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Chemical Compositions and Nutritional Evaluation of Energy Feeds For Ruminant Using *In vitro* Gas Production Technique

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Abstract: Eleven energy feed sources were evaluated for nutritive value by *in vitro* gas production technique. The rumen mixed microbe inoculums source was taken from fistulated Brahman-Thai native crossbred steers. The energy feed source were 1) broken rice 2) paddy rice 3) corn meal 4) rice bran 5) rice pollard 6) rice bran oil relate 7) cassava chip 8) mung bean meal 9) tomato pomace 10) soybean hull and 11) peanut hull. The treatments were assigned to randomize completely block design (blocked by source feedstuffs). The results indicated that soluble gas fractions (a), the fermentation of the insoluble fraction (b), rate of gas production (c) and potential of extent of gas production (|a|+b) were significantly different ($p<0.01$) among energy feed sources. The cumulative gas volume at 24, 48 and 96 h after incubation were highly significant difference ($p<0.01$) and estimated Metabolizable Energy (ME) were; 6.42, 5.37, 5.91, 6.68, 4.46, 6.59, 7.42, 5.24, 4.89, 6.18 and 4.48 MJ kg^{-1} DM, respectively. Cassava chip exhibited the greatest gas production characteristics, gas volume and estimated metabolizable energy. These results suggested that because cassava chip is available locally and inexpensive, it is the best potential energy source for beef and dairy cattle.

Key words: Chemical composition, *in vitro*, nutritive value, energy feed

Introduction

The nutritive value of ruminant feed is determined by the concentration of its chemical compositions, as well as rate and extent of digestion in the rumen. Methods previously used to determine rate and extent of digestibility were *in vivo* and *in sacco*. However, these methods were expensive, laborious, require a fistulated animal and large quantities of feed, thereby making them unsuitable for routine feed evaluation. *In vitro* gas production is an alternative technique used to determine the nutritive value of feedstuffs, since rate and extent of degradation and rumen fermentation can be easily determined by measurement of cumulative gas production (Khazaal *et al.*, 1995; Dhanoa *et al.*, 2000; Sommart *et al.*, 2000). Therefore, the gas production technique should be considered for use in nutritive evaluation in developing counties. Because it is economical, highly reproducible and an easy method of obtaining a dynamic descriptions of nutritive value of feedstuffs, while at the same time allowing for more samples to be analyzed (Herrero *et al.*, 1996). Additionally, relationships have been observed between a feed's gas production profile and in dry matter digestibility (Sommart *et al.*, 2000) and feed intake (Blummel and Ørskov, 1993; Blummel and Becker, 1997).

Energy content of ruminant feed sources is an important factor to take into consideration due to feed ration composes of energy content approximately seventy percent (Chumpawadee, 2002). When planning diet formulation, cost, chemical composition and digestibility of an energy feed source should be fully taken into account. Numerous variety of energy feeds are available in tropical zones. However, there are insufficient information available regarding the effect of feed used on kinetics of gas production.

With respect to energy feeds in Thailand, limited information is available on kinetics of gas production and nutritive value. Therefore, the aim of this study was to evaluate chemical composition and nutritive values of energy feeds in ruminants using the *in vitro* gas production technique.

Materials and Methods

Feedstuffs samples and chemical analysis: The energy feeds were collected from various feed mills and organizations in the North East of Thailand. All samples were ground through a 1 mm screen for the *in vitro* gas production technique incubation and chemical analysis. The samples were determined Dry Matter (DM), Crude Protein (CP) and ash content (AOAC, 1990). Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) of samples were assayed using the method proposed by Van Soest *et al.* (1991). Concentrations of Ca, Mg, K, Na, Mn, Cu and Fe of feedstuffs were determined by Atomic Absorption Spectrophotometer (AA 680, Shimadzu, Japan).

Table 1: Chemical composition and mineral content of energy feeds

Feed	DM (%)	CP	Ash	NDF	ADF	ADL	Ca	P	Mg	K	Na	Mn	Cu	Fe
stuffs	%DM basis						mg/gDM							
Broken rice	91.01	7.19	2.79	29.73	0.74	0.58	0.18	0.54	0.02	0.40	1.05	0.013	0.003	0.033
Paddy rice	93.27	4.88	7.81	26.53	18.23	4.92	0.38	0.26	0.33	2.01	0.99	0.117	0.003	0.053
Corn meal	89.15	7.66	1.63	21.51	2.40	1.45	0.26	0.80	0.33	4.15	1.05	0.003	0.002	0.067
Rice bran	92.50	12.77	7.65	13.32	5.42	3.00	0.12	3.93	3.77	8.65	0.50	0.114	0.003	0.066
Rice pollard	92.33	6.73	12.72	52.67	38.32	15.31	0.18	4.81	1.56	5.79	0.50	0.174	0.002	0.079
Rice bran oil relate	90.36	18.12	12.93	43.64	16.74	6.91	0.11	6.14	5.81	10.63	0.05	0.157	0.002	0.117
Cassava chip	91.90	1.63	2.97	12.30	4.91	2.46	5.07	121.00	0.26	5.35	0.92	0.024	0.002	0.068
Mung bean meal	92.05	18.05	4.66	43.50	29.66	4.45	10.30	6.01	1.45	8.48	0.94	0.006	0.007	0.087
Tomato pomace	94.16	23.69	5.99	50.04	36.62	26.74	6.28	9.33	1.82	7.14	2.44	0.072	0.011	0.227
Soybean hull	91.73	17.99	5.41	49.71	32.22	3.06	11.18	2.83	0.96	10.24	0.80	0.009	0.007	0.149
Peanut hull	96.11	18.48	4.02	27.63	13.05	5.84	6.72	3.94	1.31	6.00	0.18	0.029	0.027	0.281

Gas production technique: The experimental design was randomize completely block (eight replicates per treatment). The treatments included broken rice, paddy rice, corn meal, rice bran, rice pollard, rice bran oil relate, cassava chip, mung bean meal, tomato pomace, soybean hull and peanut hull. Strict anaerobic techniques were used in all steps during the rumen fluid transfer and incubation period. Rumen fluid inoculum was removed before the morning feeding under vacuum pressure via the rumen fistula into a 2 liter glass flask and transferred into two pre-warmed 1 liter thermos flasks which were then transported to the laboratory. The medium preparation was as described by Makkar *et al.* (1995). Mixed rumen fluid inoculums were obtained from two fistulated Brahman-Thai native crossbred steers (weighed 250±15 kg). The animals were offered rice straw *ad libitum* and 0.5% body weight of concentrate. The animals were fed twice daily, water and a mineral lick were available *ad libitum* for 14 days.

The feed sample of approximately 0.5 g on a fresh weight basis was transferred into a 50 mL serum bottle (Sommart *et al.*, 2000). The bottles were pre-warmed in a hot air oven at 39°C for about 1 h prior to injection of 40 mL of rumen fluid medium (using a 60 mL syringe) to each bottle. The bottles were stoppered with rubbers stoppers, crimp sealed and incubated in a hot air oven set at 39°C.

The rate of gas production was measured by reading and recording the amount of gas volume after incubation using a 20 mL glass syringe connected to the incubation bottle with a 23 gauge, 1.5 inch needle. Readings of gas production were recorded from 1 to 96 h (hourly from 1-12 h, every 3 h from 13-24 h, every 6 h from 25-48 h and every 12 h from 49-96 h) after incubation periods. Amount of cumulative gas volume at 2, 4, 6, 12, 24, 48, 72 and 96 h after incubations were fitted using the equation $y = a+b\ [(1-\text{Exp}(-ct)]$ (Ørskov and McDonald, 1979), where a = the intercept, which ideally reflects the fermentation of the soluble fraction, b = the fermentation of the insoluble fraction, c = rate of gas production (a+b) = potential extent of gas production, y = gas production at time 't'.

In vitro digestibility of dry matter and organic matter were measured at 24 and 96 h after incubation. The metabolizable energy will be calculated as ME, MJ kg^{-1} DM = $2.20+(0.136\times Gv)+(0.057\times\%CP)$, Menke *et al.* (1979), where Gv = gas volume at 24 hr, CP = %crude protein in feedstuffs.

Statistical analyses: All data obtained from the trials were subjected to the analysis of variance procedure of statistical analysis system (SAS, 1996) according to a randomize completely block design. Means were separated by Duncan New's Multiple Range Test. The level of significance was determined at $p<0.05$.

Results and Discussion

Chemical composition and mineral content of energy feeds source: The chemical compositions and mineral content of energy feeds are presented in Table 1. Generally, wide variations existed in the chemical composition of the investigated feedstuffs. The crude protein content ranged from 1.63% for cassava chip to 23.69% for tomato pomace. Ash content ranged from 1.63% for corn meal to 12.93% for rice bran oil relate. Neutral detergent fiber content ranged from 12.30% for cassava chip to 52.67% for rice pollard. Acid detergent fiber ranged from 0.74% for broken rice to 38.32% for rice pollard. Acid detergent lignin ranged from 0.58% for broken rice to 26.74% for tomato pomace. There are many factors affecting chemical composition and mineral content of concentrate feedstuffs such as stage of growth maturity, species or variety (Von Keyserlingk *et al.*, 1996; Agbagla-Dohnani *et al.*, 2001; Promkot and Wanapat, 2004), drying method, growth environment (Mupangwa *et al.*, 1997) and soil types (Thu and Preston, 1999). Those factors may partially explain differences in chemical composition between our study and others.

Gas production characteristics of energy feeds: Gas production from the fermentation of energy feeds were measured at 2, 4, 6, 12, 24, 48, 72 and 96 h using *in vitro* gas production technique adapted to describe the kinetics of fermentation based on the modified exponential model $y = a+b\ [(1-\text{Exp}(-c\,t)]$ (Ørskov and

Table 2: Gas production characteristics, gas volume and estimated metabolizable energy of concentrate feedstuff using *in vitro* gas production technique

	Feedstuffs[1]											
Parameters	BR	PR	CM	RB	RP	RBR	CC	MB	TP	SH	PH	SEM
Gas production characteristic parameters[2]												
a (mL)	-9.62^{cd}	-7.33^{bc}	-7.43^{bc}	-7.52^{bc}	0.86^{a}	-0.97^{a}	-13.80^{d}	1.22^{a}	-0.45^{a}	-2.71^{ab}	2.74^{a}	0.64
b (mL)	200.97^{a}	166.90^{bc}	214.00^{a}	113.40^{d}	65.88^{e}	90.32^{de}	182.66^{ab}	149.64^{c}	65.74^{e}	184.91^{ab}	60.34^{e}	5.64
c (%/h)	0.022^{c}	0.022^{c}	0.019^{c}	0.049^{a}	0.035^{b}	0.041^{ab}	0.034^{b}	0.016^{c}	0.049^{a}	0.020^{c}	0.017^{c}	0.00
|*a*|+*b* (mL)	210.66^{a}	174.45^{bc}	221.64^{a}	120.91^{d}	68.96^{e}	93.20^{de}	196.46^{ab}	155.27^{c}	66.69^{e}	189.40^{ab}	63.08^{e}	5.91
In vitro digestibility, %												
IVDMD24	48.87^{ab}	39.38^{ab}	66.40^{a}	59.11^{a}	20.50^{b}	41.65^{ab}	58.50^{a}	35.87^{ab}	45.05^{ab}	40.34^{ab}	5.25^{c}	3.79
IVOMD24	50.24^{ab}	40.97^{ab}	64.55^{a}	59.61^{a}	21.97^{b}	43.68^{ab}	65.98^{a}	36.09^{ab}	32.65^{ab}	41.71^{ab}	7.76^{c}	3.72
IVDMD96	90.36^{a}	66.52^{b}	84.40^{a}	69.69^{b}	32.79^{c}	68.63^{b}	88.55^{a}	69.61^{b}	35.38^{c}	90.42^{a}	28.69^{c}	2.71
IVOMD96	92.74^{a}	69.33^{b}	86.12^{a}	70.49^{b}	34.67^{c}	68.81^{b}	89.58^{a}	71.84^{b}	36.53^{c}	90.94^{a}	32.98^{c}	4.44
Gas volume (mL/0.5gDM)												
GV24	70.05^{b}	53.13^{cd}	60.23^{bcd}	69.13^{b}	34.50^{e}	61.80^{bc}	94.50^{a}	47.50^{d}	24.60^{e}	54.25^{cd}	22.60^{e}	2.12
GV48	132.69^{a}	109.47^{bc}	124.33^{ab}	89.80^{de}	50.60^{f}	83.10^{de}	139.30^{a}	75.40^{e}	30.24^{g}	98.88^{cd}	35.70^{fg}	3.47
GV96	161.85^{a}	131.03^{b}	160.90^{a}	97.30^{d}	60.73^{e}	100.10^{d}	159.90^{a}	114.65^{c}	28.87^{f}	133.00^{b}	51.20^{e}	4.09
ME, Mj/kg DM												
ME[3]	6.42^{bc}	5.37^{de}	5.91^{cd}	6.68^{b}	4.46^{f}	6.59^{bc}	7.42^{a}	5.24^{de}	4.89^{ef}	6.18^{bc}	4.48^{f}	0.11

Note: ^{a,b,c,d,}Means within a row different superscripts differ ($p<0.01$), [1]BR = Broken rice, PR = Paddy rice, CM = Corn meal, RB = Rice bran, RP = Rice pollard, RBR = Rice bran oil relate, CC = Cassava chip, MB = Mung bean meal, TP = Tomato pomace, SH = Soybean hull, PH = Peanut hull, [2]*a* = describe ideally reflects the fermentation of the soluble fraction, *b* = described the fermentation of the insoluble fraction, c = Rates of gas production, |*a*|+*b* = Potential extent of gas production, IVDMD = *in vitro* dry matter digestibility, IVOMD = *in vitro* organic matter digestibility, GV = Gas volume[3]ME (MJ kg^{-1} DM) = 2.20+(0.136xGv)+(0.057x%CP) (Menke *et al.*, 1979)

McDonald, 1979). The gas production characteristics are presented in Table 2. A comparison of gas production characteristics of different treatments indicated significant differences between treatments ($p<0.01$). The *a*, intercept value for all feeds were ranged from-13.80 to 0.86 mL. The intercept (*a*) was lowest in cassava chip, compared to all other energy feed sources. The values for *a*, intercept, were negative in the incubations in this study. These data suggested that a lag phase due to delay in microbial colonization of the substrate may occur in the early stage of incubation. Several authors (Khazaal *et al.*, 1993; Blummel and Becker, 1997) have also reported negative values with various substrates when using mathematical models to fit gas production kinetics. This is due to either a deviation from the exponential cause of fermentation or delays in the onset of fermentation due to the microbial colonization. It is well known that the value for absolute a (|a|), used described ideally reflect the fermentation of the soluble fraction. In this study the absolute gas production was highest for cassava chip. The soluble fraction in cassava chip was also found to be the highest. The findings were similar to those report by Nitipot and Sommart (2003).

The gas volume at asymptote (b) described the fermentation of the insoluble fraction. The fermentation of the insoluble fraction of energy feed sources were significantly different ($p<0.01$). The fermentation of insoluble fractions of broken rice 2) paddy rice 3) corn meal 4) rice bran 5) rice pollard 6) rice bran oil relate 7) cassava chip 8) mung bean meal 9) tomato pomace 10) soybean hull and 11) peanut hull were; 200.97, 166.90, 214.00, 113.40, 65.88, 90.32, 182.66, 149.64, 65.74, 184.91 and 60.34 mL, respectively. It can be seen that gas production at asymptote of rice pollard, tomato pomace and peanut hull were very low when compared to other feeds, possibly a reflection of a high level of lignin (Table 1) (Chumpawadee *et al.*, 2005). Additonally, tomato pomace and peanut hull had high protein content. The gas production is basically the result of the fermentation of carbohydrates into acetate, propionate and butyrate (Gatachew *et al.*, 1998). The protein fermentation does not lead to extensive gas production (Khazaal *et al.*, 1995). The high fermentation of the insoluble fraction were observed in broken rice, corn meal, paddy rice corn meal and cassava chip, possibly influenced by the carbohydrate fractions readily availability to the microbial population. Deaville and Givens (2001) have also reported that kinetics of gas production could be affected by carbohydrate fraction.

The fast rates of gas production (c) were observed in rice bran, rice pollard, rice bran oil relate, cassava chip and tomato pomace, possibly influenced by the soluble carbohydrate fractions readily availability to the microbial population. Slow rate of gas production were observed in broken rice, paddy rice, corn meal, mung bean meal, soybean hull and peanut hull, indicating that those feedstuffs were less readily available to the microbes in the rumen. Nitipot and Sommart (2003) who studied the *in vitro* gas production technique. They found that the rate of gas production of cassava chip was higher than that of corn meal, broken rice and others industrial by product.

Potential extents of gas production (|a|+b) expressed in mL. Corn meal had the highest potential extents of gas production. However, it was insignificant different with broken rice, cassava chip and soybean hull. It imply that these feed were high availability in the rumen. It is possible that corn meal, broken rice, cassava chip and soybean hull also had the lowest NDF content. Thus, corn meal, broken rice, cassava chip and soybean hull are more easily degradable than other energy feed sources. The present study was in agreement with Sommart *et al.* (2000) and Nitipot and Sommart (2003) because energy feed source had lower NDF were show higher potential extent of gas production (Table 1 and Table 2). In the other hand, the potential extent of gas production of rice pollard, tomato pomace and peanut hull were low, it is probably due to the carbohydrate fraction of rice pollard, tomato pomace and peanut hull have a large proportion of lignified cell walls (see also Table 1) with low fermentation and leading to low gas production. The current findings agree with Melaku *et al.* (2003) who found that fibrous constituents, especially lignin negatively influenced *in vitro* gas production.

Gas volume: Cumulative gas volumes at 24, 48 and 96 h after incubation are shown in Table 2. The results indicate that cumulative gas volumes at 24, 48 and 96 h after incubation were significantly different ($p<0.01$). The gas volumes ranked from highest to lowest were: broken rice, corn meal, cassava chip, soybean hull, paddy rice, mung bean meal, rice bran oil relate, rice bran, rice pollard, peanut hull and tomato pomace, respectively. Menke *et al.* (1979) suggested that gas volume at 24 h after incubation is indirect relationship with metabolizable energy in feedstuffs. Sommart *et al.* (2000) suggested that gas volume is a good parameter from which to predict digestibility, fermentation end-product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Additionally, *in vitro* dry matter and organic matter digestibility were shown to have high correlation with gas volume (Sommart *et al.*, 2000; Nitipot and Sommart, 2003). Gas volume has also shown to have a close relationship with feed intake (Blummel and Becker, 1997) and growth rate (Blummel and Ørskov, 1993).

***In vitro* dry mater and organic matter digestibility:** *In vitro* dry mater and organic matter digestibility at 24 and 96 h after incubation are shown in Table 2. It can be seen that *in vitro* dry mater and organic matter digestibility are in the same way. The *in vitro* dry matter and organic matter digestibility at 24 and 96 h after incubation significantly differ among the tested energy feeds ($p<0.01$). High digestibility of dry matter and organic matter at 96 h were observed in broken rice, ground corn, cassava chip and soybean hull, because of the major carbohydrate of their feedstuffs is starch, which is fermented by amylolytic bacteria and protozoa (Kotarski *et al.*, 1992). This result implies that the microbe in the rumen and animal have high nutrient uptake. The higher fiber content (Table 1) of rice pollard, tomato pomace and peanut hull probably resulted in lower *in vitro* dry matter and organic matter digestibility since high NDF and ADL content in feedstuffs result in lower fiber degradation (Van Soest, 1988). In general, the tropical forages and concentrate feedstuffs have a large proportion of lignified cell walls with low fermentation rates and digestibility, leading to low digestibility rates and limited intake (Ibrahim *et al.*, 1995; Hindrichsen *et al.*, 2001).

Estimated Metabolizable Energy (ME): Metabolizable energy predicted by the equation of Menke *et al.* (1979) is as follows ME, MJ/kgDM = $2.20 + (0.136 \times Gv) + (0.057 \times \% CP)$ where Gv = gas volume at 24 h (mL), CP = crude protein in feedstuff (%). The ME value of concentrate feedstuff are shown in Table 2. The present study estimated ME of cassava chip and corn meal were similar with those reports by Nitipot and Sommart (2003). Estimated ME of broken rice was found to be lower than that reported by Nitipot and Sommart (2003). In addition estimated ME of corn meal, broken rice and rice bran was found to be lower than that reported by NRC (2001). Menke and Steingass (1988) reported a strong correlation between ME values measured *in vivo* and predicted from 24 h *in vitro* gas production and chemical composition of feed. The *in vitro* gas production method has also been widely used to evaluate the energy value of several classes of feed (Getachew *et al.*, 1998; Getachew *et al.*, 2002; Aiple *et al.*, 1996). Krishnamoothy *et al.* (1995) also suggested *in vitro* gas production technique should be considered for estimated ME in tropical feedstuffs. Because of evaluated ME by other technique are required labor, cost, time and complexity.

Conclusions: The energy feeds showed a great variation in chemical composition and mineral content. The results of this study demonstrates that kinetics of gas production of energy feed source differed among feed. Based on this study, high ferment abilities for energy feeds use in ruminant ranked from the highest to the lowest were; corn meal, broken rice, cassava chip, soybean hull, paddy rice, mung bean meal, rice bran, rice bran oil relate, rice pollard and tomato pomace, respectively.

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