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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 feedstuffs were used to evaluate their nutritive value using an *in vitro* gas production technique. The rumen mixed microbe inoculums were taken from 2 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 brewers grain. The treatments were assigned to a completely randomized design. The results indicated that the soluble gas fraction (*a*; -7.91, -24.06, -2.11, -28.96, -10.31 and -4.35 ml, respectively), fermentation of the 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 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 suggested that coconut meal (mech-extd), palm meal (solv-extd), mung bean meal, palm meal (mech-extd) and dried brewer grain exhibit high fermentability in the rumen while luecaena meal has the lowest value.

Key words: *In vitro* - Non forage - Nutritive value

INTRODUCTION

In the tropical zone, there are many varieties of concentrate feedstuffs. They can be classified as energy feed or protein feed by their crude protein and fiber content. Some feedstuffs can not be classified as either feed sources because of their low protein and high fiber content. However, these ingredients are commonly used in ruminant feeding systems. The degree of nutrient degradation in the rumen has a major influence on the total utilization of nutrient in feedstuffs. Therefore reliable, fast and inexpensive techniques are 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 (1,2,3), as it is can be uses to measure the rate and extent of nutrient degradation (4,5). In addition, the *in vitro* gas production technique is less expensive (3), provides easy determination (6) and is suitable for use in developing countries (7).

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

MATERIALS AND METHODS

Feedstuffs Preparation and Analysis

The feedstuffs were collected from various feed mills and organizations (Kantharavichai dairy cooperatives, Khonkaen dairy cooperatives, Maha Sarakham University feed mill, Khon Kaen University feed mill, Numhenghoad feed supplier, 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 (8). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) values were determined using the method proposed by Van Soest et al (9).

Experimental Design

The experimental design was completely randomized with 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 brewers grain. Strict anaerobic techniques were used in all steps during the rumen fluid transfer and the incubation period. Rumen fluid inoculums were 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 was preparation as described by Sommart et al (10). Mixed rumen fluid inoculums were obtained from two fistulated Brahman-Thai native crossbred steers (weighing about 250$\pm$15 kg). The animals were offered rice straw *ad libitum* and fed 0.5% body weight of concentrate (concentrate mixture: 49.8% cassava chip, 17.5% rice bran, 14.6% palm meal, 7.0% soybean meal, 1.4% urea, 0.4% salt, 1.0 % mineral mix and 8.3% sugarcane molasses). They were fed twice daily, water and a mineral lick was available *ad libitum* for 14 days.

A feed sample of approximately 0.5 g on a fresh weight basis was transferred into a 50 ml serum bottle (10). The bottles were pre-warmed in a hot air oven at 39°C for about 1 h prior to the injection of 40 ml of the rumen fluid medium (using a 60 ml syringe) to each bottle. The bottles were stoppered with rubber 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. Amounts of cumulative gas at 2, 4, 6, 12, 24, 48, 72 and 96 h were fitted using the equation $y = a+b\ [(1-\text{Exp}(-ct)]$ (11), 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 (12) according to a completely randomized design. Means were separated by Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

Chemical Composition of Non Forage High Fibrous Tropical Feed 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 CP content of palm meal (mech-extd) was lower than that reported by Carvalho et al (13) but similar to those of the Department of Livestock Development (DLD) (14), and Promkot and Wannapat (15). The NDF was higher than these 3 reports (13,14,15) while ADF was in close agreement with that reported by DLD (14), but higher than Carvalho et al (13).

The CP content of palm meal (solv-extd) was similar to that reported by Hindel et al (16), NRC (17), Woods et al (18), DLD (14) and Carvalho et al (13). However, the NDF and acid detergent fiber content were higher than that reported by DLD (14) and Carvalho et al (13), but similar to Hindel et al (16), Woods et al (18). The acid detergent lignin content of palm meal (solv-extd) was lower than the values reported by Hindel et al (16), Woods et al (18) and Carvalho et al (13).

The CP content of leucaena meal was lower than that reported by DLD but NDF, ADF and ADL were higher (14). The difference of chemical composition is probably due to the maturity and leaves-stem ratio of leucaena.

The CP content of dried brewers grain was lower than those reported by Batajoo and Shaver (19) and NRC (17). The NDF content was higher than that recorded by Batajoo and Shaver (19). The ADF content was higher than that reported by NRC (17).

The results indicated that dried brewers grain has the highest CP content while leucaena meal (leaf and stem) had the lowest CP content among the feedstuffs. Palm meal (mech-extd) showed the highest NDF while mung bean meal showed the lowest NDF, ADF and ADL content. There are many factors that affect chemical composition such as the oil extraction process (20) stage of growth (15) species or variety (21), drying method, growth environment (22) and soil types (23). These factors may partially explain the differences in chemical composition between our study and others.

Gas Production Characteristics

Gas production from the fermentation of non forage high fibrous tropical feeds were measured at 2, 4, 6, 12, 24, 48, 72 and 96 h *in vitro* using adapted gas tests to describe the kinetics of fermentation based on the modified exponential model $y = a+b\ [(1\text{-Exp}(-ct)]$ (11). Although there are other models available to describe the kinetics of gas production, the Ørskov and McDonald (11) model was chosen because the relationship of its parameters with intake, digestibility and degradation characteristic of forages and concentrate feedstuffs has been well documented (2,6,10,24).

Gas production characteristics are presented in **Table 2** and **Figure 1**. A comparison of gas production characteristics of the 6 feedstuff treatments indicated significant differences among treatments ($P < 0.01$). The intercept value, a, for all feeds were negative in this study and ranged from -28.96 to -2.11 ml, being lowest for coconut meal (mech-extd) and highest for leucaena meal. The data suggest a lag phase due to the delay in microbial colonization of the substrates during the early stage of incubation. Several authors (6,25) have also reported negative values for various substrates when using mathematical models to fit gas production kinetics. It is well known that the value for absolute a ($|a|$), can be used to describe the ideal fermentation of the soluble fraction. In this study the absolute gas production was highest for coconut meal (mech-extd). The soluble fraction makes it easily attachable by ruminal microorganisms and leads to greater gas production **(Table 2)**.

The gas volumes at asymptote (b) describe the fermentation of the insoluble fraction. The b value ranked from highest to lowest were; coconut meal (mech-extd), palm meal (solv-extd), mung bean meal, palm meal (mech-extd), dried brewers grain and leucaena meal. The gas volume at asymptote has the advantage for the prediction of feed intake since it accounts for 88% of variance in intake (2). In addition, the gas volume at asymptote values for the NDF fraction were highly correlated ($r = 0.98$) with NDF degradability of corn silage (26).

Rate of gas production (c) 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 grain and leucaena meal. High rates of gas production were observed in coconut meal (mech-extd), possibly influenced by the soluble carbohydrate fractions readily available to ruminal microbes. Deaville and Givens (26) also reported that the carbohydrate fraction could affect the kinetics of gas production.

Potential extent of gas production ($a+b$) expressed in ml as ranked from the highest to the lowest were: coconut meal (mech-extd), palm meal (solv-extd), mung bean meal, palm meal (mech-extd), dried brewers grain and leucaena meal. Generally, the potential of gas production for non forage high fibrous tropical feed was high, because of the high carbohydrate fraction (particularly NDF). It is well known that gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (27). Whereas, protein fermentation does not lead to much gas production (28). 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 brewers grain. The current findings agree with *in situ* studies with the same lot of feed by Chumpawadee (unpublished data). However the potential extent of gas production of leucaena was low, this may be because the carbohydrate fraction of leucaena is mainly composed of lignified cell walls (see also **Table 1**).

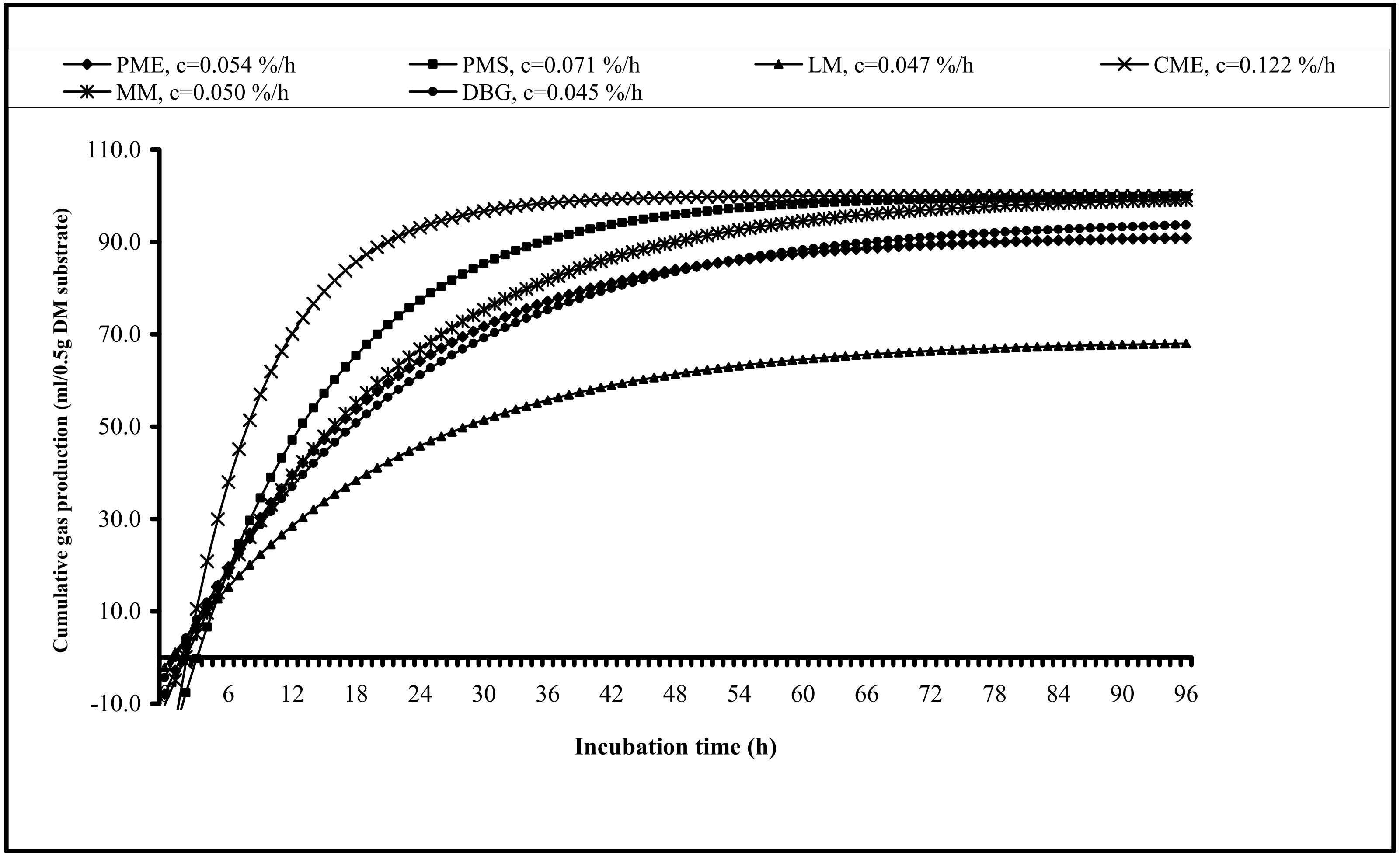

Figure 1. Cumulative gas volume estimated by y = a+b [(1-Exp(-ct)] (ml/0.5 g DM substrate) throughout 96 h. (PME = palm meal (mech-extd), PMS = palm meal (solv-extd), LM = leucaena meal, CME = coconut meal (mech-extd), MM = mung bean meal and DBG = dried brewer grain).

Table 1. Chemical composition of various high fibrous feed sources (Means±SD).

Feedstuffs[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
[1]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 brewers grain

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							
a, ml	-7.91^{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 ($P < 0.01$), [1]PME, PMS, LM, CME, MM, DBG = see table 1, [2]*a* = the intercept (ml), which ideally reflects the fermentation of the soluble fraction, *b* = the fermentation of the insoluble fraction (asymptote) (m), *c* = rate of gas production (%/h), *a*+*b* = potential extent of gas production (ml)

Gas Volume

The cumulative gas volume at 24, 48 and 96 h after incubation are shown in **Table 2** and **Figure 1**. They were significantly different ($P < 0.01$) among treatments. It can be seen that gas production reached a plateau after 48 h of fermentation, except leucaena meal. The reason might be due to the high content of lignified cell walls in leucaena which led to attachment difficulties by microorganisms. Cumulative gas volume at each sampling time was affected by a variety of feedstuffs. This finding indicated that the degradability of non forage high fibrous tropical feeds are different. The gas production is directly proportional to the rate at which substrate being degraded (29). Additionally, kinetics of gas production is dependent on the relative proportions of soluble, insoluble but degradable, and undegradable particles of the feed (27). Menke et al (30) suggested that the gas volume after 24 h of incubation has a relationship with metabolizable energy in feedstuffs. Sommart et al (10) reported that gas volume is a good parameter 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 (10,24). Gas volumes also have a close relationship with feed intake (25) and growth rate in cattle (2).

CONCLUSIONS

The non forage high fibrous tropical feeds showed a great variation in chemical composition. The results of this study demonstrated that kinetics of gas production of non forage high fibrous tropical feed differ among feeds. Based on this study, fermentability ranked from the highest to the lowest were: coconut meal (mech-extd), palm meal (solv-extd), mung bean meal, palm meal (mech-extd), dried brewers grain and leucaena meal.

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บทคัดย่อ

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การประเมินคุณค่าทางโภชนะของแหล่งอาหารที่มีเยื่อใยสูงแต่ไม่ใช่พืชอาหารสัตว์โดยใช้วิธีการวัดปริมาตรแก๊ส

การศึกษาองค์ประกอบทางเคมีและประเมินคุณค่าทางโภชนะอาหารสัตว์เคี้ยวเอื้องโดยวิธีวัดปริมาตรแก๊สของอาหารเยื่อใยที่ไม่ใช่พืชอาหารสัตว์ โดยแหล่งจุลินทรีย์ที่ใช้ในการทดลองนำมาจากของเหลวในกระเพาะหมักของโคเนื้อลูกผสมบราห์มันพื้นเมืองเพศผู้ตอน วางแผนการทดลองแบบสุ่มสมบูรณ์ โดยมีวัตถุดิบอาหารที่ทำการศึกษา 6 ชนิดคือ 1) กากปาล์มหีบน้ำมัน 2) กากปาล์มสกัดน้ำมัน 3) ใบและกิ่งกระถินบด 4) กากมะพร้าวหีบน้ำมัน 5) กากถั่วเขียว และ 6) กากเบียร์แห้ง ผลการทดลอง พบว่า ค่าจุดตัดแกน y (*a*) มีค่าเท่ากับ -7.91 -24.06 -2.11 -28.96 -10.31 และ -4.35 มิลลิลิตร ตามลำดับ (P < 0.01) ค่าปริมาตรแก๊ส ณ จุดที่เส้นกราฟราบเรียบ (*b*) มีค่าเท่ากับ 99.33 124.06 70.90 128.96 110.31 และ 99.33 มิลลิลิตร ตามลำดับ (P < 0.01) อัตราการผลิตแก๊ส (*c*) มีค่าเท่ากับ 0.054 0.071 0.047 0.122 0.050 และ 0.045 เปอร์เซ็นต์ต่อชั่วโมง ตามลำดับ (P < 0.01) ค่าศักยภาพในการผลิตแก๊ส (*a+b*) มีค่าเท่ากับ 107.26 148.13 73.02 157.93 120.62 และ 103.82 มิลลิลิตร ตามลำดับ (P < 0.01) ผลการทดลองนี้แสดงให้เห็นว่ากากมะพร้าวหีบน้ำมัน กากปาล์มสกัดน้ำมัน กากถั่วเขียว และกากเบียร์แห้ง สามารถย่อยได้สูงในกระเพาะหมัก ส่วนกระถินบดมีการย่อยได้ต่ำที่สุด

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