

Nutritional Evaluation of non Forage High Fibrous Tropical Feeds for Ruminant Using *in vitro* Gas Production Technique

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Abstract: Six non forage high fibrous tropical feeds were used to evaluate nutritive value using the *in vitro* gas production technique. The rumen mixed microbe inoculums were taken from fistulated Brahman-Thai native crossbred steers. The treatments were 1) palm meal (mech-extd), 2) palm meal (solv-extd), 3) leucaena meal (leaf and stem), 4) coconut meal (mech-extd), 5) mung bean meal and 6) dried brewer gain. The treatments were assigned to completely randomized design. The results indicate that soluble gas fraction (a; -7.91, -24.06, -2.11, -28.96, -10.31 and -4.35 mL, respectively), fermentation of insoluble fraction (b; 99.33, 124.06, 70.90, 128.96, 110.31 and 99.33 mL, respectively), rate of gas production (c; 0.054, 0.071, 0.047, 0.122, 0.050 and 0.045 %/h, respectively) and potential of extent of gas production (a+b; 107.26, 148.13, 73.02, 157.93, 120.62 and 103.82 mL, respectively) were significantly different ($P < 0.01$) among treatments. The cumulative gas volume at 24, 48 and 96 h after incubation were significantly different ($P < 0.01$). These results suggest that coconut meal (mech-extd), palm meal (solv-extd), mung bean meal, palm meal (mech-extd) and dried brewer gain are exhibited high fermentability in the rumen.

Key words: *In vitro*, non forage, nutritive value, tropical feeds

Introduction

In the tropical zone have many variety of concentrate feedstuffs. Its can be classify to energy feed sources or basal diet and protein feed sources. There are classifying by crude protein and fibrous content. Some feedstuffs can not classify to both feed sources, because of theirs are low protein and high fibrous content. However, their ingredients are commonly use in ruminant feeding systems. The degree of nutrient degradation occurring in the rumen is major influence on total utilization of nutrient in feedstuffs. Therefore reliable, fast and inexpensive technique is required to quantify both rate and extent of nutrient degradation from difference sources in the rumen. The *in vitro* gas production technique has proved to be a potentially useful technique for feed evaluation (Menke and Steingass, 1988; Blummel and Ørskov, 1993; Herrero *et al.*, 1996; Getachew *et al.*, 2004), as it is capable of measuring rate and extent of nutrient degradation (Groot *et al.*, 1996; Cone *et al.*, 1997). In addition, *in vitro* gas production technique provide less expensive (Getachew *et al.*, 2004), easily to determine (Khazaal *et al.*, 1993) and suitable for use in developing countries (Blummel *et al.*, 1997).

With respect to non forage high fibrous tropical feeds in Thailand, limited information is available on kinetic of degradation. Therefore, the aim of this study was to evaluated nutritive values of non forage high fibrous tropical feeds for ruminant using the *in vitro* gas production technique.

Materials and Methods

Feedstuffs preparation and analysis: The feedstuffs were collected from various feed mill and organizations (Kantharavichai dairy cooperation, Khonkaen dairy cooperation, Maha Sarakham University feed mill, Khon Kaen University feed mill, Numhenghoad feed suppliers, Chareon Esan commercial feed mill, Songserm Kankaset feed supplier) in the North East of Thailand. All test feed samples (Table 1) were ground to pass through a 1 mm screen for *in vitro* incubation and chemical analysis. The samples were analyzed to determine dry matter (DM), crude protein (CP) and ash content (AOAC, 1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were assayed using the method proposed by Van Soest *et al.* (1991).

Experimental design: The experimental design was completely randomized (four replicates per treatment). The treatments included palm meal (mech-extd), palm meal (solv-extd), leucaena meal (leaf and stem), coconut meal (mech-extd), mung bean meal and dried brewer gain. Strict anaerobic techniques were used in all steps during the rumen fluid transfer and incubation period. Rumen fluid inoculums 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 Sommart *et al.* (2000). Mixed

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Table 1: Chemical composition of various high fibrous feed sources

Feed stuffs ¹	DM (%)	CP	Ash	NDF	ADF	ADL
				% DM basis-----		
PME	92.58±0.39	11.31±0.17	3.58±0.07	82.47±0.41	57.23±0.20	20.32±0.61
PMS	91.04±0.02	16.68±0.08	6.94±0.04	82.70±1.70	51.41±0.88	9.09±0.02
LM	90.88±0.03	10.28±0.09	9.86±0.01	58.01±1.04	50.79±0.51	17.00±0.62
CME	92.18±0.05	10.93±0.32	3.32±0.13	67.30±0.67	42.69±1.05	8.06±1.19
MM	90.65±0.01	18.46±0.78	5.83±0.01	32.26±0.07	29.53±0.53	3.31±0.04
DBG	90.28±0.05	19.56±0.45	6.18±0.11	74.65±0.59	29.12±0.08	5.06±1.58

DM = dry matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber and ADL = acid detergent lignin.

¹PME = palm meal (mech-extd), PMS = palm meal (solv-extd), LM = leucaena meal, CME = coconut meal (mech-extd), MM = mung bean meal and DBG = dried brewer gain

rumen fluid inoculums were obtained from two fistulated Brahman-Thai native crossbred steers (weighing about 250±15 kg). The animals were offered rice straw *ad libitum* and fed 0.5 % body weight of concentrate (concentrate mixture: 49.80% cassava chip, 17.5% rice bran, 14.60% palm meal, 7.0% soybean meal, 1.40% urea, 0.4% salt, 1.0% mineral mix and 8.30% sugarcane molasses). The animals were fed twice daily, water and a mineral lick was 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 hour 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'.

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 completely randomized 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 of non forage high fibrous tropical feeds source: Chemical compositions of the six

non forage high fibrous tropical feeds are presented in Table 1. Generally, wide variations existed in the chemical composition of the investigated feedstuffs. The crude protein content of palm meal (mech-extd) was lower than that reported by Carvalho *et al.* (2005). It was similar to reports by Department of Livestock Development (2004) and Promkot and Wanapat (2004). The neutral detergent fiber and acid detergent fiber content higher than that reported by Department of Livestock Development (2004); Promkot and Wanapat (2004) and Carvalho *et al.* (2005). It was similar to reports by Shannak *et al.* (2000). The acid detergent fiber content was in close agreement with report by Department of Livestock Development (2004), but higher than that reported by Carvalho *et al.* (2005).

The crude protein content of palm meal (solv-extd) was similar to reports by Hindel *et al.* (1995); NRC (2001); Woods *et al.* (2003); Department of Livestock Development (2004) and Cavalho *et al.* (2005). However, the neutral detergent fiber and acid detergent fiber content were higher than that reported by Department of Livestock Development (2004) and Cavalho *et al.* (2005), but similar to reports by Hindel *et al.* (1995) and Woods *et al.* (2003). The acid detergent lignin content of palm meal (solv-extd) was lower than that reports by Hindel *et al.* (1995); Woods *et al.* (2003) and Cavalho *et al.* (2005). The crude protein content of leucaena meal was lower than that reported by Department of Livestock Development (2004), but neutral detergent fiber, acid detergent fiber and acid detergent lignin content were higher than that reported by Department of Livestock Development (2004). The difference of chemical composition probably due to maturity and leaves-stem ratio of leucaena.

The crude protein content of dried brewer gain was lower than those reported by Batajoo and Shaver (1998); NRC (2001). The neutral detergent fiber content was higher than that reported by Pereira *et al.* (1998) and Batajoo and Shaver (1998). The acid detergent fiber content was higher than that reported by Pereira *et al.* (1998) and NRC (2001).

The results indicate that dried brewer gain has highest crude protein content as compared to other non forage

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Table 2: Gas production characteristics and gas volume of high fibrous tropical feed sources using *in vitro* gas production technique

Parameters	Treatment ¹						SEM
	PME	PMS	LM	CME	MM	DBG	
Gas production characteristic parameters ²							
a, mL	-7.9 ^{bc}	-24.06 ^d	-2.11 ^a	-28.96 ^e	-10.31 ^c	-4.35 ^{ab}	2.15
b, mL	99.33 ^b	124.06 ^a	70.90 ^c	128.96 ^a	110.31 ^b	99.33 ^b	4.31
c, %/h	0.054 ^c	0.071 ^b	0.047 ^c	0.122 ^a	0.050 ^c	0.045 ^c	0.01
a+b, mL	107.26 ^b	148.13 ^a	73.02 ^c	157.93 ^a	120.62 ^b	103.82 ^b	6.27
Gas production (ml/0.5g)							
24 h	69.50 ^{bc}	84.00 ^{ab}	45.50 ^d	101.75 ^a	62.87 ^{cd}	60.37 ^{cd}	4.35
48 h	83.87 ^c	132.5 ^a	59.62 ^d	116.00 ^b	96.75 ^c	81.75 ^c	5.29
96 h	90.75 ^c	149.12 ^a	68.75 ^d	121.87 ^b	122.75 ^b	100.12 ^c	5.65

a, b, c, d Means within a row different superscripts differ (P<0.01). ¹PME = palm meal (mech-extd), PMS = palm meal (solv-extd), LM = leucaena meal, CME = coconut meal (mech-extd), MM = mung bean meal and DBG = dried brewer gain. ²a = the intercept (mL), which ideally reflects the fermentation of the soluble fraction, b = the fermentation of the insoluble fraction (asymptote) (mL), c = rate of gas production (%/h), a+b = potential extent of gas production (mL)

high fibrous tropical feeds source. Leucaena meal (leaf and stem) was shown to have the lowest crude protein content. Palm meal (mech-extd) showed highest neutral acid detergent fiber content as compared to other high fibrous tropical feeds source. Mung bean meal showed lowest neutral detergent fiber, acid detergent fiber and acid detergent lignin content. Many factors affect chemical composition such as oil extraction process (Mara et al., 1999) stage of growth (Promkot and Wanapat, 2004) maturity and species or variety (von Keyserlingk et al., 1996 and Agbagla-Dohnani et al., 2001), drying method and growth environment (Mupangwa et al., 1997) and soil types (Thu and Preston, 1999). These factors may partially explain differences in chemical composition between our study and others.

Gas production characteristics: Gas production from the fermentation of energy feed source was measured at 2, 4, 6, 12, 24, 48, 72 and 96 h *in vitro* using gas tests adapted to describe the kinetics of fermentation base on the modified exponential model $y = a + b [(1 - \text{Exp}(-ct))]$ (Ørskov and McDonald, 1979). Although there are other models available to describe the kinetics of gas production, the Ørskov and McDonald (1979) model was chosen because the relationship of its parameters with intake, digestibility and degradation characteristic of forages and concentrate feedstuffs had been documented (Blummel and Ørskov, 1993; Khazaal et al., 1993; Sommart et al., 2000; Nitipot and Sommart, 2003). Gas production characteristics are presented in Table 2. A comparison of gas production characteristics of different treatments indicated significant differences between treatment (P<0.01). The a, intercept value for all feeds were ranged from -28.96 to -2.11 mL. Coconut meal (mech-extd) had the lowest value for a, intercept, while leucaena meal had the highest a, intercept value.

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 coconut meal (mech-extd). The soluble fraction in coconut meal (mech-extd) was also found to be the highest. The soluble fraction makes it easily attachable by ruminal microorganisms and leads to much gas production (Table 2).

The gas volumes at asymptote (b) described the fermentation of the insoluble fraction. The gas volumes at asymptote of high fibrous tropical feed ranked from highest to lowest were; coconut meal (mech-extd), palm meal (solv-extd), mung bean meal, palm meal (mech-extd), dried brewer gain and leucaena meal. The gas volumes at asymptote have the advantage for predict feed intake. Blummel and Ørskov (1993) found that the gas volume at asymptote could account for 88% of variance in intake. In addition the gas volume at asymptote values for the NDF fraction were highly correlation (r=0.98) with NDF degradability of corn silage (Deville and Givens, 2001).

Rate of gas production expressed in %/h as ranked from the fastest to the slowest were; coconut meal (mech-extd), palm meal (solv-extd), palm meal (mech-extd), mung bean meal, dried brewer gain and leucaena meal. High rates of gas production were observed in coconut meal (mech-extd), possibly influenced by the carbohydrate fractions readily availability to the microbial

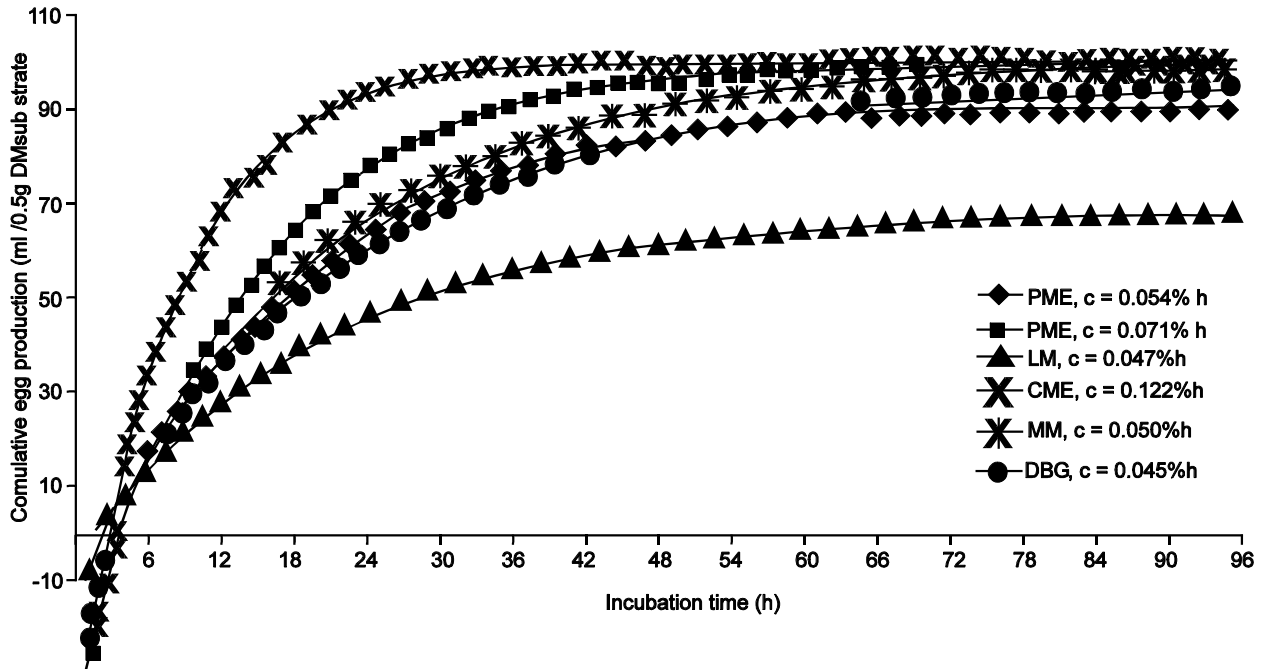


Fig. 1: Cumulative gas volume estimated by $y = a + b [(1 - \text{Exp}(-ct))]$ (mL/0.5 gDM Substrate) throughout 96 h. (PME = palm meal (mech-extd), PMS = palm meal (solv-extd), LM = leucaena meal, CME = coconut meal (mech-extd), MM= mung bean meal and DBG = dried brewer gain).

population. Deaville and Givens (2001) have also reported that carbohydrate fraction could be affected to kinetics of gas production.

Potential extent of gas production (a+b) expressed in mL as ranked from highest to the lowest were: coconut meal (mech-extd), palm meal (solv-extd), mung bean meal, palm meal (mech-extd), dried brewer gain and leucaena meal. Generally, the potential of gas production for non forage high fibrous tropical feed was high, because of their feed are abundant of carbohydrate fraction (particularly NDF). It is well known that gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Getachew *et al.*, 1998). Whereas, protein fermentation does not lead to much gas production (Khazaal *et al.*, 1995). High potential extent of gas production was observed in coconut meal (mech-extd), palm meal (solv-extd), palm meal (mech-extd), mung bean meal and dried brewer gain. It imply that their feed were high availability in the rumen. The current findings agree with *in situ* studies on non forage high fibrous tropical feed (Chumpawadee, data unpublished). However the potential extent of gas production of leucaena was low, it possibly due to the carbohydrate fraction of leucaena have a large proportion of lignified cell walls (see also Table 1) with low fermentation and leading to low gas production.

Gas volume: The cumulative gas volume at 24, 48 and 96 h after incubation are shown in Table 2. The results

indicate that cumulative gas volume at 24, 48 and 96 h after incubation were significantly different ($P < 0.01$) between treatment. Curve of cumulative gas production for each treatment are presented in Fig. 1. It can be seen that gas production reached a plateau after 48 h fermentation, exception leucaena meal. The reason for that, it possibly the carbohydrate fraction of leucaena have a large proportion of lignified cell walls and leading to difficulty attach by microorganisms. Cumulative gas volume at each sampling time was affected by variety of feedstuffs. These finding indicate that fractional of substrate and degradability of non forage high fibrous tropical feed are difference. The gas produced is directly proportional to the rate at which substrate degraded (Dhanoa *et al.*, 2000). Additionally, kinetics of gas production is dependent on the relative proportions of soluble, insoluble but degraded, and undegradable particles of the feed (Getachew *et al.*, 1998). Menke *et al.* (1979) suggested that gas volume at 24 h after incubation has been relationship with metabolizable energy in feedstuffs. Sommart *et al.* (2000) reported 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 volumes also have shown a close relationship with feed intake (Blummel

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and Becker, 1997) and growth rate in cattle (Blummel and Orskov, 1993).

Conclusions: The non forage high fibrous topical feeds showed a great variation in chemical composition. The results of this study demonstrate that kinetics of gas production of non forage high fibrous topical feed differed among feeds. Based on this study, high ferment ability of non forage high fibrous topical feed ranked from the highest to the lowest were; coconut meal (mech-extd), palm meal (solv-extd), mung bean meal, palm meal (mech-extd), dried brewer gain and leucaena meal.

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