et al*. (2002) as modified by Wanapat and Ngamsaeng (2004).

2.6 Statistical analysis

The data were analyzed by Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure of the Minitab software version 13.31, 2000. Significance between means was tested using the least significant difference (LSD) according to the following model:

$Yij = \mu + T_i + e_{ij}$ Where Y_{ij}= The criteria under study, in treatment i of time j,

μ = over all sample mean, T_i = Effect of treatment i , e_{ij} = Error

3. Results

3.1 Chemical constituents

The chemical composition of the feedstuffs are shown in Table 1.The CP content ranged from 1.3 % in bitter cucumber to 28.0 % in sesbania leaf. More than half the samples had CP, NDF and ADF contents ranging from 10 to 20%, 27.4 to 85.6% and 11.3 to 53.8%, respectively. P, K, Ca, Mg and S contents ranged from 0.03 to 0.48%, 0.85 to 6.29%, 0.06 to 2.56%, 0.04 to 0.51% and 0.05 to 0.43%, respectively. Feed resources could be divided into three groups: high, medium and low, depending on CT concentration, which ranged from 11.4 to 16.8%, 2.1 to 4.6% and 0.7 to 1.7%, respectively. Mangosteen peel had the highest and pak kayaeng the lowest CT value. However, the CT contents in fresh banana fruit, Indian mulberry fruit, banana flower and rice straw were so low as to be non-detectable.

3.2 In vitro gas production

There was a significant difference ($P< 0.001$) in gas production among substrates (Table 2). Gas production was lower in groups which had higher levels of CT, ranging from 32.2 to 181.9 ml g^{-1} DM. Furthermore, the pattern of gas production was such that it rapidly increased during the period 6 to 24 h after incubation and after that slowly increased, as shown in Figure 1. It was also observed that initial gas production was markedly slow during the first 3 h for all sources. There was a wide range in potential gas production (*b*), while rates of gas production ranged from 0.001 h^{-1} in rice straw to 0.083 in Indian mulberry fruit (Table 2).

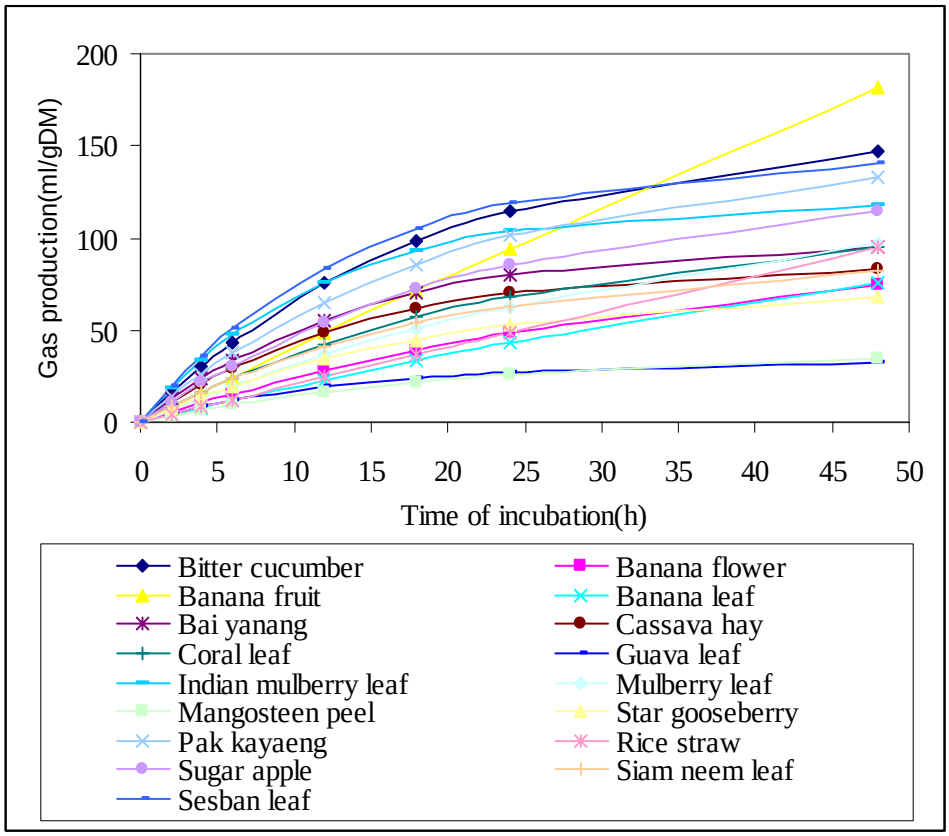

Figure 1. Pattern of in vitro gas production of local feed resources incubated in rumen fluid for 48h.

3.3 Volatile fatty acids (VFA) production

The results of VFA production are shown in Tables 3, 4,5 and 6 for 2, 4, 6, and 48 h of incubation, respectively. There were significant differences ($p<0.05$) in total (from 48 to 88 m*M* L^{-1}) and individual VFA production and acetate to propionate ratio (from 1.6 to 4.6) among substrates, but the values were variable. However, propionate production was slightly higher in the group which contained CT than without CT. The pattern of TVFAs, acetate, propionate and butyrate production and acetate : propionate ratio are presented in Figures 2, 3, 4, 5 and 6, respectively. The patterns varied among substrates during 2 to 6 h and after that changed only slightly until 48 h after incubated.

Table 3. Total ($mM1^{-1}$) and individual (mol%) volatile fatty acid production and acetate to propionate ratio (A:P) in buffered rumen fluid after 2 h incubation of 200 mg of feed sample

Substrates	Total	Acetate	Propionate	Butyrate	A:P
Bitter cucumber	135.5^{a}	66.7^{efg}	31.1^{b}	2.0^{kl}	2.1^{hij}
Banana flower	98.1^{b}	70.9^{cdef}	20.3^{de}	8.7^{cde}	3.4^{efg}
Banana fruit	38.6^{i}	72.6^{cde}	21.0^{de}	6.2^{gf}	3.5^{efg}
Banana leaf	58.9^{ef}	85.5^{a}	12.4^{fg}	0.9^{l}	6.9^{b}
Bai yanang	65.2^{e}	74.1^{bcd}	16.1^{ef}	9.6^{c}	5.0^{c}
Cassava hay	65.3^{e}	73.7^{bcd}	20.3^{de}	5.9^{fgh}	3.6^{ef}
Coral leaf	73.9^{d}	66.8^{efg}	28.2^{bc}	4.8^{ghij}	2.3^{ghij}
Guava leaf	53.1^{fg}	65.6^{fg}	25.4^{cd}	8.9^{cd}	2.6^{efghi}
Indian mulbery fruit	60.5^{ef}	76.3^{bc}	20.4^{de}	3.2^{jk}	3.8^{ed}
Mulberry leaf	45.1^{hi}	62.6^{gh}	31.8^{b}	5.4^{fghi}	2.0^{hij}
Mangosteen peel	50.9^{gh}	79.7^{b}	16.4^{ef}	3.8^{hijk}	4.8^{cd}
Star gooseberry leaf	58.9^{ef}	57.9^{hi}	31.7^{b}	10.3^{c}	1.8^{ji}
Pak kayaeng	42.1^{i}	86.2^{a}	10.1^{g}	3.5^{ijk}	8.5^{a}
Sugar apple leaf	39.6^{i}	66.1^{fg}	27.1^{bc}	6.7^{efg}	2.4^{fghi}
Siam neem leaf	86.6^{c}	45.0^{j}	38.8^{a}	16.0^{b}	1.1^{j}
Sesbania leaf	59.9^{ef}	53.1^{i}	24.2^{cd}	12.6^{a}	2.2^{hij}
Rice straw	41.0^{i}	68.6^{defg}	24.3^{cd}	7.0^{def}	3.0^{efgh}
SEM	**2.6**	**2.0**	**1.9**	**0.7**	**0.4**

Means in the same column with different letters are significantly different ($P<0.05$)

Table 4. Total ($mM1^{-1}$) and individual (mol%) volatile fatty acid production and acetate to propionate ratio (A:P) in buffered rumen fluid after 4 h incubation of 200 mg of feed sample

Substrates	Total	Acetate	Propionate	Butyrate	A:P
Bitter cucumber	90.5^{a}	62.6^{de}	34.1^{ab}	3.2^{ijk}	1.8^{gh}
Banana flower	85.5^{ab}	70.4^{bc}	20.1^{efg}	9.4^{cdef}	3.5^{c}
Banana fruit	41.9^{kl}	58.4^{e}	32.8^{ab}	8.6^{defg}	1.8^{gh}
Banana leaf	76.3^{bcd}	63.4^{cde}	17.9^{fgh}	18.5^{a}	3.5^{c}
Bai yanang	64.5^{defgh}	68.3^{bcd}	23.3^{def}	8.2^{defg}	3.2^{cde}
Cassava hay	38.6^{l}	73.2^{b}	13.7^{gh}	12.9^{bc}	5.3^{b}
Coral leaf	70.5^{cdef}	72.7^{b}	24.6^{cde}	2.5^{jk}	2.9^{cdefg}
Guava leaf	78.1^{bc}	57.7^{e}	28.3^{bcd}	13.8^{b}	2.0^{fgh}
Indian mulbery fruit	73.4^{bcde}	72.6^{b}	23.6^{def}	3.7^{hijk}	3.1^{cdef}
Mulberry leaf	48.4^{ijkl}	64.3^{cde}	29.7^{abcd}	5.9^{fghij}	2.3^{defgh}
Mangosteen peel	58.5^{fghi}	71.8^{b}	17.9^{fgh}	10.1^{cde}	4.1^{c}
Star gooseberry leaf	52.6^{hijk}	58.0^{e}	35.0^{a}	6.8^{efgh}	1.6^{h}
Pak kayaeng	55.8^{hij}	70.3^{bc}	18.6^{efgh}	10.9^{bcd}	3.7^{k}
Sugar apple leaf	63.6^{efgh}	82.1^{a}	12.6^{h}	5.1^{ghijk}	6.5^{a}
Siam neem leaf	58.2^{ghi}	67.3^{bcd}	30.6^{abc}	1.9^{k}	2.1^{efgh}
Sesbania leaf	69.5^{cdefg}	66.5^{bcd}	19.4^{efg}	13.9^{b}	3.4^{cd}
Rice straw	44.6^{jkl}	72.7^{b}	21.1^{ef}	6.1^{fghi}	3.6^{c}
SEM	**4.2**	**2.4**	**2.2**	**1.2**	**0.4**

Means in the same column with different letters are significantly different ($P<0.05$)

Table 5. Total ($mM1^{-1}$) and individual (mol%) volatile fatty acid production and acetate to propionate ratio (A:P) in buffered rumen fluid after 6 h incubation of 200 mg of feed sample

Substrates	Total	Acetate	Propionate	Butyrate	A:P
Bitter cucumber	77.7^{b}	61.3^{gh}	33.8^{ab}	4.8^{ef}	1.8^{f}
Banana flower	77.7^{b}	73.5^{bcd}	20.9^{ef}	5.4^{de}	3.5^{c}
Banana fruit	42.8^{n}	65.7^{fg}	25.6^{c}	8.6^{bc}	2.6^{de}
Banana leaf	65.5^{c}	76.3^{b}	21.0^{ef}	2.5^{fg}	3.6^{c}
Bai yanang	55.8^{def}	71.6^{cde}	22.9^{cdef}	5.4^{de}	3.1^{cd}
Cassava hay	41.3^{h}	75.2^{bc}	16.9^{g}	7.7^{cd}	4.4^{b}
Coral leaf	58.8^{cde}	70.4^{de}	23.6^{cde}	5.8^{de}	2.9^{cd}
Guava leaf	59.6^{cde}	59.8^{h}	34.1^{a}	5.9^{de}	1.7^{f}
Indian mulbery fruit	77.1^{b}	76.7^{b}	21.9^{def}	1.3^{g}	3.4^{c}
Mulberry leaf	56.0^{def}	71.3^{cde}	22.9^{cdef}	5.6^{de}	3.1^{cd}
Mangosteen peel	59.7^{cde}	72.4^{bcde}	20.4^{efg}	7.0^{cde}	3.6^{c}
Star gooseberry leaf	53.8^{efg}	54.6^{i}	35.9^{a}	9.4^{abc}	1.5^{f}
Pak kayaeng	54.0^{ef}	68.7^{ef}	19.7^{fg}	11.5^{a}	3.5^{c}
Sugar apple leaf	48.8^{fgh}	83.8^{a}	11.2^{h}	4.8^{ef}	7.5^{a}
Siam neem leaf	62.8^{cd}	70.6^{de}	24.7^{cd}	4.6^{ef}	2.9^{cd}
Sesbania leaf	86.1^{a}	59.0^{h}	30.5^{b}	10.3^{ab}	1.9^{ef}
Rice straw	45.9^{gh}	73.7^{bcd}	20.6^{ef}	5.5^{de}	3.6^{c}
SEM	**2.8**	**1.5**	**1.2**	**0.8**	**0.2**

Means in the same column with different letters are significantly different ($P<0.05$)

Table 6. Total ($mM1^{-1}$) and individual (mol%) volatile fatty acid production and acetate to propionate ratio (A:P) in buffered rumen fluid after 48 h incubation of 200 mg of feed sample

Substrates	Total	Acetate	Propionate	Butyrate	A:P
Bitter cucumber	88.0^{a}	57.6^{gh}	34.6^{a}	7.7^{cdef}	1.6^{fg}
Banana flower	66.2^{efg}	65.8^{cde}	23.2^{de}	10.9^{b}	2.8^{bcde}
Banana fruit	74.6^{bcd}	57.9^{gh}	26.6^{bcd}	15.4^{a}	2.1^{defg}
Banana leaf	72.8^{cde}	58.6^{fgh}	34.1^{a}	7.2^{def}	1.7^{fg}
Bai yanang	68.0^{def}	73.1^{ab}	19.9^{ef}	6.8^{ef}	3.7^{ab}
Cassava hay	49.0^{i}	65.4^{cde}	25.3^{cde}	9.2^{bcde}	3.2^{bc}
Coral leaf	56.7^{h}	66.7^{bcd}	23.4^{de}	9.7^{bcd}	2.8^{bcde}
Guava leaf	59.5^{gh}	52.1^{h}	31.3^{ab}	16.4^{a}	1.6^{g}
Indian mulbery fruit	67.8^{def}	60.9^{defg}	30.9^{abc}	8.1^{cdef}	1.9^{fg}
Mulberry leaf	62.6^{fgh}	67.6^{bcd}	24.1^{de}	8.2^{cdef}	2.8^{efg}
Mangosteen peel	80.6^{b}	59.9^{efg}	33.8^{a}	6.1^{f}	1.7^{fg}
Star gooseberry leaf	55.8^{hi}	64.7^{cdef}	26.7^{bcd}	8.5^{bcdef}	2.4^{cdefg}
Pak kayaeng	74.9^{bcd}	75.3^{a}	16.2^{f}	8.4^{bcdef}	4.6^{a}
Sugar apple leaf	66.2^{efg}	65.1^{cdef}	24.6^{de}	10.1^{bc}	2.6^{cdef}
Siam neem leaf	48.5^{i}	67.5^{bcd}	23.2^{de}	9.1^{bcde}	2.9^{bcde}
Sesbania leaf	79.3^{bc}	69.0^{abc}	22.7^{de}	8.1^{cdef}	3.0^{bcd}
Rice straw	66.5^{efg}	66.6^{bcde}	26.5^{bcd}	6.8^{ef}	2.5^{cdefg}
SEM	**2.5**	**2.3**	**2.1**	**0.9**	**0.3**

Means in the same column with different letters are significantly different ($P<0.05$)

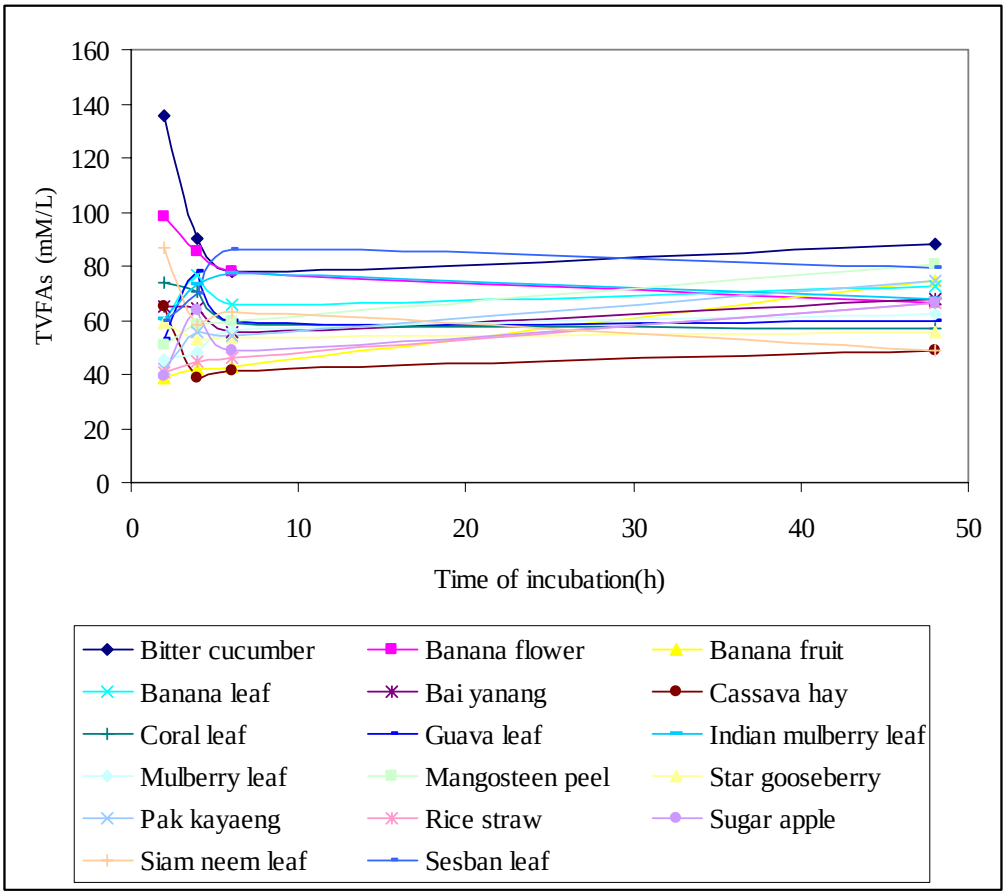

Figure 2. Pattern of total volatile fatty acids (TVFs) of local feed resources incubated in rumen fluid for 48h

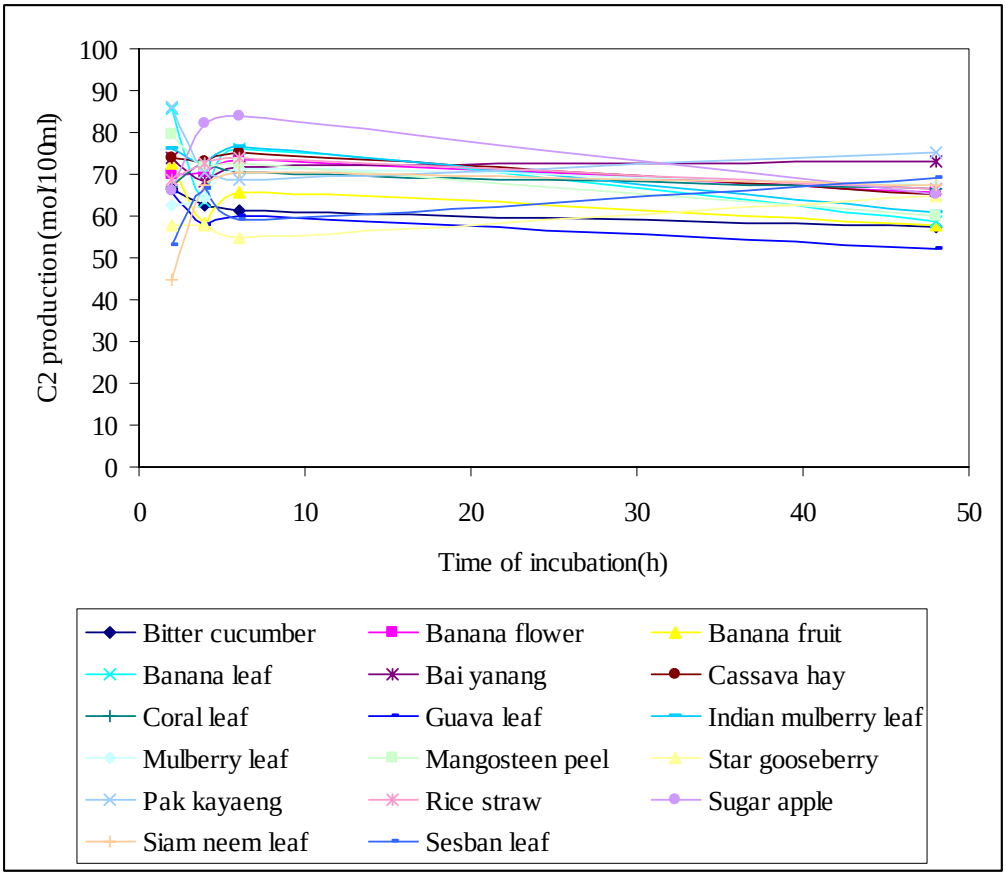

Figure 3. Pattern of acetate (C2) production of local feed resources incubated in rumen fluid for 48h.

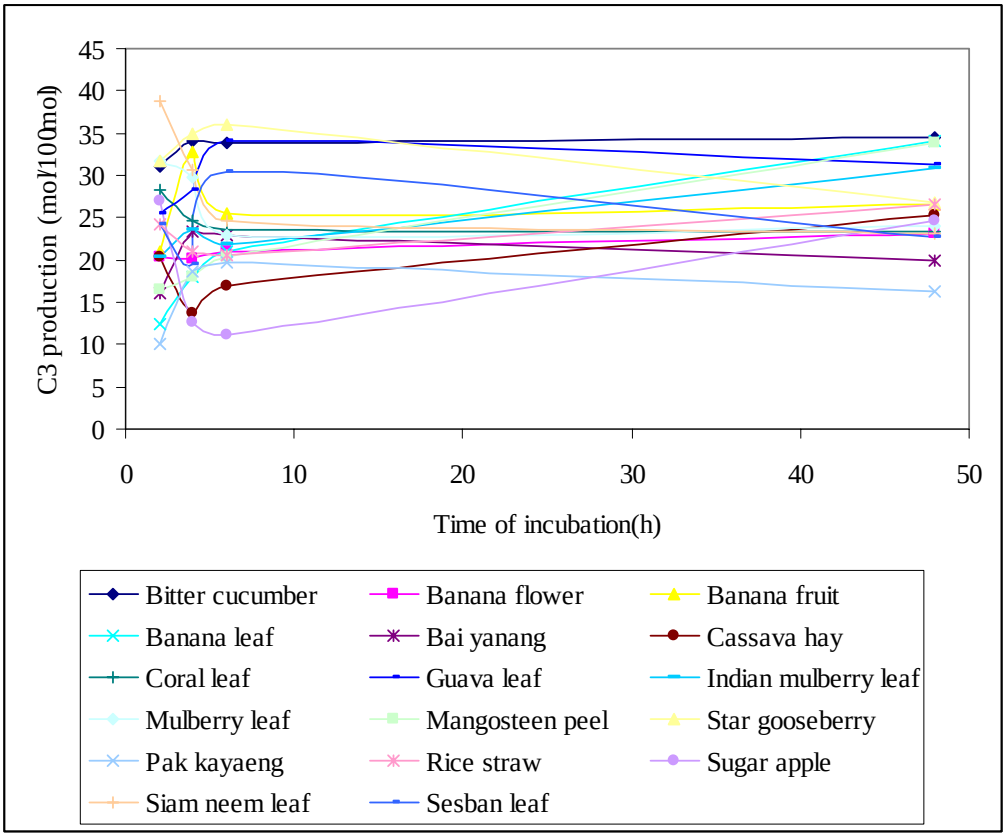

Figure 4. Pattern of propionate (C3) production of local feed resources incubated in rumen fluid for 48h.

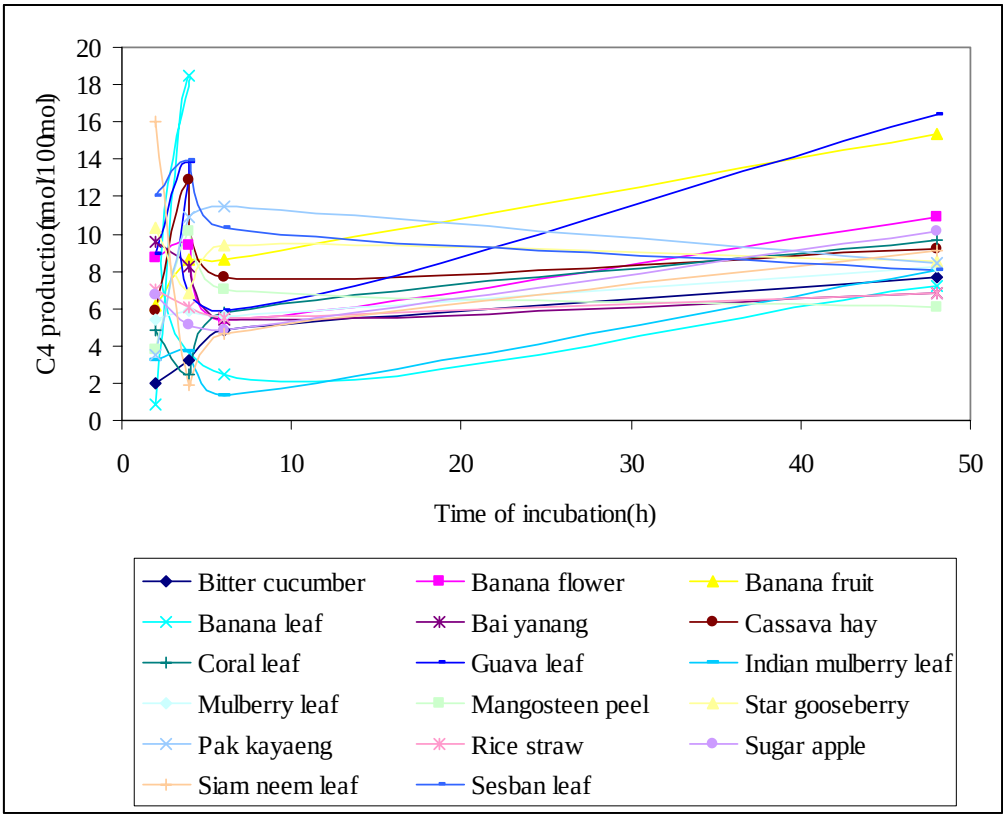

Figure 5. Pattern of butyrate (C4) production of local feed resources incubated in rumen fluid for 48h.

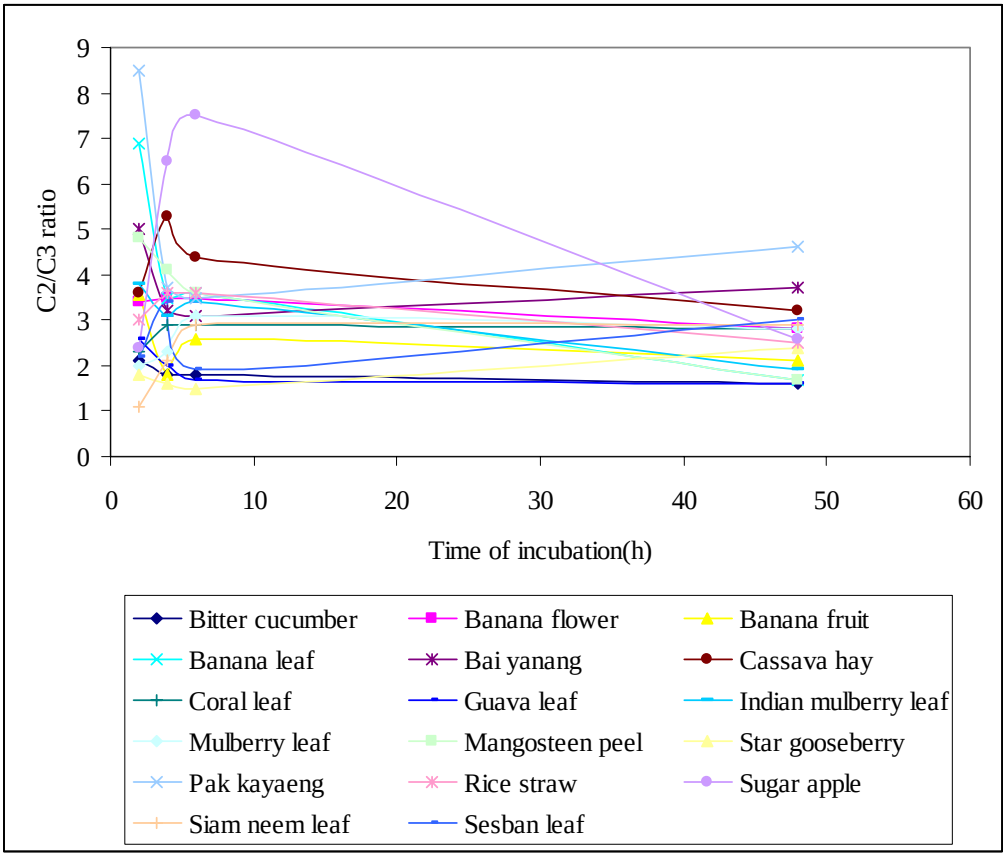

Figure 6. Pattern of acetate : propionate ratio (C2/C3) of local feed resources incubated in rumen fluid for 48h.

3.4 Relationship between gas and VFA production

The relationships between gas and TVFA and individual VFA production are shown in Table 7. The correlation coefficients (*r*) were relatively low between gas production after 48 h incubation and with total VFA (0.39), acetate (0.16) and butyrate (0.05) production. Moreover, negative correlations were obtained between gas and propionate production (-0.20). Overall there appeared to be no correlations between gas production volume and rumen fermentation parameters among these tropical feed resources.

Table 7. Correlation (*r*) between gas production (ml g^{-1} DM) and Total volatile fatty acids(TVFAs)(mMl^{-1}), individual VFA production (mol%) for feed samples incubated in buffered rumen fluid

Item	Gas	P <
TVFAs		
2	0.12	0.363
4	0.18	0.187
6	0.38	0.004
48	0.39	0.003
Acetate		
2	-0.07	0.618
4	0.08	0.561
6	-0.10	0.462
48	0.16	0.259
Propionate		
2	-0.01	0.901
4	0.00	0.989
6	0.12	0.386
48	-0.20	0.139
Butyrate		
2	0.17	0.222
4	-0.12	0.374
6	-0.00	0.983
48	0.05	0.715

4. Discussion

4.1 Chemical composition

The CP content of the cassava hay (21.7%) was lower than the standard mean value of 23.6%, as reported by Wanapat (2003), and 25.5% in cassava whole plant by Moore and Cock (1985). However, the value was within the range reported by Poungchompu *et al*. (2001) (20.6-22.0%). In addition, Vongsamphanh *et al*. (2003) reported that CP level can increase to 27.3% if harvested at 3 months after planting and growing in soils with high fertility. However, the NDF and ADF values were within ranges reported in earlier studies. The levels of condensed tannins in cassava hay were slightly lower than those reported by Wanapat and Poungchompu (2001), but values for mangosteen peel, guava leaves and sesbania leaves were similar to those in previous reports (Getachew *et al*., 2002; Hossain and Becker, 2002).

4.2 In vitro gas production

In vitro gas production values at 48 h from this experiment were lower than in several previous studies (Menke *et al*., 1979; Doane *et al*., 1997; Blummel *et al*., 1999; Liu *et al*., 2002; Getachew *et al*., 2002). Nevertheless, this could be due to differences in the chemical composition of the feeds, especially CP and NDF contents. Pell and Schofield (1993) reported high correlations between gas production and NDF disappearance, (r = 0.99) or gas production and DM disappearance (r = 0.95) (Prasad *et al*., 1994). As total gas production is the result of various fractions being fermented at the same time, but at different rates, this leads to complex multiple rates that are difficult to partition. Pell and Schofield (1993) suggested that plotting the rate of gas production as a function of time, calculated by subtracting gas volumes at adjacent time, can be used to identify pools that are digested at different rates. In addition, gas production was reported to be linearly related to energy value in compound feeds (Menke *et al*., 1979; Blummel *et al*., 1993; Aiple *et al*., 1996), feed intake (Khazaal *et al*., 1993; Blummel and Bullerdieck, 1997), production of short chain fatty acids (Makkar *et al* ., 1995), methane production *in vivo* (Moss and Givens,1997) and microbial protein synthesis (Krishnamoorthy *et al*., 1991b). In this experiment, it appeared that gas production was minimally produced after 48 h after incubation suggesting less fermentation activity in all substrates.

4.3 Volatile fatty acid (VFA) production

Based on this study, the VFAs especially proportion of acetate, propionate and butyrate were different among substrates. The proportion of propionate production was slightly higher and C2/C3 ratio was lower in the group which contained CT than without CT. This could have been due to differences in the chemical composition of the feeds, particularly CT content. The effect of CT on TVFAs and molar proportions of individual VFA production could be due to reduced protozoal and increased bacterial populations, since acetate and butyrate are the major fermentation end-products of protozoa (Jouany, 1994). This result agrees with reports by Hossain and Becker (2002) and Getachew *et al*. (2002). The molar proportion of different VFAs produced is dependent on the type of substrate. Therefore, the molar ratio of acetate : propionate has been used to evaluate the substrates. Rapidly fermentable carbohydrates yield relatively higher propionate as compared to acetate, and the reverse takes place when slowly fermentable carbohydrates are incubated (Makkar *et al*., 1995). In addition to carbohydrate fermentation, protein degradation also leads to a proportionally smaller amount of VFAs. The carbon skeleton arising from deamination gives rise to a variety of VFA (Allison, 1970). For example, fermentation of glycine can lead to ammonia and acetic acid without the release of CO_2, and that of leucine, isoleucine and valine to isovaleric acid, 2-methylbutyric acid, and isobutyric acids, respectively (Blackburn, 1965; Allison, 1970.). D-threonine can yield acetic acid, and DL-aspartic acid degradation leads primarily to propionic acid (Blackburn, 1965). The extent of VFA production from proteins is dependent upon on the amino acid composition of the feeds and the extent of rumen deamination of these amino acids.

4.4 Relationship between gas and VFAs production

The relatively weak correlations obtained between 48 h gas production and TVFAs (r = 0.39) and individual VFA production were lower than in a previous study with browse species (Getachew *et al*., 2002) and industrial by-product feeds (r = 0.76)(Getachew *et al*., 2004). This could be due to proportionally higher condensed tannins and/or saponins in feeds and available carbohydrates. Generally, gas is produced mainly when substrate is fermented to acetate and butyrate, while propionate was found to be relatively higher in all feeds, especially at 48h. Substrate fermentation to propionate yields gas only from buffering of the acid because an extra carbon atom in propionate would otherwise have appeared as CO_2 (Wolin, 1960). Therefore, relatively lower gas production is associated with propionate production as is shown by the negative correlation in this study (r = -0.20). One of the most challenging problems associated with using gas production methods is that the amount of gas produced varies with different molar proportions of VFAs as obtained in this study.

4.5 Effects of condensed tannins on gas and VFA production

Lower production of gas and changes in proportion of VFAs production, observed in substrates that contained high CT content was consistent with Getachew *et al*. (2002) and Wina *et al*. (2003). Pell and Schofield (1993) reported high correlations between gas production and NDF disappearance (r = 0.99) or gas production and DM disappearance (r = 0.95) (Prasad *et al*., 1994), and higher concentration of tannins in the diet is associated with reduction in organic matter digestibility (Silanikove *et al*., 1997; Waghorn and Shelton, 1997). Therefore, substrates especially, mangosteen peel and guava leaves, which had high CT contents, resulted in low gas production in this case. Barry *et al*. (1986) suggested a level of CT in diet of 30-40 g/kg DM for efficient utilization by ruminants. Moreover, a relatively weak correlation between CT and percent increase in gas production (r = 0.23) was observed in a previous study (Wood and Plumb, 1995; Abdulrazak *et al*., 2000), which could be due to the variation in structural and biological activity of tannins. CT values determined by the Vanillin-HCl method do not appear to reflect the biological activity. Changing the pattern of fermentation towards higher molar proportion of propionate could have been due to the changing microbial composition in the rumen.

5. Conclusions and recommendations

Based on this experiment, it can be concluded as follows;

- Local feed resources have variable in CT content, and can be divided into three groups as follows: High (>10%), including mangosteen peel, guava leaf and siam neem leaf; medium (2 to 10%), including sesban leaf, sugar apple leaf, star gooseberry leaf, coral leaf, bai yanang, cassava hay and bitter cucumber and low (<2%), including banana leaf, mulberry leaf and pak kayaeng.

- High group results in low gas production and due to the high molar proportion of propionate low acetate : propionate ratios.
- The correlation (*r*) coefficient was relatively low between gas and VFA production in local plans containing CT.
- Local feed resources which had high CT contents can be used as alternative dietary supplements to improve rumen ecology and feed utilization in ruminants.
- However, animal trials should be conducted in order to more clearly understand the optimal levels of CT and their effect on rumen ecology, especially the mode of action of CT on ruminal microbes.

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Table 1.Chemical composition of local feed resources used in *in vitro* gas production incubated in buffered rumen fluid

Substrate	DM	CP	Ash	NDF	ADF	P	K	Ca	Mg	S	CT[1]	CS[2]
	%	……..%DM……										
Mangosteen peel	93.0	18.3	2.8	56.8	51.3	0.03	1.23	0.16	0.04	0.05	16.8	10.0
Guava leaf	91.0	10.1	6.4	54.0	29.1	0.12	1.67	0.95	0.25	0.10	15.8	2.8
Siam neem leaf	89.9	14.9	6.3	52.1	30.0	0.12	1.43	1.47	0.40	0.14	11.4	2.8
Sesbania leaf	89.5	28.0	10.3	27.4	14.6	0.30	2.93	1.51	0.46	0.22	4.6	2.0
Sugar apple leaf	90.1	18.6	7.7	49.9	23.1	0.14	1.80	1.99	0.23	0.12	3.8	c.d
Star gooseberry leaf	89.3	17.4	7.4	54.1	28.9	0.17	1.81	1.34	0.35	0.25	3.4	c.d
Coral leaf	87.2	19.2	11.9	50.5	31.1	0.24	0.96	2.56	0.38	0.20	2.6	1.8
Bai yanang	90.5	16.4	7.1	62.3	37.1	0.16	1.57	0.94	0.21	0.20	2.3	1.3
Cassava hay	88.9	21.7	9.9	54.0	31.2	0.17	0.85	1.33	0.51	0.16	2.2	1.7
Bitter cucumber	85.8	1.3	8.8	50.9	30.1	0.48	3.29	0.20	0.26	0.12	2.1	4.1
Banana leaf	89.4	13.8	10.1	78.2	35.6	0.28	3.41	0.27	0.25	0.23	1.7	1.3
Mulberry leaf	87.1	15.2	10.8	56.6	18.6	0.21	1.36	1.82	0.27	0.12	1.6	2.3
Pak kayaeng	87.6	14.9	16.7	63.1	53.8	0.16	2.04	1.34	0.37	0.43	0.7	1.3
Fresh banana fruit	83.4	2.3	2.9	45.4	11.3	0.07	1.30	0.06	0.10	0.06	c.d	1.9
Indian mulberry fruit	88.8	7.1	6.3	49.8	39.9	0.14	2.24	0.44	0.16	0.23	c.d	3.1
Banana flower	89.2	12.4	14.1	68.5	52.8	0.39	6.29	0.21	0.39	0.17	c.d	c.d
Rice straw	90.6	3.0	13.5	85.6	53.2	0.10	2.04	0.47	0.17	0.13	c.d	c.d

[1]CT = Condensed tannins; [2]CS = Crude saponins; c.d = not detectable

Table 2. ***In vitro*** **gas production of feed samples incubated in buffered rumen fluid**

Substrates	Gas production (ml g $^{-1}$ DM)							Gas production constants[a]	
h	2	4	6	12	18	24	48	*b*	*c*
Bitter cucumber	16.2^{b}	30.7^{b}	43.7^{b}	75.4^{b}	98.3^{ab}	115.0^{a}	146.9^{b}	159.2^{ab}	0.053^{c}
Banana flower	5.3^{gh}	10.4^{fg}	15.2^{g}	28.1^{ij}	39.2^{hi}	48.8^{gh}	75.0^{hi}	105.9^{fghi}	0.025^{e}
Banana fruit	8.1^{ef}	16.2^{e}	24.3^{e}	48.1^{ef}	71.5^{d}	94.5^{cd}	181.9^{a}	166.2^{a}	0.003^{f}
Banana leaf	4.0^{hi}	8.0^{gh}	11.8^{gh}	22.8^{jkl}	33.1^{i}	42.7^{h}	75.5^{hi}	137.3^{bcde}	0.010^{f}
Bai yanang	12.7^{cd}	23.8^{cd}	33.4^{cd}	55.5^{d}	70.2^{d}	79.8^{e}	95.2^{ef}	98.8^{ghi}	0.069^{b}
Cassava hay	11.3^{d}	21.0^{d}	29.6^{d}	49.0^{e}	61.7^{e}	70.0^{f}	83.0^{fgh}	86.1^{ij}	0.070^{b}
Coral leaf	8.4^{ef}	16.3^{e}	23.6^{ef}	42.2^{fg}	56.9^{ef}	68.6^{f}	95.5^{ef}	113.0^{efgh}	0.039^{d}
Guava leaf	4.5^{hi}	8.3^{gh}	11.7^{gh}	19.2^{kl}	24.2^{j}	27.3^{i}	32.2^{j}	33.4^{k}	0.072^{b}
Indian mulbery fruit	18.5^{a}	34.0^{a}	47.2^{ab}	75.8^{b}	93.1^{bc}	103.6^{b}	118.1^{d}	120.7^{defg}	0.083^{a}
Mulberry leaf	6.9^{fg}	13.3^{ef}	19.5^{f}	36.3^{gh}	50.8^{fg}	63.2^{f}	97.7^{e}	139.7^{bcd}	0.025^{e}
Mangosteen peel	3.4^{i}	6.5^{h}	9.3^{h}	16.4^{l}	21.8^{j}	25.9^{i}	34.6^{j}	39.2^{k}	0.045^{cd}
Star gooseberry leaf	7.3^{ef}	13.8^{e}	19.8^{f}	34.2^{hi}	44.8^{gh}	52.6^{g}	67.8^{i}	74.1^{j}	0.052^{c}
Pak kayaeng	13.7^{c}	26.1^{e}	37.3^{c}	65.1^{c}	85.8^{c}	101.2^{bc}	132.6^{c}	147.0^{abc}	0.049^{c}
Sugar apple leaf	11.2^{d}	21.5^{d}	30.8^{d}	54.2^{de}	72.1^{d}	85.6^{de}	114.5^{d}	129.7^{cdef}	0.045^{cd}
Siam neem leaf	8.6^{e}	16.4^{e}	23.4^{ef}	40.7^{g}	53.5^{f}	63.0^{f}	82.2^{gh}	91.0^{hij}	0.050^{c}
Sesbania leaf	19.2^{a}	35.8^{a}	50.3^{a}	83.2^{a}	104.7^{a}	118.8^{a}	140.6^{bc}	145.5^{abc}	0.071^{b}
Rice straw	4.1^{hi}	8.1^{gh}	12.2^{gh}	24.3^{jk}	36.3^{i}	48.2^{gh}	95.1^{efg}	167.2^{a}	0.001^{b}
SEM	0.5	1.0	1.4	2.2	2.7	3.1	4.5	8.5	0.003

Means on the same a column with different letters are significantly different (P<0.05).

[a] *b* : potential gas production (ml g^{-1} DM); *c* : fractional rate of gas production (h^{-1}).