THE NATIONAL RESEARCH COUNCIL OF THAILAND
RESEARCH LIBRARY

CHAPTER III

EXPERIMENT I: NUTRITIVE EVALUATION OF SOME TROPICAL FEEDSTUFFS

3.1 EXPERIMENT 1.1: NUTRITIVE EVALUATION OF SOME TROPICAL FORAGE GRASS AND ALTERNATIVE FORAGE FEEDSTUFFS

3.1.1 Introduction

Forage grass is a major roughage source for ruminant livestock such as beef and dairy cattle in Thailand. There are many tropical forage grasses including natural pastures e.g. Communist grass (*Pennisetum pedicellatum*), Large crab grass (*Digitaria adscendens*), Crowfoot grass (*Dactyloctenium aegyptium* (L.) Wild) (WTSR, 2008), and cultivated forages e.g. Purple guinea grass (*Panicum maximum* TD58), Dwarf napier grass *(Pennisetum purpureum* cv. Mott), King grass *(Pennisetum purpureum* cv. Kinggrass) and Pangola grass *(Digitaria eriantha)*, although those natural pastures and cultivated forages are available only in the rainy season. But ruminant livestock farmers are facing a critical feed supply shortage in both quantity and quality in the dry season, leading to the search for alternative feed resources such as sugarcane (*Saccharum officinarum*) and sweet sorghum *(Sorghum bicolor)*.

Sugarcane is locally available in the dry season and inexpensive. The high biomass and drought tolerance of sugarcane make it a vailable option for ruminant feed (Sommart et al., 2005a,b). The nutritive value of grasses generally declines with maturity, while sugar cane cells have their highest content at maturity (Van et al., 2002). Another example, sorghum is showing potential and attracting interest as a forage crop for ruminants in many regions of the world such as India (Blummel et al., 2003), Israel (Miron et al., 2007) and Argentina (Di Marco et al., 2009). Sweet sorghum has high resistance to drought, making it a suitable forage crop for semi-arid areas, especially in light of its higher productivity under limited irrigation conditions (Yosef et al., 2009). Although these two forages are not considered as conventional forage, in terms of their potential properties, sugarcane and sweet sorghum are considered high potential alternative roughage sources for beef and dairy cattle in the dry season.

Chemical composition, nutritive value, digestibility and energy content of the above grasses and alternative forages are essential data for farmers to use for diet formulation and feeding management. Chemical composition such as proximate analysis (AOAC, 1990) and fiber (Van Soest, 1991) are routinely determined in many laboratories and available from many sources such as the WTSR (2008) tables. However, there is meagre digestibility and very limited energy content information, especially metabolizable energy (ME).

Determining digestibility and energy content require considerable resources of time, labor, and money. Evaluation of feedstuffs for the whole tract and ruminal digestion through feeding experiments is expensive and requires sophisticated laboratory and animal facilities (Krishnamoorthy et al., 1995). *In vitro* gas production technique has proved to be a potentially useful rapid technique for feed evaluation, as it is capable of measuring rate and extent of nutrient degradation. In addition, it is less expensive, easy to determine, and suitable for use in developing countries (Chumpawadee et al., 2006a). Moreover, this technique could be used to determine ME content by prediction equation using chemical composition and gas production (Menke et al., 1979; Menke and Steingass, 1988). It is a useful technique for screening tests to search for potential feedstuffs for ruminants.

The objectives of this experiment, therefore, were to determine chemical composition, nutritive values and to compare digestibility, kinetics of gas production and metabolizable energy of selected forage grasses and alternative forages using *in vitro gas* production technique.

3.1.2 Materials and Methods

3.1.2.1 Experimental design and treatment

The experiment was arranged into randomized complete block design. There were 4 replicates for kinetics of gas production determination, 8 replicates for *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) evaluation and 4 replicates for *in vitro* neutral detergent fiber digestibility (IVNDFD) evaluation.

Twelve forage grass feedstuffs in the experiment were collected from farmers by Laboratory of Khon Kaen Animal Nutrition Research and Development Center, Khon Kaen, Thailand. They were Atratum grass (*Paspalum atratum)*, Purple

guinea grass (*Panicum maximum* TD58), Para grass *(Brachiaria mutica)*, Dwarf napier grass *(Pennisetum purpureum* cv. Mott), King grass *(Pennisetum purpureum* cv. Kinggrass), Pangola grass *(Digitaria eriantha)* (2 samples), Plicatulum grass *(Paspalum plicatulum)*, Ruzi grass *(Brachiaria ruziziensis)* (2 samples), Rhodes grass *(Chloris gayana),* and Creeping signal grass *(Brachiaria humidicola)*. These feedstuff treatments were categorized as forage grasses according to their botanical family (Kellems and Church, 2002). Two alternative forages, the 1 year cutting age whole sugarcane *(Saccharum officinarum)* and 84 day cutting interval (dci) sweet sorghum *(Sorghum bicolor)*, were collected from Nampong district, Khon Kaen Province and Experimental Station, Faculty of Agriculture, Khon Kaen University, respectively.

3.1.2.2 Animals

Three male crossbred Holstein Friesians of body weight approximately 200 kg were used as the source of rumen inoculum. The animals were maintained on dried chopped sweet sorghum *(Sorghum bicolor)* as a roughage source at a Department of Animal Science's experimental station, Faculty of Agriculture, Khon Kaen University. Rumen fluid was removed under vacuum pressure via stomach tube into a 2 liter suction flask and transferred into two pre-warmed 1.8 liter vacuum flasks which were then transported to the laboratory for preparation of rumen inoculum.

3.1.2.3 Feedstuff samples and chemical analysis

All test feedstuff samples were dried at 60˚C in hot air oven for 48 h and ground through a 1 mm screen for chemical analysis and *in vitro* gas production technique. The feedstuff 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 determined using the method proposed by Van Soest et al. (1991).

Feed samples of 500 mg (fresh weight basis) were transferred into 50 ml serum bottles (Sommart et al., 2000). The bottles were stoppered with rubber stopper, crimp sealed and incubated in a hot air oven with water bath set at 39˚C. Bottles were pre-warmed for 1 hour at 39˚C and were injected with 40 ml of rumen inoculum prepared as following Sommart et al. (2000).

3.1.2.4 Data collections

1) Gas volume (Gv)

The rate of gas production was measured by reading and recording the 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. Cumulative gas volumes after incubations were fitted using the equation $y = a + b\ (1\text{-}e^{-ct})$ (Ørskov and McDonald, 1979), where y = gas production at time 't', a = the intercept, which ideally reflects the fermentation of the soluble fraction, b= the fermentation of the insoluble fraction, c = rate of gas production, and ($|a|+b$) = potential extent of gas production.

2) Digestibility

After 24 hour of incubation, a 12 bottle sample of each treatment was taken and kept in a freezer to inhibit microbial activity. Subsequently, 8 bottles of each treatment were subjected to *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) determination and 4 bottles were subjected to neutral detergent fiber digestibility (IVNDFD) determination.

To determine the IVDMD, residues of incubated samples in bottles were filtered through a groose crucible and dried at 105˚C in a hot air oven for DM determination. Thereafter, the DM residues were subjected to incineration in a muffle furnace at 550˚C for ash determination in order to evaluate the IVOMD. To determine the IVNDFD, residues of incubated samples in bottles were filtered through a groose crucible. Then, the residues were analyzed for NDF and lastly for ash. The obtained residue DM, ash and NDF data were used for calculation of digestibility according to the following equations:

$$\text{IVDMD (\%)} = \frac{[\text{DM initial incubation (g)} - \text{DM after incubation (g)}] \times 100}{\text{DM initial incubation (g)}}$$

$$\text{IVOMD (\%)} = \frac{[\text{OM initial incubation (g)} - \text{OM after incubation (g)}] \times 100}{\text{OM initial incubation (g)}}$$

$$\text{IVNDFD (\%)} = \frac{[\text{NDF initial incubation (g)} - \text{NDF after incubation (g)}] \times 100}{\text{NDF initial incubation (g)}}$$

3) Estimation of metabolizable energy (ME)

Estimation of ME content was calculated following the equations of Menke et al. (1979); ME (MJ/kg DM) = 2.20+(0.136×Gv)+(0.057×CP) (Eq1) and Menke and Steingass (1988); ME (MJ/kg DM) = 2.20+(0.1357×Gv) + (0.0057×CP) + (0.0002859×EE^2) (Eq2) by using gas volume produced at 24 h of incubation (Gv, ml), crude protein (CP, %) and ether extract (EE, %) as factors of prediction.

Since gas production volume, in this study, was collected from incubation of a 0.5 g of DM, the gas volume was adjusted to be equal to 0.2 g of DM for ME calculation in the above equations. The equation for adjusting is shown as follows:

Gv produced from 0.2 g DM = (0.2× Gv produced from 0.5 g DM)/0.5

3.1.2.5 Statistical analysis

All data obtained from the trials were subjected to the analysis of variance procedure of statistical analysis system (SAS, 1996) according to a Randomized Complete Block Design (RCBD). Means were compared by using the LSMEANS and STDERR statement in PROC GLM. Mean separation were determined using the PDIFF statement in PROC GLM with a $P < 0.05$ significance level. The original model includes the treatment and block effects as follows:

$$\mathbf{Yij} = \mu + \tau i + \beta j + \varepsilon ij$$

Where, **Yij** = observation value in feedstuff sample i, batch of injected rumen inoculum j

μ = overall mean

τi = effect of feedstuff sample i when i = 1, 2,.., 14

βj = effect of batch of injected rumen inoculum j when j = 1, 2, 3 and 4

εij = residual error

3.1.3 Results and Discussion

3.1.3.1 Chemical composition

Chemical composition of selected grass and alternative forages are showed in Table 3.1.1. The CP content of 30 day cutting interval (dci) Creeping signal grass *(Brachiaria humidicola)* (13.73 %) was highest in the grass group. It was higher than CP content of the same cutting age Creeping signal grass in WTSR (2008) table (8.40 %). Also, it was higher than low quality signal grass (84 dci) in the study of Sturm et al. (2007) and higher than 100 dci fertilized Signal grass in report of Nogueira Fiho et al. (2000) (4.3 and 7.4 %, respectively). The CP content of 30 dci Dwarf napier grass *(Pennisetum purpureum* cv. Mott) and King grass *(Pennisetum purpureum* cv. Kinggrass) in this study were similar to the 100 dci *Pennisetum purpureum* cv. Napier (9.6 %) in the investigation of Nogueira Filo et al. (2000).

In this study, there were two samples of Pangola grass. The CP content of Pangola grass 2 *(Digitaria eriantha)* (4.32 %) was lowest among selected forage grasses while the other, 45 dci Pangola grass 1 *(Digitaria eriantha)*, had superior CP content (10.47%). Pangola grass 2 *(Digitaria eriantha)* in this study was considered a low quality Pangola cultivar similar to villages grazing site Pangola grass in South Africa where CP content ranged from 2.9 to 5.2 % (Matlebyane et al., 2009). Suzuki et al. (2008a) found that CP content of Pangola grass was 9.5 %. WTSR (2008) reported that CP content of 45 dci Pangola grass varied from 9.5 to 10.5 %. Moreover, data in WTSR (2008) table indicated that the CP content of Pangola grass decreased with longer cutting interval. Based on this information, it is indicated that to keep the quality of forage grasses high, a suitable cutting interval needs to be considered.

Table 3.1.1 Chemical composition of forage grasses and alternative forages

Feedstuff	Cutting Age, d	Chemical composition						
		DM[1],%	CP	EE	Ash	NDF	ADF	ADL
			% of DM basis					
Forage grasses								
Atratum grass *(Paspalum atratum)*	30	91.00	6.22	1.21	8.18	73.23	46.16	5.49
Purple guinea grass (*Panicum maximum* TD58)	30	93.77	12.93	2.26	14.45	72.40	42.65	3.40
Para grass *(Brachiaria mutica)*	30	95.80	12.23	2.31	11.81	66.91	39.74	4.19
Dwarf napier grass *(Pennisetum purpureum* cv.Mott)	30	96.28	8.28	2.72	12.29	57.34	39.78	2.99
King grass *(Pennisetum purpureum* cv. Kinggrass)	30	91.34	10.50	0.30	10.00	74.39	42.24	4.64
Pangola grass 1 *(Digitaria eriantha)*	45	90.45	10.47	2.63	6.50	79.98	43.22	5.16
Pangola grass 2 *(Digitaria eriantha)*	-	93.60	4.32	1.90	7.67	66.83	41.76	5.10
Plicatulum grass *(Paspalum plicatulum)*	30	92.73	11.14	1.99	8.95	71.97	43.64	5.99
Ruzi grass 1 *(Brachiaria ruziziensis)*	30	94.69	7.05	1.42	7.60	79.79	46.10	4.84
Ruzi grass 2 *(Brachiaria ruziziensis)*	-	90.14	6.05	0.90	6.24	64.97	44.10	6.96
Rhodes grass *(Chloris gayana)*	30	95.94	6.15	1.72	7.99	70.91	42.95	4.54
Creeping signal grass *(Brachiaria humidicola)*	30	92.77	13.73	3.14	8.04	73.99	40.07	4.49
Alternative forages								
Sugarcane *(Saccharum officinarum)*	1 year	91.08	2.92	1.01	7.88	44.32	32.20	5.99
Sweet sorghum *(Sorghum bicolor)*	84	90.40	8.88	1.13	7.19	59.56	38.07	4.93

[1]DM=dry matter, CP= crude protein, EE = ether extract, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin

The CP content of some selected grasses such as Atratum grass *(Paspalum atratum)*, Pangola grass 2 *(Digitaria eriantha)*, Ruzi grass 2 *(Brachiaria ruziziensis)* and Rhodes grass *(Chloris gayana)*, of the same cutting interval, were slightly lower than reported by Angthong et al. (2001) where grasses were meticulously cultivated in an experimental station. This different CP content result could be due to different soils and fertilizer treatments influencing the nutritive value of forage grass (McDonald et al., 2002).

Among alternative forages, the CP content of whole sugarcane was lower than that of sweet sorghum (2.92 and 8.88 %, respectively). The whole sugarcane used in this study (1 year cutting age) had CP content slightly higher than a 6 month (Van et al., 2007) and a 1 year (Sommart et al., 2005a) cutting age sugarcane (2.5 and 2.62 %, respectively) but lower than a 3 and 6 month cutting age sugarcane (Sommart et al., 2005b) (ranged from 4.02 to 7.09 %). The study of Sommart et al. (2005b) indicated that CP content of sugarcane decreased when cutting interval increased. The CP content of whole sugarcane in this study was slightly higher than sugarcane stalk in study of Kawashima et al. (2002) and Odai et al. (2005) (1.4-2.0 and 2.2 %, respectively).

Sommart et al. (2005a) found that CP content in sugarcane tops was relatively higher than in other aerial parts such as old leaves and stalk.

The CP content of 84 dci sweet sorghum (*Sorghum bicolor*) (8.88 %) in this study was higher than the 90 dci sweet sorghum (5.10 % CP) (WTSR, 2008) and the 94 dci of FS-5 *Sorghum bicolor* and BMR-101 *Sorghum bicolor* (5.07 and 5.83 % CP, respectively) (Miron et al., 2007). But it was comparable to 60 dci sorghum grass (*Sorghum almum*) (WTSR, 2008) and sorghum silage (NRC, 2000) where CP content is 8.00 and 9.39 %, respectively. Jaisil and Snitchon (2005) reported that CP content of sweet sorghum gradually decreased from 14.20 to 4.21 % when cutting interval increased from 8 to 18 week. These data show that cutting interval affects CP content in both sugarcane and sweet sorghum. The range of CP content was comparable to that of forage grass in Experiment 1.1 which ranged from 4.32 to 13.73 %.

Fat (EE) content of selected grass feedstuffs was quite low. It ranged from 0.30 % in King grass *(Pennisetum purpureum* cv. Kinggrass) to 3.14 % in Creeping signal grass *(Brachiaria humidicola).* These forages were considered low fat feedstuffs. This range of fat content agrees with WTSR (2008) table which states that fat content in grasses cut at various intervals (30 to 75 days) varied from 0.9 to 5.9 %.

The NDF content of selected forage grasses ranged from 57.34 % in Dwarf napier grass *(Pennisetum purpureum* cv.Mott) to 79.79 % in Ruzi grass 1 *(Brachiaria ruziziensis).* The NDF content of 30 dci Dwarf napier grass in this study was lower than in the experiment of Sarwar et al. (1999) where NDF content of 40 and 60 dci Dwarf napier grass ranged from 70.6 to 73.6 and 78.3 to 79.1 %, respectively. The NDF content of Ruzi grass 1 *(Brachiaria ruziziensis)* in this study was similar to the experiment of Pamo et al. (2007) who found that dry season Ruzi grass in Central Africa had NDF content of 76.0 % which was higher than rainy season Ruzi grass (70.5 %). As found in previous reports, NDF content of grasses gradually increases when cutting interval (Mbwile and Uden, 1997 and Sarwar et al., 1999) or stage of growth (Fukushima and Dehority, 2000) increased.

The NDF content of whole sugarcane (44.32 %) was lower than sweet sorghum (59.56 %) even though sugarcane had longer cutting interval than sorghum. Sommart et al. (2005b) found that NDF content of sugarcane tended to decrease when cutting age increased. Jaisil and Snitchon (2005) reported that NDF content of

sweet sorghum gradually decreased from 86.76 to 54.47 % when cutting interval increased from 8 to 18 week. It might be due to cell content of both sugarcane and sweet sorghum increasing when stage of growth increases.

The ADF content of selected grasses varied within a narrow range of 39.74 to 46.16 %. It was highest in Atratum grass (*Paspalum atratum)* while lowest in Para grass *(Brachiaria mutica).* WTSR (2008) reported that ADF content of 30 to 75 dci forage grasses varied from 28.5 to 49.4 %.

The ADF content of whole sugarcane was slightly lower than sweet sorghum (32.20 and 38.07 %, respectively). The ADF content of 1 year cutting interval whole sugarcane was moderate compared to studies of Van et al. (2002) (24.6 %) and Sommart et al. (2005a) (46.11 %). However, it was relatively higher than a 3 and 6 month cutting interval whole sugarcane in the study of Sommart et al. (2005b) (28.63-33.60 %) and Van et al. (2007) (23.9 %). The ADF content of sweet sorghum was higher than that found by Sukho (2008) (46.14 %). Jaisil and Snitchon (2005) reported that ADF content of a 84 dci sweet sorghum was 36.13 %. Furthermore, they found that it gradually decreased from 41.48 to 27.52 % when cutting interval increased from 8 to 18 week.

Lignin (ADL) content was lowest in Dwarf napier grass *(Pennisetum purpureum* cv.Mott) (2.99 %) while Ruzi grass 2 *(Brachiaria ruziziensis)* (6.96 %) was the highest. Lignin content of 30 dci Dwarf napier grass in this study was similar to 45 dci Dwarf napier grass in WTSR (2008) table (2.8 %) but lower than that reported by Sarwar et al. (1999) where ADL content of 40 and 60 dci Dwarf napier grass ranged from 7.4 to 8.3 and 10.9 to 12.1 %, respectively.

Lignin (ADL) content of whole sugarcane was slightly higher than sweet sorghum (5.99 and 4.93 %, respectively). Lignin content of 1 year cutting interval whole sugarcane was lower than previously reported by Sommart et al. (2005a) (9.85 %). They found that lignin content in aerial parts of sugarcane was highest in old leaves. Data of Sommart et al. (2005b) indicated that a 3 month cutting interval had lignin content higher than 6 month cutting interval. Compared to the present study, both of them had lignin content higher than in 1 year cutting interval whole sugarcane. Lignin content of the 84 dci sweet sorghum in this study was lower than that reported by Sukho (2008) (7.79 %). It was higher than the same cutting age (2.30 %) but comparable to a 98 dci (4.86 %) in the study of Jaisil and Snitchon, 2005. They found that lignin content of sweet

sorghum increased when cutting age increased from 8 to 14 weeks and declined from 14 to 18 weeks.

Forage grasses used in this study were mostly cut at 30 dci. However, the nutritive values of the forage grass group varied, the nutritive values of whole sugarcane differed from sweet sorghum, and their differences are repeated when compared to other studies. These grass and alternative forage nutritive values were influenced by many factors such as stage of growth, species, soils and fertilizer treatment, grazing system, climate and season (McDonald et al., 2002). These factors may partially explain differences in chemical composition between this study and others.

3.1.3.2 *In vitro* digestibility

In vitro dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD) and *in vitro* neutral detergent digestibility (IVNDFD) of feedstuffs were determined at post-24 h of incubation. The digestibility of grass feedstuffs is shown in Table 3.1.2. The data suggest that IVDMD and IVOMD values of each feedstuff are similar.

The IVDMD and IVOMD in selected forage grasses varied over a wide range from 25.72 to 48.30 and 25.35 to 45.87 %, respectively. The IVDMD and IVOMD were highest in Dwarf napier grass *(Pennisetum purpureum* cv.Mott) while lowest in Plicatulum grass *(Paspalum plicatulum)*.

The IVNDFD varied in the forage grass group. It ranged from 9.97 to 30.72 %. It was lowest in Plicatulum grass *(Paspalum plicatulum)* and highest in Creeping signal grass *(Brachiaria humidicola)*. The IVNDFD of King grass *(Pennisetum purpureum* cv. Kinggrass) and Dwarf napier grass *(Pennisetum purpureum* cv.Mott) in this study were comparable to elephant grass (*Penissetum purpureum* cv. Cameroun) (23.29 %) in the study of Magalhaes et al. (2010) who investigated the 24 h *in sacco* incubation.

Among alternative forages, whole sugarcane showed greater IVDMD and IVOMD than that of sweet sorghum ($P<0.01$). Whole sugarcane exhibited the higher IVDMD and IVOMD than that of selected forage grasses while sweet sorghum was in the range of those selected forage grasses. There was no significant difference in IVNDFD among whole sugarcane and sweet sorghum. Data indicates that both of them have lower IVNDFD than selected forage grasses. Compared to previous studies, the

IVNDFD of whole sugarcane in this study is less than the 24 h *in sacco* incubation of elephant grass (*Penissetum purpureum* cv. Cameroun) (29.87 %) (Magalhaes et al., 2010).

Table 3.1.2 *In vitro* digestibility, gas production kinetics and calculated ME of forage grasses and alternative forages

Feedstuffs	Digestibility, %[1]			Kinetic of gas production				ME [2] (MJ/kg DM)	
	IVDMD	IVOMD	IVNDFD	a, ml	b, ml	|a|+b, ml	c, %/h	Eq1	Eq2
Forages grasses									
Atratum grass *(Paspalum atratum)*	30.02^{g}	30.90^{f}	18.21^{g}	-3.42^{c}	167.16^{bc}	170.70^{bc}	0.017^{fg}	5.05^{def}	4.73^{ef}
Purple guinea grass (*Panicum maximum* TD58)	44.28^{c}	40.64^{c}	29.97^{abc}	-10.87^{h}	151.22^{ef}	162.09^{cd}	0.021^{e}	5.42^{de}	4.76^{ef}
Para grass *(Brachiaria mutica)*	39.01^{d}	35.35^{de}	25.27^{cdef}	-6.48^{de}	128.90^{h}	135.37^{e}	0.022^{de}	5.30^{de}	4.67^{ef}
Dwarf napier grass *(Pennisetum purpureum* cv.Mott)	48.30^{b}	45.87^{b}	27.09^{cde}	-8.90^{fgh}	170.93^{b}	179.83^{b}	0.024^{cd}	6.10^{bc}	5.67^{bc}
King grass *(Pennisetum purpureum* cv. Kinggrass)	33.21^{fg}	31.04^{f}	23.39^{defg}	-8.00^{defg}	163.36^{bcd}	171.22^{bc}	0.015^{gh}	4.65^{g}	4.10^{g}
Pangola grass 1 *(Digitaria eriantha)*	35.82^{def}	34.87^{de}	33.14^{ab}	5.89^{b}	117.39^{i}	123.27^{f}	0.029^{b}	6.19b^{c}	5.65^{bc}
Pangola grass 2 *(Digitaria eriantha)*	36.97^{de}	36.89^{d}	23.09^{efg}	-7.68^{defg}	153.67^{def}	161.20^{cd}	0.022^{de}	4.75^{fg}	4.52^{fg}
Plicatulum grass *(Paspalum plicatulum)*	25.72^{h}	25.35^{g}	9.97^{h}	-5.75^{de}	161.35^{cd}	167.10^{c}	0.014^{h}	4.68^{fg}	4.11^{g}
Ruzi grass 1 *(Brachiaria ruziziensis)*	38.39^{de}	36.11^{de}	28.14^{bcd}	-9.89^{gh}	182.50^{a}	192.40^{a}	0.016^{g}	4.97^{efg}	4.60^{ef}
Ruzi grass 2 *(Brachiaria ruziziensis)*	45.30^{bc}	45.29^{b}	27.60^{cde}	-1.86^{c}	140.43^{g}	143.74^{e}	0.030^{b}	6.40^{b}	6.08^{b}
Rhodes grass *(Chloris gayana)*	38.51^{de}	36.98^{d}	21.66^{fg}	-7.48^{def}	159.69^{cde}	167.17^{c}	0.021^{e}	5.35^{def}	5.03^{de}
Creeping signal grass *(Brachiaria humidicola)*	38.41^{de}	37.04^{d}	33.7^{2a}	-9.27^{fgh}	155.13d^{ef}	164.40^{cd}	0.023^{cd}	5.95^{c}	5.24^{cd}
Alternative forages									
Sugarcane *(Saccharum officinarum)*	53.43^{a}	50.49^{a}	5.91^{h}	8.37^{a}	115.87^{i}	124.26^{f}	0.076^{a}	7.63^{a}	7.47^{a}
Sweet sorghum *(Sorghum bicolor)*	35.00^{ef}	33.14^{ef}	4.66^{h}	-7.50^{def}	149.93^{f}	157.42^{d}	0.018^{f}	5.12^{def}	4.66^{ef}
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
SEM	1.2555	1.2222	1.5539	0.7418	2.9851	3.2341	0.0007	0.1550	0.1552

[1]IVDMD, IVOMD and IVNDFD= *in vitro* DM, OM and NDF digestibility respectively,
[2]calculated ME follows equation of Menke et al. (1979) (Eq1) and Menke and Steingass (1988) (Eq2)
$^{a, b, c, d, e, f, g, h, I, j, k, l}$ means within column with different superscripts differ significantly ($P<0.05$)

3.1.3.3 Kinetics of gas production

The *in vitro* gas production technique was adapted to describe the kinetics of fermentation that were based on the modified exponential model $y = a + b\ (1\text{-}e^{-ct})$ (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 compatibility of its parameters with intake, digestibility and degradation characteristic of feedstuffs had been documented (Blummel and Ørskov, 1993; Khazaal et al., 1993; Sommart et al., 2000; Nitipot and Sommart, 2003).

The kinetics of gas production are shown in Table 3.1.2 and Figure 3.1.1 (a) and (b). A comparison of the gas production characteristics of different treatments indicated significant differences between treatments. The value *a*, intercept, varied from -10.87 to 5.89 ml/0.5g DM substrate in forage grass feedstuffs. There were both positive and negative values. 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 a lag in microbial colonization (Chumpawadee, 2006).

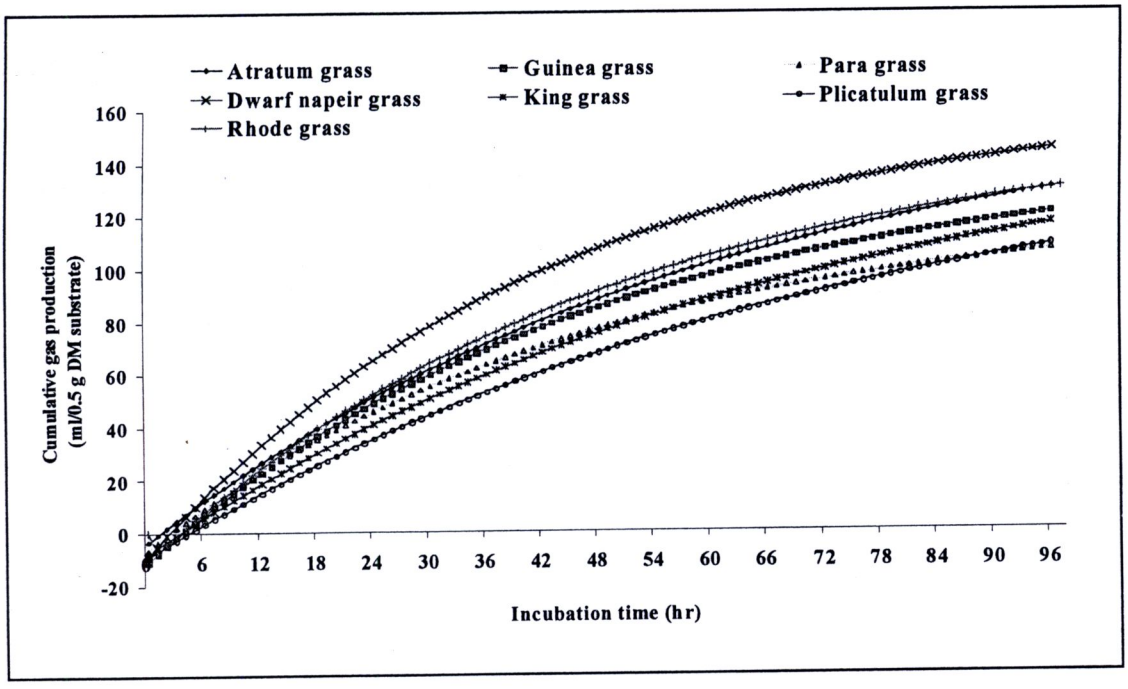
In this study, the *a* value was lowest for Purple guinea grass (-10.87 ml/0.5g DM substrate) which was not different ($P>0.05$) from Ruzi grass 1 and Creeping signal grass (-9.89 and -9.27 ml/0.5g DM substrate, respectively). The value *a* was highest for Pangola grass 1 (5.89 ml/0.5g DM substrate) which was different ($P<0.01$) from the other Pangola grass 2 (-7.68 ml/0.5g DM substrate). However, it is well known that the value for absolute *a* ($|a|$), ideally reflects the soluble fraction fermentation. Therefore, the fermentation of the soluble fraction was highest in Purple guinea grass and lowest in Pangola grass 1.

The value *a*, intercept, of whole sugarcane was higher ($P<0.01$) than sweet sorghum (8.37 and -7.50 ml/0.5g DM substrate, respectively). The $|a|$ of the 1 year cutting interval whole sugarcane in this study was similar to the same cutting interval sugarcane in study of Sommart et al. (2005a) but it was higher than a 3 and 6 month cutting age whole sugarcane (Sommart et al., 2005b)

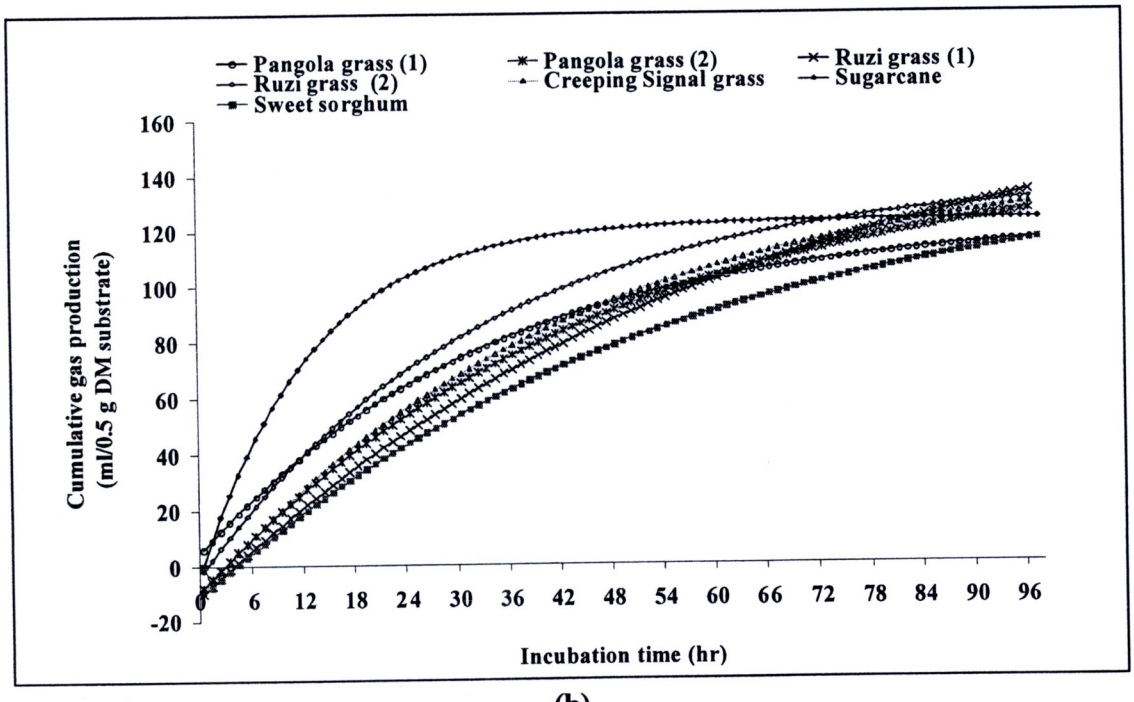
The gas volume at asymptote (*b*) describes the fermentation of the insoluble fraction. The *b* value varied among forage grasses and ranged between 117.39 to 182.50 ml/0.5g DM substrate. Ruzi grass 1 exhibited the highest ($P<0.01$) fermentation of insoluble fraction while Pangola grass 1 showed the lowest ($P<0.01$). Also, this pattern was observed in the potential extent of gas production ($|a|+b$). Thus, Ruzi grass 1 (192.40 ml/0.5g DM substrate) exhibited the highest ($P<0.01$) potential extent while Pangola grass 1 (123.27 ml/0.5g DM substrate) showed the lowest ($P<0.01$).

The *b* value of whole sugarcane is lower (P<0.01) than sweet sorghum (115.87 and 149.93 ml/0.5g DM substrate, respectively). In the same way as gas volume at asymptote, the potential extent of gas production (|*a*|+*b*) of whole sugarcane is lower (P<0.01) than sweet sorghum (124.26 and 157.42 ml/0.5g DM substrate, respectively).

In rate of gas production (*c*, %/h), whole sugarcane exhibited the fastest (P<0.01) rate (0.076 %/h) followed by Ruzi grass 2 and Pangola grass 1 (0.030 and 0.029 %/h, respectively). On the other hand, Plicatulum grass and King grass showed the lowest (P<0.01) rate of gas production (0.014 and 0.015 %/h, respectively). Rate of gas production of the 1 year cutting interval whole sugarcane in this study was higher than found by Sommart et al. (2005a) (0.054 %/h) but it was similar to the 6 month cutting age whole sugarcane (Sommart et al., 2005b). This variation might be due to the amount of cell content in the sugarcanes used in all these studies. Cell content of sugarcane is mainly composed of sugar which is rapidly utilized by microbes. Rate of gas production is possibly due to the carbohydrate fraction being readily available to the microbial population, which indicates that variation in cell content influences rate of gas production.



(a)



(b)

Figure 3.1.1 Cumulative gas volume estimated by $y = a + b\ (1\text{-}e^{-ct})$ (ml/0.5g DM substrate) throughout 96 h of incubation of selected forage grass feedstuffs

3.1.3.4 Calculated metabolizable energy

Estimation of ME content was calculated following equation of Menke et al. (1979); ME (MJ/kg DM) = 2.20+(0.136×Gv)+(0.057×CP) (Eq1) and Menke and Steingass (1988); ME (MJ/kg DM)=2.20+(0.1357×Gv)+(0.0057×CP)+ (0.0002859×EE^2) (Eq2) by using gas volume produced at 24 h of incubation (Gv, ml), crude protein (CP, %) and ether extract (EE, %) as factors of prediction. From the equation, therefore, Gv at 24 h of incubation, CP and EE content have a positive effect on estimated ME.

The calculated metabolizable energy of grass feedstuffs are presented in Table 3.1.2. The estimated metabolizable energy using Eq1 and Eq2 varied among grass feedstuffs and ranged between 4.65 to 6.40 and 4.11 to 6.08 MJ/kg DM, respectively. Ruzi grass (2) and Pangola grass (1) exhibited the highest estimated metabolizable energy which reflects their amount of gas produced at 24 h of incubation and/or CP and EE content. Compared to the results of Nogueira Filo et al. (2000) who investigated the estimated metabolizable energy in forage grasses using equations proposed by Menke and Steingass (1988) by using gas volume produced at 24 h of incubation (Gv, ml), crude protein (CP, %), the estimated metabolizable energy of 30 dci Creeping signal grass *(Brachiaria humidicola)* in this study was similar to the 100 dci fertilized signal grass (5.91 MJ/kg DM) in their report.

The estimated metabolizable energy of 30 dci Dwarf napier grass *(Pennisetum purpureum* cv. Mott) and King grass *(Pennisetum purpureum* cv. Kinggrass) in this study were lower than the 100 dci *Pennisetum purpureum* cv. Napier (9.12 MJ/kg DM) in the investigation of Nogueira Filo et al. (2000), but comparable to the result of Magalhaes et al. (2010) who investigated the estimated metabolizable energy of elephant grass (*Penissetum purpureum* cv. Cameroun) (5.66 MJ/kg DM) by using the same variable of prediction.

Both metabolizable energy values of whole sugarcane estimated by using Eq1 and Eq2 were significantly higher than that of sweet sorghum ($P<0.05$). Data indicated that although sweet sorghum had the higher CP than whole sugarcane, gas volume produced at 24 h of incubation of whole sugarcane was higher (data not shown). The higher digestibility is thus reflected in the higher estimated metabolizable energy value. Magalhaes et al. (2010) used the same variable of prediction and found a lower estimated metabolizable energy of sugarcane (6.21 MJ/kg DM) than this present study.

This variation of the observed estimated metabolizable energy is related to the difference in CP content and gas production at 24 h of incubation which is stated in the predictive equation.

3.1.4 Conclusion

Data in this study indicate that the chemical composition of selected forage grasses and alternative forages varied among feedstuffs. For digestibility, the obtained data suggest that IVDMD, IVOMD and IVNDFD values varied among species of grasses. For digestibility of alternative forages, whole sugarcane showed greater IVDMD and IVOMD than sweet sorghum but they did not differ in IVNDFD. Data indicate that both of them have lower IVNDFD than selected forage grasses. The kinetics of gas production of selected forage grasses and alternative forages also differed between feeds. The estimated metabolizable energy of feedstuffs was varied. The 30 dci of Ruzi grass and Dwarf napier grass showed the highest estimated metabolizable energy. The results of this study demonstrate that whole sugarcane and sweet sorghum are high potential alternative roughage sources for beef and dairy cattle in the dry season where feed supplies become critically low in both quantity and quality.

3.2 EXPERIMENT 1.2: NUTRITIVE EVALUATION OF TROPICAL FORAGE LEGUME FEEDSTUFFS

3.2.1 Introduction

A major constraint to livestock production in tropical areas is the fluctuating quantity and quality of the year-round feed supply. Particularly during the dry season, the natural pastures drop in quantity and quality, especially in energy and nitrogen content. Tropical forages are low in protein and have high cell wall contents resulting in low digestibility. Supplementation of tropical grasses with legumes has been reported to result in increased dry matter intake (Mupangwa et al., 2000). Herbaceous tropical forage legumes such as Cavalcade *(Centrosema pascuorum* cv. Cavalcade), Verano stylo *(Stylosanthes hamata* cv.Verano) and Thapra stylo *(Stylosanthes guianensis* CIAT 184) have great potential as protein supplements to low quality roughages and they are promoted by the Department of Livestock Develoment of Thailand. However, as expansion of crop areas (due to population growth) has reduced grazing lands, there is increased competition for forage area. To alleviate feed shortage in the dry season, some

farmers collect crop residues such as peanut straw *(Arachis hypogaea)* and store them for later use in stall feeding. Thus, it is one example of the potential use of legume crop residues.

Chemical compositions such as proximate analysis (AOAC, 1990) and fiber (Van Soest, 1991) are available in many sources such as the WTSR (2008) table. However, there is meagre digestibility data and very limited energy content information, especially metabolizable energy (ME). In order to determine feed digestibility and energy content, evaluation of feedstuffs for the whole tract and ruminal digestion through feeding experiments is possible, but is expensive and requires sophisticated laboratory and animal facilities (Krishnamoorthy et al., 1995). *In vitro* gas production technique has proved to be a potentially useful rapid technique for feed evaluation, as it is capable of measuring rate and extent of nutrient degradation. In addition, it is less expensive and results are obtained easily (Chumpawadee et al., 2006b). Moreover, this technique could be used to determine ME content by prediction equations using chemical composition and gas production (Menke et al., 1979; Menke and Steingass, 1988).

The objectives of this experiment, therefore, were to determine chemical composition, nutritive values and to compare digestibility, kinetics of gas production and metabolizable energy of selected forage legumes using *in vitro gas* production technique.

3.2.2 Materials and Methods

3.2.2.1 Experimental design and treatment

The experiment was arranged into randomized complete block design. There were 4 replicates for kinetics for gas production determination, 8 replicates for *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) evaluation and 4 replicates for *in vitro* neutral detergent fiber digestibility (IVNDFD) evaluation.

Five forage legume feedstuffs in the experiment were collected from Laboratory of Khon Kaen Animal Nutrition Research and Development Center, Khon Kaen. The selected forage legumes were Cavalcade *(Centrosema pascuorum* cv. Cavalcade) (2 samples), Verano stylo *(Stylosanthes hamata* cv.Verano) and Thapra Stylo *(Stylosanthes guianensis* CIAT 184). These feedstuff treatments were categorized as forage legumes following their taxonomic family (Kellems and Church, 2002). Althought peanut straw *(Arachis hypogaea)* is not actually classified as a forage legume, in this

experiment, however, it was included as a forage legume treatment because taxonomically, it is appropriate, and locally available.

3.2.2.2 Animals

Three male crossbred Holstein Friesians of body weight approximately 200 kg were used as the source of rumen inoculum. The animals were maintained on dried chopped sweet sorghum *(Sorghum bicolor)* as a roughage source at a Department of Animal Science's experimental station, Faculty of Agriculture, Khon Kaen University. Rumen fluid was removed under vacuum pressure via stomach tube into a 2 liter suction flask and transferred into two pre-warmed 1.8 liter vacuum flasks which were then transported to the laboratory for preparation of rumen inoculum.

3.2.2.3 Feedstuff samples and chemical analysis

All test feedstuff samples were dried at 60◦C in hot air oven for 48 h and ground through a 1 mm screen for chemical analysis and *in vitro* gas production technique. The feedstuff 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 determined using the method proposed by Van Soest et al. (1991).

Feed samples of 500 mg (fresh weight basis) were transferred into 50 ml serum bottles (Sommart et al., 2000). The bottles were stoppered with rubber stopper, crimp sealed and incubated in a hot air oven with water bath set at 39˚C. Bottles were pre-warmed for 1 hour at 39˚C and were injected with 40 ml of rumen inoculum prepared as following Sommart et al. (2000).

3.2.2.4 Data collections

1) Gas volume (Gv)

The rate of gas production was measured by reading and recording the 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. Cumulative gas volumes after incubations were fitted using the equation $y = a + b\ (1\text{-}e^{-ct})$ (Ørskov and McDonald, 1979), where y = gas production at time 't', a = the intercept, which ideally reflects the

fermentation of the soluble fraction, b= the fermentation of the insoluble fraction, c = rate of gas production, and ($|a|+b$) = potential extent of gas production.

2) Digestibility

After 24 hour of incubation, a 12 bottle sample of each treatment was taken and kept in a freezer to inhibit microbial activity. Subsequently, 8 bottles of each treatment were subjected to *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) determination and 4 bottles were subjected to neutral detergent fiber digestibility (IVNDFD) determination.

To determine the IVDMD, residues of incubated samples in bottles were filtered through a groose crucible and dried at 105◦C in a hot air oven for DM determination. Thereafter, the DM residues were subjected to incineration in a muffle furnace at 550◦C for ash determination in order to evaluate the IVOMD. To determine the IVNDFD, residues of incubated samples in bottles were filtered through a groose crucible. Then, the residues were analyzed for NDF and lastly for ash. The obtained residue DM, ash and NDF data were used for calculation of digestibility according to the following equations:

$$\text{IVDMD (\%)} = \frac{[\text{DM initial incubation (g)} - \text{DM after incubation (g)}] \times 100}{\text{DM initial incubation (g)}}$$

$$\text{IVOMD (\%)} = \frac{[\text{OM initial incubation (g)} - \text{OM after incubation (g)}] \times 100}{\text{OM initial incubation (g)}}$$

$$\text{IVNDFD (\%)} = \frac{[\text{NDF initial incubation (g)} - \text{NDF after incubation (g)}] \times 100}{\text{NDF initial incubation (g)}}$$

3) Estimation of metabolizable energy (ME)

Estimation of ME content was calculated following the equations of Menke et al. (1979); ME (MJ/kg DM) = $2.20+(0.136\times Gv)+(0.057\times CP)$ (Eq1) and Menke and Steingass (1988); ME (MJ/kg DM) = $2.20+(0.1357\times Gv) + (0.0057\times CP) + (0.0002859\times EE^2)$ (Eq2) by using gas produced at 24 h of incubation (Gv, ml), crude protein (CP, %) and ether extract (EE, %) as factors of prediction.

Since gas, in this study, was collected from incubation of a 0.5 g of DM, the gas volume was adjusted to be equal to 0.2 g of DM for ME calculation in the above equations. The equation for adjusting is shown as follows:

Gv produced from 0.2 g DM = (0.2× Gv produced from 0.5 g DM)/0.5

3.2.2.5 Statistical analysis

All data obtained from the trials were subjected to the analysis of variance procedure of statistical analysis system (SAS, 1996) according to a Randomized Complete Block Design (RCBD). Means were compared by using the LSMEANS and STDERR statement in PROC GLM. Mean separation were determined using the PDIFF statement in PROC GLM with a $P < 0.05$ significance level. The original model includes the treatment and block effects as follows:

$$\mathbf{Yij} = \mu + \tau i + \beta j + \varepsilon ij$$

Where, **Yij** = observation value in feedstuff sample i, batch of injected rumen inoculum j

μ = over all mean

τi = effect of feedstuff sample i when i = 1, 2,.., 5

βj = effect of batch of injected rumen inoculum j when j = 1, 2, 3 and 4

εij = residual error

3.2.3 Results and Discussion

3.2.3.1 Chemical composition

The chemical composition of selected forage legumes are shown in Table 3.2.1. Generally, there were wide variations in the chemical composition of the investigated feedstuffs.

The CP content ranged from 9.94 to 17.76 %. The CP content of 30 day cutting interval (dci) Cavalcade was greater than reports of Snitwong et al. (2004), Chumpawadee et al. (2006b), Mungman (2007) and Chaokaur and Sommart (2009) (14.8, 11.0, 13.4 and 12.92 %, respectively). It may be due to the earlier cutting age of Cavalcade in this study. Verano stylo in this study had CP content similar to 45 dci Verano stylo (17.8 % CP) in the study of Namsilee et al., 2002, but slightly higher than

60 dci (15.2 % CP) which reported in WTSR (2008) table. Thapra stylo in this study had the higher CP content than in report of Hue et al. (2008) (50 to 60 dci; 15.4 % CP) and Aumont et al. (1995) (28 to 77 dci; 13.3 % CP), but it was lower than the study of Mupangwa et al. (2000) (140 dci; 25.3 % CP). Although peanut straw was post-harvest aerial part, it had higher CP content (10.98 %) than some forage grasses in experiment 1.1. In terms of CP content, Cavalcade *(Centrosema pascuorum* cv. Cavalcade), Verano stylo *(Stylosanthes hamata* cv.Verano) and Thapra stylo *(Stylosanthes guianensis* CIAT 184) are considered to be high quality roughages for ruminants.

Ether extract (EE) or fat content of selected forage legumes was quite low, ranging from 1.07 % in peanut straw to 2.61 % in Cavalcade hay (1). These forages were considered low fat feedstuffs, and this range of fat content agrees with WTSR (2008) table that fat content in various forages legumes varies from 0.5 to 5.3 %.

Table 3.2.1 Chemical composition of selected forage legume sources

Feedstuff	Cutting	Chemical composition						
	Age, d	DM[1],%	CP	EE	Ash	NDF	ADF	ADL
			% of DM					
Cavalcade *(Centrosema pascuorum* cv. Cavalcade) *(1)*	30	90.33	17.41	2.61	8.64	46.66	28.16	6.70
Cavalcade *(Centrosema pascuorum* cv. Cavalcade) *(2)*	-	91.74	9.94	1.41	4.79	61.21	39.08	9.35
Verano stylo *(Stylosanthes hamata* cv.Verano)	-	93.68	17.76	1.46	7.58	69.24	49.44	10.97
Thapra stylo *(Stylosanthes guianensis* CIAT 184)	-	91.89	17.68	1.43	8.84	55.13	39.60	7.79
Peanut straw *(Arachis hypogaea)*	-	89.28	10.98	1.07	11.78	46.81	43.84	8.46

[1]DM=dry matter, CP= crude protein, EE = ether extract, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin

The NDF content ranged from 46.66 to 69.24 %, which is a similar range to previous studies. The NDF content of Cavalcade 1 (46.66 %), 30 dci agrees with a 45 to 120 dci Cavalcade hay (49.8 to 56.5 %) (WTSR, 2008) and 110 dci (46.66 %) (Snitwong et al., 2004). The Cavalcade 2 had higher NDF content (61.21 %) than the other, but it was slightly lower than reported by Mungman (2007) and Chaokaur and Sommart (2009) (68.7 and 72.6 %, respectively). Peanut straw had NDF, ADF and ADL slightly lower (but with similar differences) than reported by Chaokaur and Sommart (2009) (59.90, 56.10 and 10.84%, respectively). The ADF and Lignin content (ADL)

ranged from 28.16 to 49.44 and 6.70 to 10.97 %, respectively. Both of them were lowest in 30 dci Cavalcade 1.

These forage legume nutritive values were influenced by many factors such as stage of growth, species, soils and fertilizer treatment, including climate and season (McDonald et al., 2002). These factors may partially explain differences in chemical composition between this study and others.

3.2.3.2 *In vitro* digestibility

Except for Cavalcade 1, the IVDMD and IVOMD of selected forage legumes varied within a narrow range from 43.68 to 49.47 and 44.35 to 48.93 %, respectively (Table 3.2.2). The 30 dci Cavalcade 1 showed the highest ($P<0.01$) IVDMD and IVOMD (62.88 and 63.25 %, respectively).

The IVNDFD of Cavalcade 1 and Verano stylo were not significantly different (41.14 and 38.59 %, respectively), but both of them were highest ($P<0.01$) in these selected forage legumes.

Table 3.2.2 *In vitro* digestibility, gas production kinetics and calculated ME of selected forage legume feedstuffs

Feedstuffs	Digestibility, %[1]			Kinetic of gas production				ME [2] (MJ/kg DM)	
	IVDMD	IVOMD	IVNDFD	a, ml	b, ml	|a|+b, ml	c, %/h	Eq1	Eq2
Cavalcade *(Centrosema pascuorum cv.* Cavalcade) *(1)*	62.88^{a}	63.25^{a}	41.14^{a}	-3.15^{b}	113.81^{a}	116.96^{a}	0.057^{a}	7.66^{a}	6.76^{a}
Cavalcade *(Centrosema pascuorum cv.* Cavalcade) *(2)*	44.22^{c}	45.33^{cd}	25.14^{c}	-0.63^{a}	106.26^{bc}	107.85^{b}	0.046^{b}	6.59^{b}	6.08^{b}
Verano stylo *(Stylosanthes hamata* cv. Verano)	43.68^{c}	44.35^{d}	38.59^{a}	-8.43^{c}	101.61^{c}	110.04^{b}	0.039^{c}	6.35^{b}	5.43^{c}
Thapra stylo *(Stylosanthes guianensis* CIAT 184)	49.47^{b}	48.93^{b}	30.26^{b}	-8.40^{c}	110.09^{ab}	118.49^{a}	0.040^{c}	6.69^{b}	5.76^{bc}
Peanut straw *(Arachis hypogaea)*	45.20^{c}	46.23^{c}	16.61^{d}	-9.07^{c}	105.20^{bc}	114.27^{ab}	0.045^{b}	6.43^{b}	5.86^{b}
P value	<.0001	<.0001	<.0001	<.0001	0.0172	0.0173	<.0001	0.0001	0.0003
SEM	0.5837	0.5818	0.8566	0.7975	2.1800	2.0972	0.0015	0.1380	0.1368

[1]IVDMD, IVOMD and IVNDFD= *in vitro* DM, OM and NDF digestibility, respectively,
[2]calculated ME following equation of Menke et al. (1979) (Eq1) and Menke and Steingass (1988) (Eq2),
$^{a, b, c, d, e, f, g, h, I, j, k, l}$ means within column with different superscripts differ significantly ($P<0.05$)

Although peanut straw *(Arachis hypogaea)*, the post-harvest by-product, had the lowest ($P<0.01$) IVNDFD (16.61 %), its IVDMD and IVOMD were not significantly different ($P>0.05$) from Cavalcade *(Centrosema pascuorum cv.* Cavalcade) *(2)*.

3.2.3.3 Kinetics of gas production

The kinetics of gas production are shown in Table 3.2.2 and Figure 3.2.1. A comparison of the gas production characteristics indicated significant differences between treatments.

The value *a*, intercept, varied from -9.07 to -0.63 ml/0.5 g DM. They were all negative values. 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 a lag in microbial colonization (Chumpawadee, 2006). It is well known that the value for absolute *a* (|*a*|), described ideally, reflects the fermentation of the soluble fraction.

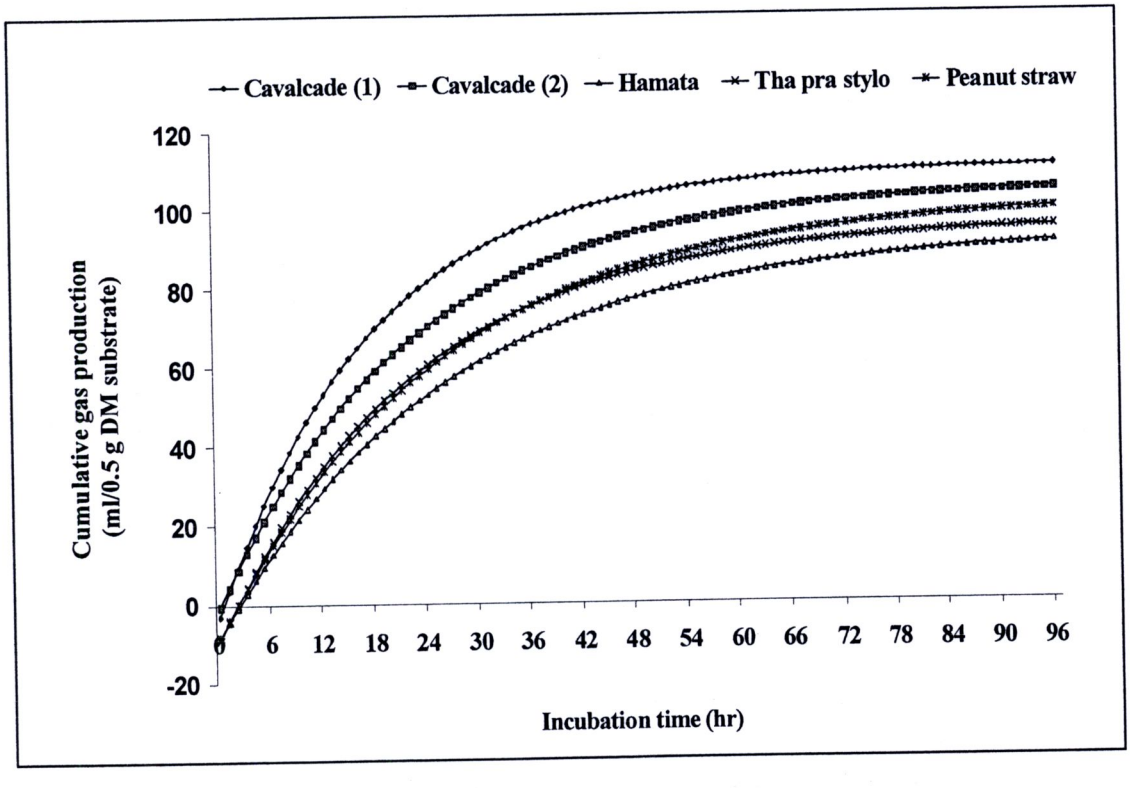


Figure 3.2.1 Cumulative gas volume estimated by $y = a + b\,(1\text{-}e^{-ct})$ (ml/0.5g DM substrate) throughout 96 h of incubation of selected forage legume feedstuffs.

The value *a* was lowest (P<0.01) for Verano stylo, Thapra stylo and peanut straw. Conversely, Cavalcade 2 had the highest (P<0.01) followed by Cavalcade 1 (-0.63 and -3.15 ml/0.5g DM substrate, respectively). However, the |*a*| ideally reflects the fermentation of the soluble fraction. Therefore, the fermentation of the soluble fraction was highest in Verano stylo, Thapra stylo and peanut straw followed by Cavalcade 1 and Cavalcade 2, respectively.

The gas volume at asymptote (*b*) describes the fermentation of the insoluble fraction. The *b* value varied among forage legumes and ranged between 105.20 to 113.28 ml/0.5g DM substrate. Cavalcade 1 exhibited the highest (P<0.01) fermentation of the insoluble fraction. Its gas volume at asymptote (*b*) may reflect its digestibility (IVDMD, IVOMD and IVNDFD) and also it is possibly due to the high CP content but low NDF, ADF and lignin (ADL) content. On the other hand, although Verano stylo had high CP content, it showed low fermentation of the insoluble fraction. It might be due to the high NDF, ADF and lignin content which are reflected in the low dry matter and organic matter digestibility. Cavalcade 1 exhibited the high potential extent of gas production (|*a*|+*b*) (116.96 ml/0.5g DM substrate). However, it was not significantly different (P>0.05) from Thapra stylo and peanut straw (118.49 and 114.27 ml/0.5g DM substrate, respectively). Rumen fermentation potential for selected forage legumes ranked from the highest to the lowest is Thapra stylo, Cavalcade 1, peanut straw, Verano stylo and Cavalcade 2.

A comparison of rate of gas production (*c*, %/h) indicated significant differences between treatments. It varied within a narrow range of 0.039 to 0.057%/h. Rate of gas production possibly reflects the carbohydrate fraction readily available to the microbial population. Cavalcade 1 had the higher cell content and CP component than any other, thus it exhibited the fastest (P<0.01) rate of gas production (0.057%/h). By contrast, Verano stylo and Thapra stylo showed the slowest rate of gas production (0.039 and 0.40 %/h, respectively).

3.2.3.4 Calculated metabolizable energy

The calculated metabolizable energy of legume forage feedstuffs are presented in Table 3.2.2. Estimation of ME content was calculated following the equation of Menke et al. (1979); ME (MJ/kg DM) = 2.20+(0.136×Gv)+(0.057×CP) (Eq1) and Menke and Steingass (1988); ME (MJ/kg DM) = 2.20+(0.1357×Gv) + (0.0057×CP)

+ ($0.0002859 \times EE^2$) (Eq2) by using gas produced at 24 h of incubation (Gv, ml), crude protein (CP, %) and ether extract (EE, %) as factors of prediction. From the equations, therefore, Gv at 24 h of incubation, CP and EE content have a positive effect on estimated ME.

The estimated metabolizable energy of forage legume feedstuffs using Eq1 and Eq2 are shown in Table 3.2.2. For equation 1 (Eq1), it varied from 6.35 to 7.66 MJ/kg DM. Cavalcade 1 had higher estimated metabolizable energy than the other four samples. Using Equation 2 (Eq2), the estimated metabolizable energy of Cavalcade 1 was highest. These estimated metabolizable energy values reflect the amount of gas volume produced at 24 h of incubation and/or CP and EE content.

3.2.4 Conclusion

Based on this study, there were variations in the chemical composition of the investigated feedstuffs. In terms of CP content, Cavalcade, Verano stylo and Thapra stylo are considered to be high quality roughages for ruminants. The result in this study demonstrates that the 30 dci Cavalcade had the highest IVDMD, IVOMD and IVNDFD. The kinetics of gas production of selected forage legumes differs among feeds. Rumen fermentation potential for selected forage legumes ranked from the highest to the lowest are, Thapra stylo, Cavalcade 1, peanut straw, Verano stylo and Cavalcade 2. Cavalcade 1 shows the highest estimated metabolizable energy.

3.3 EXPERIMENT 1.3: NUTRITIVE EVALUATION OF ENERGY SOURCE FEEDSTUFFS

3.3.1 Introduction

Energy content of feed stuffs is one of the most important nutritive values for animals and it should comprise roughly seventy percent of feed rations (Chumpawadee, 2006). In tropical areas such as Thailand, traditionally the farmers feed their cattle with green forage from natural pasture as the main feed resource (Sruamsiri, 2008). They face a shortage in both quantity and quality of roughage, especially in the dry season. Feeding low quality roughage solely provides not enough energy for animals to meet their requirement for both maintenance and production. To improve animal production, an energy supplement is needed either separate or mixed into the ration in this feeding situation.

Cassava chip, ground corn and rice mill by-product such as broken rice and rice bran are well known and widely used as animal energy sources. Nowadays, cassava starch industrial by-products such as cassava pulp and cassava peel have potential as ruminant energy feed sources (Nitipot and Sommart, 2003; Napasirth et al., 2005). Although chemical compositions such as proximate analysis (AOAC, 1990) and detergent fiber system (Van Soest, 1991) are available in the WTSR (2008) table and another reports, there is meagre digestibility data and very limited energy content information, especially metabolizable energy (ME).

In order to determine feed digestibility and energy content, evaluation of feedstuffs for the whole tract and ruminal digestion through feeding experiments is expensive and requires sophisticated laboratory and animal facilities (Krishnamoorthy et al., 1995). *In vitro* gas production technique is widely used to evaluate feed digestibility and kinetics of gas production. Moreover, this technique can be used to determine ME content by prediction equations using chemical composition and gas production (Menke et al., 1979; Menke and Steingass, 1988). It is a useful technique to use as screening test in searching for potential feedstuffs for ruminants which are less expensive, easy and suitable for use in developing countries (Chumpawadee et al., 2007a).

With respect to energy feed sources, limited information is available on the digestibility, kinetics of gas production and metabolizable energy. The objectives of this experiment, therefore, were to determine chemical composition and nutritive values and to compare digestibility, kinetics of gas production and metabolizable energy of some energy feed sources using *in vitro gas* production methodology.

3.3.2 Materials and Methods

3.3.2.1 Experimental design and treatment

The experiment was arranged into randomized complete block design. There were 4 replicates for kinetics of gas production determination, 8 replicates for *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) evaluation and 4 replicates for *in vitro* neutral detergent fiber digestibility (IVNDFD) evaluation.

Six energy source feedstuffs in the experiment were collected from Nam Pong Dairy Co-operative Limited, Khon Kaen Province and Laboratory of Khon Kaen Animal Nutrition Research and Development Center, Khon Kaen. They were

broken rice, cassava chip, cassava peel, cassava pulp, ground corn, and rice bran. These energy source feedstuffs were classified as energy feed sources by their CP content which is lower than 20 % and cell wall (NDF) content which is lower than 35 % following the guideline of Harris et al. (1982) and WTSR (2008).

3.3.2.2 Animals

Three male crossbred Holstein Friesians of body weight approximately 200 kg were used as the source of rumen inoculum. The animals were maintained on dried chopped sweet sorghum *(Sorghum bicolor)* as a roughage source at a Department of Animal Science's experimental station, Faculty of Agriculture, Khon Kaen University. Rumen fluid was removed under vacuum pressure via stomach tube into a 2 liter suction flask and transferred into two pre-warmed 1.8 liter vacuum flasks which were then transported to the laboratory for preparation of rumen inoculum.

3.3.2.3 Feedstuff samples and chemical analysis

All test feedstuff samples were dried at 60◦C in hot air oven for 48 h and ground through a 1 mm screen for chemical analysis and *in vitro* gas production technique. The feedstuff 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 determined using the method proposed by Van Soest et al. (1991).

Feed samples of 500 mg (fresh weight basis) were transferred into 50 ml serum bottles (Sommart et al., 2000). The bottles were stoppered with rubber stopper, crimp sealed and incubated in a hot air oven with water bath set at 39˚C. Bottles were pre-warmed for 1 hour at 39˚C and were injected with 40 ml of rumen inoculum prepared as following Sommart et al. (2000).

3.3.2.4 Data collections

1) Gas volume (Gv)

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. Cumulative gas volumes after incubations were fitted using the equation $y = a + b\ (1\text{-}e^{-ct})$

(Ørskov and McDonald, 1979), where y = gas production at time 't', a = the intercept, which ideally reflects the fermentation of the soluble fraction, b= the fermentation of the insoluble fraction, c = rate of gas production, and ($|a|+b$) = potential extent of gas production.

2) Digestibility

After 24 hours of incubation, a 12 bottle sample of each treatment was taken and kept in a freezer to inhibit microbial activity. Subsequently, 8 bottles of each treatment were subjected to *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) determination and 4 bottles were subjected to neutral detergent fiber digestibility (IVNDFD) determination.

To determine the IVDMD, residues of incubated samples in bottles were filtered through a groose crucible and dried at 105◦C in a hot air oven for DM determination. Thereafter, the DM residues were subjected to incineration in a muffle furnace at 550◦C for ash determination in order to evaluate the IVOMD. To determine the IVNDFD, residues of incubated samples in bottles were filtered through a groose crucible. Then, the residues were analyzed for NDF and lastly for ash. The obtained residue DM, ash and NDF data were used for calculation of digestibility according to the following equations:

$$\text{IVDMD (\%)} = \frac{[\text{DM initial incubation (g)} - \text{DM after incubation (g)}]}{\text{DM initial incubation (g)}} \times 100$$

$$\text{IVOMD (\%)} = \frac{[\text{OM initial incubation (g)} - \text{OM after incubation (g)}]}{\text{OM initial incubation (g)}} \times 100$$

$$\text{IVNDFD (\%)} = \frac{[\text{NDF initial incubation (g)} - \text{NDF after incubation (g)}]}{\text{NDF initial incubation (g)}} \times 100$$

3) Estimation of metabolizable energy (ME)

Estimation of ME content was calculated following the equations of Menke et al. (1979); ME (MJ/kg DM) = 2.20+(0.136×Gv)+(0.057×CP) (Eq1) and Menke and Steingass (1988); ME (MJ/kg DM) = 2.20+(0.1357×Gv) + (0.0057×CP) + (0.0002859×EE^2) (Eq2) by using gas volume produced at 24 h of incubation (Gv, ml), crude protein (CP, %) and ether extract (EE, %) as factors of prediction.

Since gas production volume, in this study, was collected from incubation of a 0.5 g of DM, the gas volume was adjusted to be equal to 0.2 g of DM for ME calculation in the above equations. The equation for adjusting is shown as follows:

Gv produced from 0.2 g DM = (0.2× Gv produced from 0.5 g DM)/0.5

3.3.2.5 Statistical analysis

All data obtained from the trials were subjected to the analysis of variance procedure of statistical analysis system (SAS, 1996) according to a Randomized Complete Block Design (RCBD). Means were compared by using the LSMEANS and STDERR statement in PROC GLM. Mean separation were determined using the PDIFF statement in PROC GLM with a $P < 0.05$ significance level. The original model includes the treatment and block effects as follows:

$$\mathbf{Yij} = \mu + \tau i + \beta j + \varepsilon ij$$

Where, **Yij** = observation value in feedstuff sample i, batch of injected rumen inoculum j

μ = over all mean

τi = effect of feedstuff sample i when i = 1, 2,.., 6

βj = effect of batch of injected rumen inoculum j when j = 1, 2, 3 and 4

εij = residual error

3.3.3 Results and Discussion

3.3.3.1 Chemical composition

Chemical compositions of energy feedstuffs are presented in Table 3.3.1. Their classification as energy feed stuffs followed the guideline of Harris et al. (1982) and WTSR (2008), the CP and cell wall (NDF) content of these energy sources were lower than 20 and 35 %, respectively. Rice bran had the highest CP content (14.38 %) while ground corn was at the moderate (8.31 %) and cassava chip, cassava peel and cassava pulp had the lower CP content (1.51, 4.15 and 1.72 %, respectively). Rice bran had CP content slightly higher than reported by Suksombat et al. (2006) and Chumpawadee (2008) (12.10 and 12.77 %, respectively). Cassava chip and cassava by-products such as cassava peel and pulp had CP content below 3 % and these values are

similar to previous reports of Nitipot and Sommart (2003), Suksombat et al. (2006) and Yimmongkol et al. (2007).

Rice bran had a dramatically higher EE content (17.94 %) than any of the other feeds. It had the higher EE content because it consists mainly of pericarp and germ. Other selected energy feedstuffs had a lower EE content and ranged from 0.38 to 5.00 %.

Table 3.3.1 Chemical compositions of energy feed sources

Feedstuff	Chemical composition						
	DM[1],%	CP	EE	Ash	NDF	ADF	ADL
		% of DM					
Broken rice	89.84	7.21	1.21	0.45	3.88	0.66	0.07
Cassava chip	91.40	1.51	0.67	3.87	19.42	8.50	2.50
Cassava peel	93.97	4.15	1.40	6.83	30.50	26.97	12.59
Cassava pulp	91.24	1.72	0.38	6.47	23.15	18.94	5.55
Ground corn	89.28	8.31	5.00	1.35	14.95	2.92	0.10
Rice bran	92.06	14.38	17.94	6.97	17.69	6.84	1.83

[1]DM=dry matter, CP= crude protein, EE = ether extract, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin

The NDF component of broken rice, cassava chip, ground corn and rice bran are below 20 %. In particular, the NDF contents of broken rice (3.88 %) was the lowest in these selected energy feed sources. Broken rice is small fragments of rice kernels separated from milled rice which has been processed through brushing of the grain to polish the kernel of edible rice. Cassava peel and cassava pulp had the higher level of fiber content, and the NDF and ADF were highest for cassava peel, followed by cassava pulp. The NDF and ADF content of cassava peel was similar to that reported by Aregheore (2000) and Nitipot and Sommart (2003) (29.27 to 32.0 and 21.0 to 24.58%, respectively). Cassava peel had especially high lignin content (ADL). The peel of cassava is the outer part attaching to soil, and is normally contaminated. Cassava pulp had NDF and ADF content similar to the study of Nitipot and Sommart (2003) (25.65 and 17.91 %, respectively), but different from Suksombat et al. (2006) (37.6 and 9.8 %, respectively).

Based on this study and reviewed data, the chemical compositions of available energy feedstuffs differ greatly according to the botanical origin (Fevier et al.,

2001) and the processing method of starch extraction (Rakshit, 2003). These factors may partially explain differences in chemical composition between this study and others.

3.3.3.2 *In vitro* digestibility

The digestibility of selected energy feed sources is showed in Table 3.3.2. The IVDMD and IVOMD of energy feedstuffs varied within a narrow range (65.10 to 88.99 and 68.49 to 89.06 %, respectively). The IVDMD and IVOMD were highest ($P<0.01$) in broken rice while it was lowest ($P<0.01$) in cassava peel.

The fiber digestibility (IVNDFD) of energy feedstuffs varied over a wide range from 16.34 to 60.53 %. The IVNDFD of cassava chip was highest ($P<0.01$), cassava peel and broken rice (16.34 and 16.43 %, respectively) were the lowest ($P<0.01$) while cassava pulp, ground corn and rice bran (38.11, 37.15 and 41.53%, respectively) were at the intermediate level.

Table 3.3.2 *In vitro* digestibility, gas production kinetics and calculated ME of energy feed sources

Feedstuffs	Digestibility, %[1]			Kinetic of gas production				ME [2] (MJ/kg DM)	
	IVDMD	IVOMD	IVNDFD	a, ml	b, ml	|a|+b, ml	c, %/h	Eq1	Eq2
Broken rice	88.99^{a}	89.06^{a}	16.43^{c}	-19.14^{e}	222.27^{a}	241.40^{a}	0.045^{d}	9.62^{b}	9.23^{bc}
Cassava chip	84.88^{b}	87.02^{a}	60.53^{a}	-11.42^{c}	193.79^{b}	205.21^{b}	0.084^{b}	10.38^{a}	10.28^{a}
Cassava peel	65.10^{e}	68.49^{c}	16.34^{c}	-10.26^{bc}	158.05^{d}	168.31^{d}	0.082^{b}	9.10^{c}	8.87^{c}
Cassava pulp	76.53^{d}	79.01^{b}	38.11^{b}	-9.00^{b}	180.45^{c}	189.44^{c}	0.066^{c}	9.29^{bc}	9.18^{c}
Ground corn	80.21^{c}	80.22^{b}	37.15^{b}	-15.60^{d}	185.32^{c}	200.92^{b}	0.067^{c}	10.08^{a}	9.64^{b}
Rice bran	77.95^{cd}	78.32^{b}	41.53^{b}	-1.39^{a}	116.36^{e}	118.17^{e}	0.093^{a}	8.52^{d}	7.87^{d}
P value	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
SEM	0.8371	0.8029	2.1874	0.5778	2.7990	3.0936	0.0019	0.1387	0.1378

[2]IVDMD, IVOMD and IVNDFD= *in vitro* DM, OM and NDF digestibility, respectively, [3]calculated ME follow equation of Menke et al. (1979) (Eq1) and Menke and Steingass (1988) (Eq2), $^{a, b, c, d, e, f, g, h, I, j, k, l}$ means within column with different superscripts differ significantly ($P<0.05$)

3.3.3.3 Kinetics of gas production

The kinetics of gas production of selected energy feed sources are presented in Table 3.3.2 and Figure 3.3.1. The *in vitro* gas production technique adapted to describe kinetics of fermentation are based on the modified exponential model $y = a + b\ (1\text{-}e^{-ct})$ (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 compatibility of its parameters with intake, digestibility and

degradation characteristic of feedstuffs had been documented (Blummel and Ørskov, 1993; Khazaal et al., 1993; Sommart et al., 2000; Nitipot and Sommart, 2003).

The value *a*, intercept, of selected energy feed sources varied and ranged from -19.14 to -1.39 ml/0.5g DM substrate. 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 a lag in microbial colonization (Chumpawadee. 2006). It is well known that the value for absolute *a* (|*a*|), ideally reflects the fermentation of the soluble fraction. The feed ranked from highest |*a*| to lowest were broken rice, ground corn, cassava chip, cassava peel, cassava pulp and rice bran.

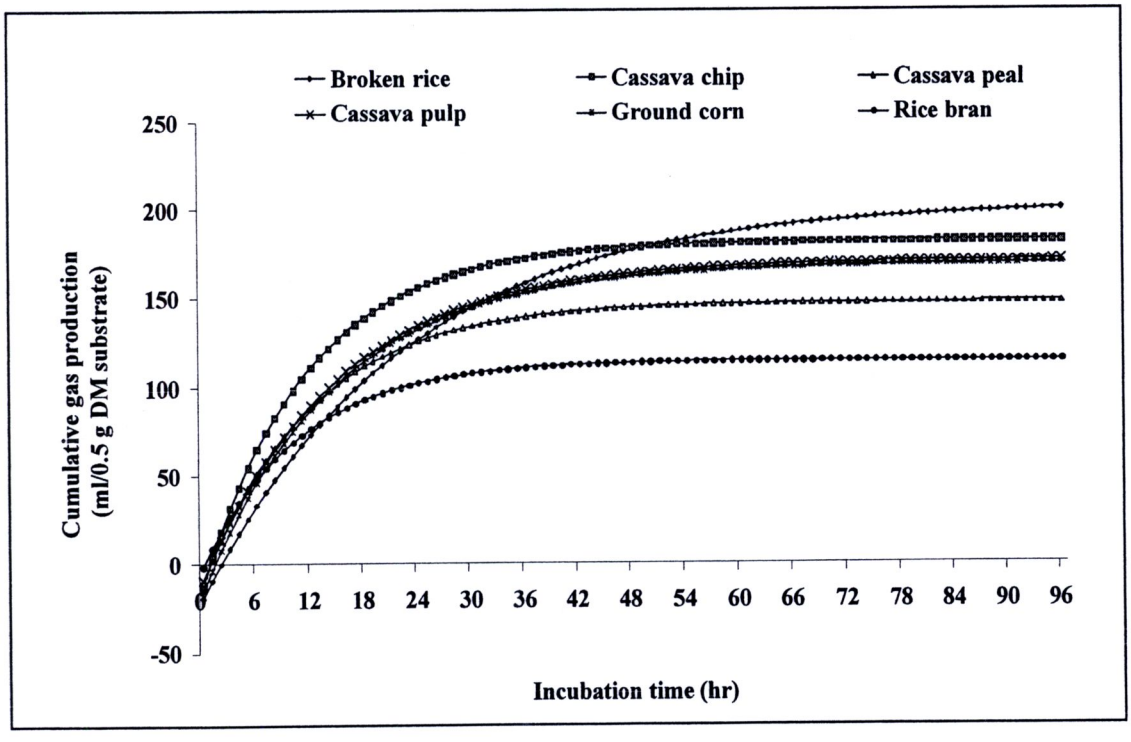


Figure 3.3.1 Cumulative gas volume estimated by $y = a + b\ (1-e^{-ct})$ (ml/0.5g DM substrate) throughout 96 h of incubation of selected energy feed sources

The gas volume at asymptote (*b*) describes the fermentation of the insoluble fraction. Broken rice showed highest ($P<0.01$) *b* value (222.27 ml/0.5g DM substrate) followed by cassava chip, ground corn, cassava pulp and cassava peel whilst rice bran had very low gas volume. This result reflects their chemical composition and digestibility as discussed above.

Potential extent of gas production ($|a|+b$) of energy feed sources, which is the summation of the fermentation of the soluble and insoluble fraction, resulted in a similar pattern to their *b* values.

Rate of gas production (*c*, %/h), is possibly influenced by the carbohydrate fraction's ready availability to the microbial population. Rate of gas production of rice bran was highest in the selected energy feed sources, and in broken rice was the lowest. Cassava chip and cassava peel were not different ($P>0.05$) in their rate of gas production while cassava pulp had a similar rate of gas production to ground corn.

3.3.3.4 Calculated metabolizable energy

The calculated metabolizable energy of energy source feedstuffs is presented in Table 3.3.2. Estimation of ME content was calculated following the equations of Menke et al. (1979); ME (MJ/kg DM) = $2.20+(0.136\times Gv)+(0.057\times CP)$ (Eq1) and Menke and Steingass (1988); ME (MJ/kg DM) = $2.20+(0.1357\times Gv) + (0.0057\times CP) + (0.0002859\times EE^2)$ (Eq2) by using gas volume produced at 24 h of incubation (Gv, ml), crude protein (CP, %) and ether extract (EE, %) as factors of prediction. From the equations, therefore, Gv at 24 h of incubation, CP and EE content have a positive effect on estimated ME.

The calculated metabolizable energy of cassava chip was not different ($P>0.05$) from ground corn. Both of them had the highest calculated metabolizable energy in the selected energy feed sources. Although broken rice had the highest IVDMD, IVOMD, fermentation of the insoluble fraction (*b*) and potential extent of gas production ($|a|+b$), it had lower calculated metabolizable energy than cassava chip and broken rice. This might be due to the slower rate of gas production (*c*) providing less gas volume at 24 h of incubation. However, broken rice had greater calculated metabolizable energy than cassava peel and rice bran.

The ME content of cassava chip was the highest, even though it is slightly lower than that reported by Holzer et al. (1997) (12.80 MJ/ kg DM). However, it was higher than calculated ME in the study of Suksombat et al. (2006) (10.04 MJ/ kg DM). The ME content of cassava pulp in this study was higher than that reported by Suksombat et al. (2006) who observed calculated ME as 9.75 MJ/ kg DM.

3.3.4 Conclusion

Chemical composition differs among the different energy feed sources. The CP and cell wall content (NDF) of these energy sources were lower than 20 and 35 %, respectively, classifying them as energy feed stuffs. The digestibility of selected energy feedstuffs, IVDMD and IVOMD were highest in broken rice while the lowest was cassava peel. The IVNDFD was highest in cassava chip. Kinetics of gas production also differs among the different energy feed sources, and the potential extent of gas production, ranked from the highest to the lowest were; broken rice, cassava chip, ground corn, cassava pulp, cassava peel and rice bran. The calculated metabolizable energy of cassava chip was highest and not different from ground corn, and followed by broken rice, cassava pulp, cassava peel and rice bran. Because cassava chip, cassava pulp and cassava peel are available locally and are inexpensive, they are the best potential energy source for beef and dairy cattle.

3.4 EXPERIMENT 1.4: NUTRITIVE EVALUATION OF PROTEIN SOURCE FEEDSTUFFS

3.4.1 Introduction

Protein or the nitrogen component is one of the limiting factors in ruminant metabolism. The nitrogen component of the diet supports the protein metabolism of the rumen organisms and their host. It is involved in the maintenance and formation of all the body tissues and organs, and other physiological and biochemical processes in the body (Yuangklang, 2008) and the primary component associated with the majority of animal products (Kellems and Church, 2002). In tropical areas such as Thailand, traditionally the farmers feed their cattle with green forage in natural pasture as the main feed resource (Sruamsiri, 2008). They face a shortage of both quantity and quality of roughage, especially in the dry season. Low quality roughage fed solely might contain inadequate protein for animals to meet their requirement. Also, most energy

supplements supply some protein, but usually not enough to meet total needs. Thus a protein source is needed to supplement or to mix into the ration.

Brewery waste, kapok seed meal, tomato pomace, and soybean meal are available in markets and used by farmers as protein source feedstuffs. Their chemical compositions and nutritive values such as digestibility and energy content are essential data for farmers for diet formulation to meet animal requirements. The chemical compositions such as proximate analysis (AOAC, 1990) and fiber (Van Soest, 1991) are available in many sources such as the WTSR (2008) table. However, there is meagre digestibility data and very limited energy content information, especially metabolizable energy (ME). In order to determine feed digestibility and energy content, evaluation of feedstuffs for the whole tract and ruminal digestion through feeding experiments is possible, but is expensive and requires sophisticated laboratory and animal facilities (Krishnamoorthy et al., 1995).

In vitro gas production technique, nowadays, is widely used to evaluate feed digestibility and kinetics of gas production. Moreover, this technique can be used to determine ME content by prediction equations using chemical composition and gas production (Menke et al., 1979; Menke and Steingass, 1988). It is a useful technique to use as a screening test for potential feedstuffs for ruminants, and is less expensive, easy and suitable for use in developing countries (Chumpawadee et al., 2006b). The objectives of this experiment, therefore, were to determine chemical composition, nutritive values and to compare digestibility, kinetics of gas production and metabolizable energy of selected protein source feedstuffs using *in vitro gas* production technique.

3.4.2 Materials and Methods

3.4.2.1 Experimental design and treatment

The experiment was arranged into randomized complete block design. There were 4 replicates for kinetics of gas production determination, 8 replicates for *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) evaluation and 4 replicates for *in vitro* neutral detergent fiber digestibility (IVNDFD) evaluation.

Five feedstuffs in the experiment were collected from 1) Nam Pong Dairy Co-operative Limited, Khon Kaen Province, and 2) Laboratory of Khon Kaen Animal Nutrition Research and Development Center, Khon Kaen. The selected protein

source feedstuffs were dried brewery waste, wet brewery waste, kapok seed meal, tomato pomace, and soybean meal. Feedstuff treatments were considered to be protein source feedstuffs following the guideline of Harris et al. (1982) and WTSR (2008) where they are classified by their crude protein (CP) content which is higher than 20 % of dry matter.

3.4.2.2 Animals

Three male crossbred Holstein Friesians of body weight approximately 200 kg were used as the source of rumen inoculum. The animals were maintained on dried chopped sweet sorghum *(Sorghum bicolor)* as a roughage source at a Department of Animal Science's experimental station, Faculty of Agriculture, Khon Kaen University. Rumen fluid was removed under vacuum pressure via stomach tube into a 2 liter suction flask and transferred into two pre-warmed 1.8 liter vacuum flasks which were then transported to the laboratory for preparation of rumen inoculum.

3.4.2.3 Feedstuff samples and chemical analysis

All test feedstuff samples were dried at 60◦C in hot air oven for 48 h and ground through a 1 mm screen for chemical analysis and *in vitro* gas production technique. The feedstuff 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 determined using the method proposed by Van Soest et al. (1991).

Feed samples of 500 mg (fresh weight basis) were transferred into 50 ml serum bottles (Sommart et al., 2000). The bottles were stoppered with rubber stopper, crimp sealed and incubated in a hot air oven with water bath set at 39°C. Bottles were pre-warmed for 1 hour at 39°C and were injected with 40 ml of rumen inoculum prepared as following Sommart et al. (2000).

3.4.2.4 Data collections

1) Gas volume (Gv)

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. Cumulative gas volumes after incubations were fitted using the equation $y = a + b\ (1\text{-}e^{-ct})$

(Ørskov and McDonald, 1979), where y = gas production at time 't', a = the intercept, which ideally reflects the fermentation of the soluble fraction, b= the fermentation of the insoluble fraction, c = rate of gas production, and ($|a|+b$) = potential extent of gas production.

2) Digestibility

After a 24 hour of incubation, a 12 bottle sample of each treatment was taken and kept in a freezer to inhibit microbial activity. Subsequently, 8 bottles of each treatment were subjected to *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) determination and 4 bottles were subjected to neutral detergent fiber digestibility (IVNDFD) determination.

To determine the IVDMD, residues of incubated samples in bottles were filtered through a groose crucible and dried at 105◦C in a hot air oven for DM determination. Thereafter, the DM residues were subjected to incineration in a muffle furnace at 550◦C for ash determination in order to evaluate the IVOMD. To determine the IVNDFD, residues of incubated samples in bottles were filtered through a groose crucible. Then, the residues were analyzed for NDF and lastly for ash. The obtained residue DM, ash and NDF data were used for calculation of digestibility according to the following equations:

$$\text{IVDMD (\%)} = \frac{[\text{DM initial incubation (g)} - \text{DM after incubation (g)}] \times 100}{\text{DM initial incubation (g)}}$$

$$\text{IVOMD (\%)} = \frac{[\text{OM initial incubation (g)} - \text{OM after incubation (g)}] \times 100}{\text{OM initial incubation (g)}}$$

$$\text{IVNDFD (\%)} = \frac{[\text{NDF initial incubation (g)} - \text{NDF after incubation (g)}] \times 100}{\text{NDF initial incubation (g)}}$$

3) Estimation of metabolizable energy (ME)

Estimation of ME content was calculated following the equations of Menke et al. (1979); ME (MJ/kg DM) = 2.20+(0.136×Gv)+(0.057×CP) (Eq1) and Menke and Steingass (1988); ME (MJ/kg DM) = 2.20+(0.1357×Gv) + (0.0057×CP) + (0.0002859×EE^2) (Eq2) by using gas produced at 24 h of incubation (Gv, ml), crude protein (CP, %) and ether extract (EE, %) as factors of prediction.

Since gas volume, in this study, was collected from incubation of a 0.5 g of DM, the gas volume was adjusted to be equal to 0.2 g of DM for ME calculation in the above equations. The equation for adjusting is shown as follows:

Gv produced from 0.2 g DM = (0.2× Gv produced from 0.5 g DM)/0.5

3.4.2.5 Statistical analysis

All data obtained from the trials were subjected to the analysis of variance procedure of statistical analysis system (SAS, 1996) according to a Randomized Complete Block Design (RCBD). Means were compared by using the LSMEANS and STDERR statement in PROC GLM. Mean separation were determined using the PDIFF statement in PROC GLM with a $P < 0.05$ significance level. The original model includes the treatment and block effects as follows:

$$\mathbf{Yij} = \mu + \tau i + \beta j + \varepsilon ij$$

Where, **Yij** = observation value in feedstuff sample i, batch of injected rumen inoculum j

μ = over all mean

τ**i** = effect of feedstuff sample i when i = 1, 2,.., 5

ßj = effect of batch of injected rumen inoculum j when j = 1, 2, 3 and 4

ε**ij** = residual error

3.4.3 Results and Discussion

3.4.3.1 Chemical composition

Chemical compositions of selected protein source feedstuffs are presented in Table 3.4.1. These feedstuffs are classified as protein sources following the guideline of Harris et al. (1982) and WTSR (2008) where protein source feedstuffs have CP content higher than 20 %.

Soybean meal had the highest CP content (50.32 %), exceeding that in WTSR (2008) table (approximately 45 to 47 %). However, these values agree with Kellems and Church (2002) who stated that protein content of soybean meal is standardized at 44 to 50 % as fed basis. The dried and wet brewery waste had CP content approximately 30 %. It was higher than reported by Kellems and Church (2002) and

WTSR (2008) table (25.0 and 26.0 %, respectively), but similar to NRC (2000) and Chumpawadee (2009) (29.2 and 29.1%, respectively). Kapok seed meal had CP content similar to WTSR (2008) table (31.9 %). Tomato pomace had the lowest CP content (21.01 %) in the selected protein source feedstuffs and agrees with NRC (2001) and Chumpawadee (2009) (19.3 and 23.7 %, respectively).

All selected protein source feedstuffs had high fiber content (NDF, ADF and ADL), except for soybean meal which had the lowest fiber content (13.51, 10.05 and 0.06 %, respectively). The dried and wet brewery waste had NDF content of 64.24, and 73.78 %, respectively. It was dramatically higher than NRC (2000) and WTSR (2008) table (48.7 and 50.7 %, respectively). But its ADF content was similar to these sources of information (31.2 and 22.8%, respectively). Tomato pomace had NDF, ADF and ADL content higher than in NRC (2001) table (60.0, 47.6 and 13.3 %, respectively) and the report of Chumpawadee (2008) (50.0, 36.6 and 26.7 %, respectively). Kapok seed meal had NDF, ADF and ADL content at a moderate level (39.97, 34.25 and 20.89 %) in selected protein sources. It was higher than the values in WTSR (2008) table (31.4, 27.8 and 14.2 %, respectively).

In this study and reviewed data, the chemical compositions of some commercially available feeds differ greatly according to the species and the processing, method of manufacture, temperature and duration of heating. Furthermore, nutritive values of meals can differ significantly from the mean value given in tables of feedstuff composition (Fevier et al., 2001). These factors may partially explain differences in chemical composition between this study and others.

Table 3.4.1 Chemical composition of protein source feedstuffs

Feedstuff	Chemical composition						
	DM[1],%	CP	EE	Ash	NDF	ADF	ADL
		% of DM					
Brewery waste (dried)	92.71	31.95	7.70	4.38	64.24	24.27	3.49
Brewery waste (wet)	95.89	32.58	12.29	2.49	73.78	22.95	3.63
Kapok seed meal	91.55	30.73	7.36	8.06	39.97	34.25	20.89
Soybean meal	92.81	50.32	1.13	4.30	13.51	10.05	0.06
Tomato pomace	93.40	21.01	0.97	4.49	62.98	44.95	21.11

[1]DM=dry matter, CP= crude protein, EE = ether extract, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin

3.4.3.2 *In vitro* digestibility

The digestibility of selected protein feedstuffs is shown in Table 3.4.2. The IVDMD, IVOMD and IVNDFD of protein feedstuffs varied over a wide range from 20.95 to 81.65, 21.23 to 83.30 and 7.10 to 59.45 %, respectively. The digestibility (IVDMD, IVOMD and IVNDFD) of soybean meal was highest (P<0.01) followed by tomato pomace (49.10, 49.93 and 29.11 %, respectively). The IVDMD and IVOMD of dried brewery waste (33.09 and 33.66 %, respectively) were higher (P<0.01) than that of wet brewery waste (20.95 and 21.23 %, respectively), however, the IVNDFD of these feedstuffs (22.19 and 21.45 %, respectively) was not significantly different. The IVDMD and IVOMD of kapok seed meal were at the moderate level (38.29 and 38.52 %, respectively) but its IVNDFD (7.10 %) was lowest (P<0.01).

Table 3.4.2 *In vitro* digestibility, gas production kinetics and calculated ME of protein source feedstuffs

Feedstuffs	Digestibility, %[1]			Kinetic of gas production				ME[2] (MJ/kg DM)	
	IVDMD	IVOMD	IVNDFD	a, ml	b, ml	|a|+b, ml	c, %/h	Eq1	Eq2
Brewery waste (dried)	33.09^{d}	33.66^{d}	22.19^{c}	-0.79^{a}	72.66^{b}	75.13^{b}	0.034^{e}	5.99^{b}	4.36^{bc}
Brewery waste (wet)	20.95^{e}	21.23^{e}	21.45^{c}	-0.19^{a}	39.91^{c}	40.51^{c}	0.040^{d}	5.42^{b}	3.79^{c}
Kapok seed meal	38.29^{c}	38.52^{c}	7.10^{d}	1.59^{a}	44.85^{c}	46.44^{c}	0.071^{a}	5.98^{b}	4.42^{bc}
Soybean meal	81.65^{a}	83.30^{a}	59.45^{a}	0.26^{a}	118.01^{a}	119.34^{a}	0.059^{b}	9.18^{a}	6.59^{a}
Tomato pomace	49.10^{b}	49.93^{b}	29.11^{b}	-3.96^{b}	82.82^{b}	87.118^{b}	0.051^{c}	6.58^{b}	5.49^{ab}
P value	<.0001	<.0001	0.0001	0.0088	<.0001	<.0001	<.0001	0.0002	0.0022
SEM	0.7959	0.7082	2.1122	0.8207	4.0148	4.6284	0.0016	0.3935	0.3938

[1]IVDMD, IVOMD and IVNDFD= *in vitro* DM, OM and NDF digestibility, respectively,
[2]calculated ME follow equation of Menke et al. (1979) (Eq1) and Menke and Steingass (1988) (Eq2),
$^{a, b, c, d, e, f, g, h, I, j, k, l}$ means within column with different superscripts differ significantly (P<0.05)

3.4.3.3 Kinetics of gas production

The kinetics of gas production of selected protein feed sources are presented in Table 3.4.2 and Figure 3.4.1. The *in vitro* gas production technique has been adapted to describe kinetics of fermentation based on the modified exponential model $y = a + b\ (1\text{-}e^{-ct})$ (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 compatibility of its parameters with intake, digestibility

and degradation characteristic of feedstuffs has been documented (Blummel and Ørskov, 1993; Khazaal et al., 1993; Sommart et al., 2000; Nitipot and Sommart, 2003).

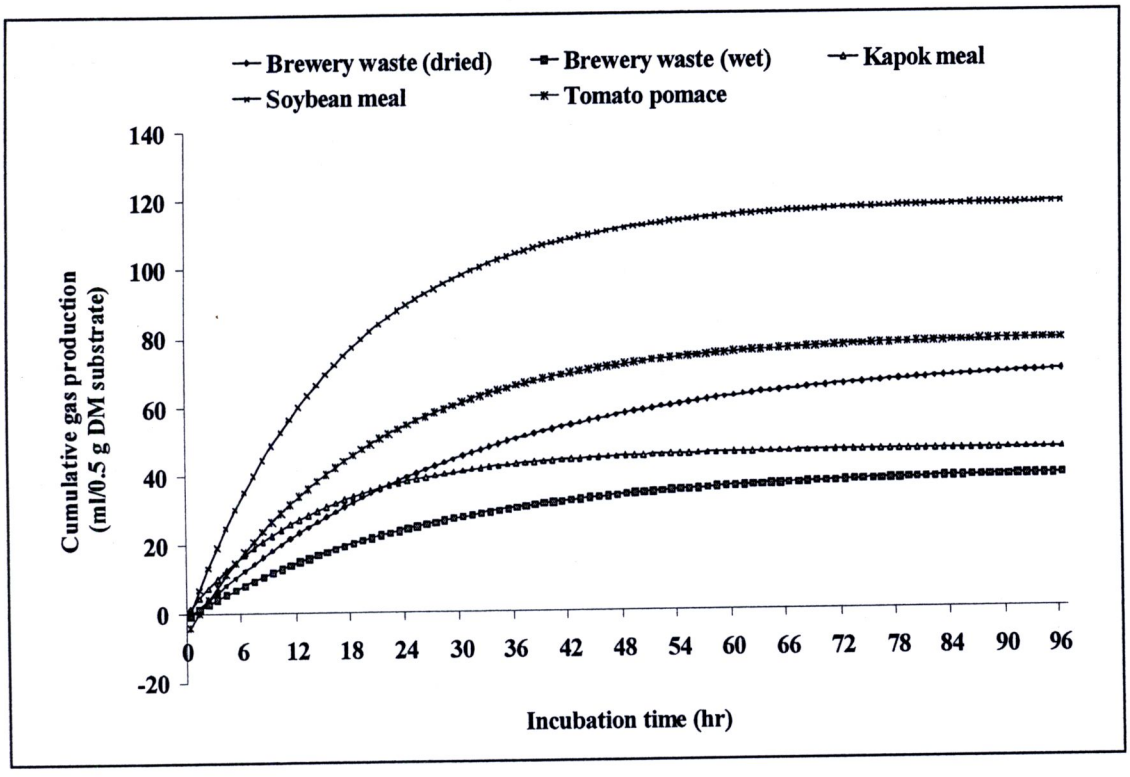


Figure 3.4.1 Cumulative gas volume estimated by $y = a + b\ (1\text{-}e^{-ct})$ (ml/0.5g DM substrate) throughout 96 h of incubation of protein source feedstuffs.

The value *a*, intercept, of selected protein source feedstuffs varied over a narrow ranged from -3.96 to 1.59 ml/0.5g DM substrate. 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 a lag in microbial colonization (Chumpawadee, 2006). It is well known that the value for absolute *a* (|*a*|), described ideally, reflects the fermentation of the soluble fraction. The |*a*| value was highest in tomato pomace There were no significant differences among dried and wet brewery waste, kapok seed meal and soybean meal.

THE NATIONAL RESEARCH COUNCIL OF THAILAND
RESEARCH LIBRARY

The gas volume at asymptote (*b*) describes the fermentation of the insoluble fraction. Soybean meal showed highest ($P<0.01$) *b* value (118.01 ml/0.5g DM substrate) followed by tomato pomace and dried brewery waste whilst wet brewery waste and kapok seed meal had the lowest gas volume.

Potential extent of gas production ($|a|+b$) of these selected protein feeds found by the summation of the fermentation of the soluble and insoluble fractions resulted in a pattern similar to their *b* values.

3.4.3.4 Calculated metabolizable energy

The calculated metabolizable energy of protein source feedstuffs is presented in Table 3.4.2. Estimation of ME content was calculated following the equations of Menke et al. (1979); ME (MJ/kg DM) = 2.20+(0.136×Gv)+(0.057×CP) (Eq1) and Menke and Steingass (1988); ME (MJ/kg DM) = 2.20+(0.1357×Gv) + (0.0057×CP) + (0.0002859×EE^2) (Eq2) by using gas produced at 24 h of incubation (Gv, ml), crude protein (CP, %) and ether extract (EE, %) as factors of prediction. From the equations, therefore, Gv at 24 h of incubation, CP and EE content positively affect estimated ME.

Soybean meal had the highest ($P<0.01$) calculated metabolizable energy (9.18 MJ/kg DM). All of the others were not significant different. The higher calculated metabolizable energy of soybean meal reflected the higher digestibility and rate of gas production (*c*) which affected the gas volume at 24 h of incubation. Both gas volume at 24 h of incubation and CP and EE content positively affect estimated ME.

The estimated metabolizable energy of soybean meal and tomato pomace in this study is higher than previous studies using the same procedure by Chumpawadee et al. (2007a,b) (8.10 and 4.89 MJ/kg DM, respectively). However, the estimated metabolizable energy of dried and wet brewery waste in this study is similar to the finding of Chumpawadee et al. (2007b) (5.39 MJ/kg DM). Compared to Chumpawadee et al. (2007a,b) experiments, it can be seen that the investigated feedstuffs in this study had a similar CP content but differed in gas produced at 24 h (data not shown). This difference and variation among laboratories may cause the variation in calculated metabolizable energy across studies.

3.4.4 Conclusion

Chemical compositions differ among the different protein source feedstuffs. These selected feed sources had CP content higher than 20% and were classified as protein feedstuffs. Soybean meal had the lowest fiber content whilst the other feeds had high fiber content. In terms of digestibility, soybean meal showed the highest in DM, OM and NDF digestibility. Kapok seed meal showed the lowest IVNDFD. This study demonstrates that kinetics of gas production of protein feeds differs among feeds. Based on this study, potential of fermentation for protein feeds used for ruminants ranked from the highest to the lowest are; soybean meal, tomato pomace, dried brewery waste, wet brewery waste and kapok seed meal. Soybean meal demonstrated a higher estimated metabolizable energy than the others.

3.5 EXPERIMENT 1.5: NUTRITIVE EVALUATION OF WHOLE OILSEED FEEDSTUFFS

3.5.1 Introduction

Oilseeds are high in protein and fat and able to be used as an alternative protein/energy source for cattle. Feeding cattle directly without processing may be beneficial for farmers both in terms of nutritive value and market price. Arieli (1998) reviewed that oilseed such as whole cotton seed has advantages in reducing methane production and heat increment. The low heat increment of whole cotton seed makes it potentially valuable in hot weather. In Thailand, likely oilseeds are included not only whole cotton seed but also kapok seed, soybean seed and peanut seed with husk. Although utilization of whole cotton seed as ruminant feed has been investigated (Solaiman et al., 2002; Zhang et al 2007; Sommart et al. and 1999 Wongnen, 2009), for the remainder, there is very limited information about their nutritive values.

Chemical compositions, nutritive value, digestibility and energy content of feedstuffs are essential data for farmers to use for diet formulation to meet animal requirements. Chemical compositions such as proximate analysis (AOAC, 1990); and fiber (Van Soest, 1991) are available in many sources such as the NRC (2000), NRC (2001) and WTSR (2009) table. However, there is meagre digestibility data and very limited energy content information, especially metabolizable energy (ME). In order to

determine digestibility and energy content, much investment in terms of time, labor and finance is required.

In vitro gas production technique, nowadays, is widely used for evaluating feed digestibility and kinetics of gas production. Moreover, this technique can be used to determine ME content by prediction equation using chemical composition and gas production (Menke et al., 1979; Menke and Steingass, 1988). It is a useful technique to use as screening test in searching for potential feedstuffs for ruminants, being less expensive, easy and suitable for use in developing countries (Chumpawadee et al., 2006b). The objectives of this experiment, therefore, were to determine chemical composition and nutritive values and to compare digestibility, kinetics of gas production and metabolizable energy of selected oilseeds using *in vitro gas* production technique.

3.5.2 Materials and Methods

3.5.2.1 Experimental design and treatment

The experiment was arranged into randomized complete block design. There were 4 replicates for kinetics of gas production determination, 8 replicates for *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) evaluation and 4 replicates for *in vitro* neutral detergent fiber digestibility (IVNDFD) evaluation.

Whole cotton seed, kapok seed, peanut seed with pod husk, and soybean seed used in the experiment were collected from Experimental Station, Department of Animal Science, Faculty of Agriculture, Khon Kaen University. Peanut seed with pod husk used in this experiment had different physical characteristics from the other treatments, where it was covered with pod husk as at harvest. However, this pod husk can be utilized in ruminants as a fiber source, and may be suitable to use as ruminant feed. This feed was included and run as an oilseed feedstuff.

3.5.2.2 Animals

Three male crossbred Holstein Friesians of body weight approximately 200 kg were used as the source of rumen inoculum. The animals were maintained on dried chopped sweet sorghum *(Sorghum bicolor)* as a roughage source at a Department of Animal Science's experimental station, Faculty of Agriculture, Khon Kaen University. Rumen fluid was removed under vacuum pressure via stomach tube into a 2 liter suction

flask and transferred into two pre-warmed 1.8 liter vacuum flasks which were then transported to the laboratory for preparation of rumen inoculum.

3.5.2.3 Feedstuff samples and chemical analysis

All test feedstuff samples were dried at 60◦C in hot air oven for 48 h and ground through a 1 mm screen for chemical analysis and *in vitro* gas production technique. The feedstuff 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 determined using the method proposed by Van Soest et al. (1991).

Feed samples of 500 mg (fresh weight basis) were transferred into 50 ml serum bottles (Sommart et al., 2000). The bottles were stoppered with rubber stopper, crimp sealed and incubated in a hot air oven with water bath set at 39˚C. Bottles were pre-warmed for 1 hour at 39˚C and were injected with 40 ml of rumen inoculum prepared as following Sommart et al. (2000).

3.5.2.4 Data collections

1) Gas volume (Gv)

The rate of gas production was measured by reading and recording the 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. Cumulative gas volumes after incubations were fitted using the equation $y = a + b\ (1\text{-}e^{-ct})$ (Ørskov and McDonald, 1979), where y = gas production at time 't', a = the intercept, which ideally reflects the fermentation of the soluble fraction, b= the fermentation of the insoluble fraction, c = rate of gas production, and ($|a|+b$) = potential extent of gas production.

2) Digestibility

After a 24 hour of incubation, a 12 bottle sample of each treatment was taken and kept in a freezer to inhibit microbial activity. Subsequently, 8 bottles of each treatment were subjected to *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) determination and 4 bottles were subjected to neutral detergent fiber digestibility (IVNDFD) determination.

To determine the IVDMD, residues of incubated samples in bottles were filtered through a groose crucible and dried at 105◦C in a hot air oven for DM determination. Thereafter, the DM residues were subjected to incineration in a muffle furnace at 550◦C for ash determination in order to evaluate the IVOMD. To determine the IVNDFD, residues of incubated samples in bottles were filtered through a groose crucible. Then, the residues were analyzed for NDF and lastly for ash. The obtained residue DM, ash and NDF data were used for calculation of digestibility according to the following equations:

$$\text{IVDMD (\%)} = \frac{[\text{DM initial incubation (g)} - \text{DM after incubation (g)}] \times 100}{\text{DM initial incubation (g)}}$$

$$\text{IVOMD (\%)} = \frac{[\text{OM initial incubation (g)} - \text{OM after incubation (g)}] \times 100}{\text{OM initial incubation (g)}}$$

$$\text{IVNDFD (\%)} = \frac{[\text{NDF initial incubation (g)} - \text{NDF after incubation (g)}] \times 100}{\text{NDF initial incubation (g)}}$$

3) Estimation of metabolizable energy (ME)

Estimation of ME content was calculated following the equations of Menke et al. (1979); ME (MJ/kg DM) = 2.20+(0.136×Gv)+(0.057×CP) (Eq1) and Menke and Steingass (1988); ME (MJ/kg DM) = 2.20+(0.1357×Gv) + (0.0057×CP) + (0.0002859×EE^2) (Eq2) by using gas volume at 24 h of incubation (Gv, ml), crude protein (CP, %) and ether extract (EE, %) as factors of prediction.

Since gas volume, in this study, was collected from incubation of a 0.5 g of DM, the gas volume was adjusted to be equal to 0.2 g of DM for ME calculation in the above equations. The equation for adjusting is shown as follows:

Gv produced from 0.2 g DM = (0.2× Gv produced from 0.5 g DM)/0.5

3.5.2.5 Statistical analysis

All data obtained from the trials were subjected to the analysis of variance procedure of statistical analysis system (SAS, 1996) according to a Randomized Complete Block Design (RCBD). Means were compared by using the LSMEANS and STDERR statement in PROC GLM. Mean separation were determined using the PDIFF

statement in PROC GLM with a $P < 0.05$ significance level. The original model includes the treatment and block effects as follows:

$$\mathbf{Yij = \mu + \tau i + \beta j + \varepsilon ij}$$

Where, **Yij** = observation value in feedstuff sample i, batch of injected rumen inoculum j

μ = over all mean

τi = effect of feedstuff sample i when i = 1, 2,.., 5

ßj = effect of batch of injected rumen inoculum j when j = 1, 2, 3 and 4

εij = residual error

3.5.3 Results and Discussion

3.5.3.1 Chemical composition

Chemical compositions of selected oilseeds are presented in Table 3.5.1. The CP contents of whole cotton seed, kapok seed, peanut seed with pod husk and soybean seed were 33.59, 28.52, 28.75 and 38.91 %, respectively. The CP content of whole cotton seed is greater than previously reported in Thailand such as Chumpawadee et al. (2005a), WTSR (2008) and Wongnen (2008) (21.8, 19.8 and 21.3 %, respectively). Also, it was greater than foreign reports of Luginbuhl et al, (2000) and Solaiman et al. (2002) (24.0 and 23.5 %, respectively). These values agree with Zhang et al. (2007) who reviewed that CP content of whole cotton seed ranged from 25.1 to 31.9 %. The CP content of kapok seed was slightly lower than kapok seed meal in previous reports of Chumpawadee et al. (2007b) (24.01 %). Raw soybean seed had CP content slightly higher than the study of Nasri et al. (2008) (36.25 %) but similar to Banta et al. (2008) (39.0 %).

Table 3.5.1 Chemical composition of selected oilseeds

Feedstuff	Chemical composition						
	DM[1],%	CP	EE	Ash	NDF	ADF	ADL
		% of DM					
Whole cotton seed	95.60	33.59	27.80	8.14	22.54	15.09	6.59
Kapok seed	96.51	28.52	22.71	6.18	32.65	29.81	16.87
Peanut seed with pod husk	95.07	28.75	32.03	3.79	27.63	21.02	6.06
Soybean seed	97.03	38.91	20.83	5.48	15.46	10.57	0.40

[1]DM=dry matter, CP= crude protein, EE = ether extract, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin

Ether extract (EE) content of selected oilseeds was higher than 20 %. The EE content as ranked from the lowest to the highest were soybean seed, kapok seed, whole cotton seed and peanut with husk (20.83, 22.71, 27.80 and 32.03 %, respectively). The EE content of raw soybean seed was similar to Banta et al. (2008) and WTSR (2008) (21.4 and 19.8 %, respectively) but higher than Nasri et al. (2008) (18.0 %). It agrees with the work of Kellems and Church (2002) who reported that the whole soybean seed contained 15 to 20 % oil, which normally was removed by solvent extraction during the preparation of the meal. Whole cotton seed had EE contents higher than those reported by Mabjeesh et al. (1998) (20.4 %), Zhang et al. (2007) (18.9-26.5 %) and Wongnen (2009) (20.2 %)

Kapok seed had the highest NDF, ADF and ADL content (32.65, 29.81 and 16.87 %, respectively) while these were lowest in soybean seed (15.46, 10.57 and 0.40 %, respectively). Although peanut seed was included with husk, the fiber content was lower than kapok seed. The NDF and ADF content of whole cotton seed in this study (22.54 %) was higher than found by Chumpawadee et al. (2005a) (52.3 %) and Zhang et al. (2007) who found that it ranged from 36.3 to 55.4 5 and 27.2 to 44.9 %, respectively. Soybean seed had NDF content lower than reported by Nasri et al. (2008) while ADF component was similar (23.0 and 11.0 %, respectively).

3.5.3.2 *In vitro* digestibility

The *in vitro* digestibility of selected oilseed feedstuffs is shown in Table 3.5.2. The IVDMD and IVOMD varied over a wide range from 49.54 to 93.01 and 51.07 to 93.27 %, respectively. The IVDMD and IVOMD were highest ($P<0.01$) in soybean seed, and lowest ($P<0.01$) in whole cotton seed and kapok seed. The IVDMD

and IVOMD results in this study are dramatically higher than those of Chumpawadee et al. (2007a) for essentially the same full fat soybean feed (57.42 and 57.55 %, respectively). On the other hand, the IVDMD and IVOMD of kapok seed were similar to previous studies of Chumpawadee et al. (2007b) (55.20 and 57.35 %, respectively). Kapok seed had the highest NDF ADF and ADL content, especially ADL which is considered to be an indigestible fraction and prohibits the digestion of other nutrients. This might cause the low digestibility. Moreover, results suggested that even though peanut used in this study included seed and husk, its IVDMD and IVOMD were not less than that of whole cotton seed and kapok seed which are whole oilseeds.

The IVNDFD also varied over a wide range from 6.41 to 57.61 %. It was highest (P<0.01) in soybean seed while it was lowest (P<0.01) in kapok seed which was not significantly differ from peanut seed with husk (7.02 %). As suggested above, the high ADL of Kapok seed may be the cause the low NDF digestibility.

Table 3.5.2 *In vitro* digestibility, gas production kinetics and calculated ME of selected oilseeds

Feedstuffs	Digestibility, %[2]			Kinetic of gas production				ME [3] (MJ/kg DM)	
	IVDMD	IVOMD	IVNDFD	*a*, ml	*b*, ml	|*a*|+*b*, ml	*c*, %/h	Eq1	Eq2
Whole cotton seed	49.34^{c}	51.07^{c}	26.88^{b}	5.04^{a}	44.57^{b}	49.32^{b}	0.040^{b}	5.76^{b}	4.26^{c}
Kapok seed	51.90^{c}	52.13^{c}	6.41^{c}	3.41^{b}	29.92^{d}	33.33^{d}	0.068^{a}	5.19^{c}	3.87^{d}
Peanut seed with husk	63.68^{b}	64.37^{b}	7.02^{c}	3.62^{b}	39.78^{c}	43.39^{c}	0.068^{a}	5.65^{b}	4.47^{b}
Soybean seed	93.01^{a}	93.27^{a}	57.61^{a}	1.40^{c}	88.77^{a}	90.17^{a}	0.065^{a}	8.17^{a}	6.29^{a}
P value	<.0001	<.0001	0.0001	0.0002	<.0001	<.0001	0.0013	<.0001	<.0001
SEM	1.0913	0.9945	1.8672	0.2497	1.3101	1.2041	0.0030	0.0530	0.0527

[1]IVDMD, IVOMD and IVNDFD= *in vitro* DM, OM and NDF digestibility, respectively,
[2]calculated ME follow equation of Menke et al. (1979) (Eq1) and Menke and Steingass (1988) (Eq2),
[a, b, c, d, e, f, g, h, I, j, k, l] means within column with different superscripts differ significantly (P<0.05)

3.5.3.3 Kinetics of gas production

The kinetics of gas production are presented in Table 3.5.2 and Figure 3.5.1. The *in vitro* gas production technique is adapted to describe kinetics of fermentation that are based on the modified exponential model $y = a + b(1-e^{-ct})$ (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

compatibility of its parameters with intake, digestibility and degradation characteristic of feedstuffs had been documented (Blummel and Ørskov, 1993; Khazaal et al., 1993; Sommart et al., 2000; Nitipot and Sommart, 2003).

The value *a* ideally reflects the fermentation of the soluble fraction. All selected oilseeds exhibited a positive *a* value. The value *a* of selected oilseeds as ranked from lowest to highest were soybean seed, kapok seed, peanut seed with husk and whole cotton seed.

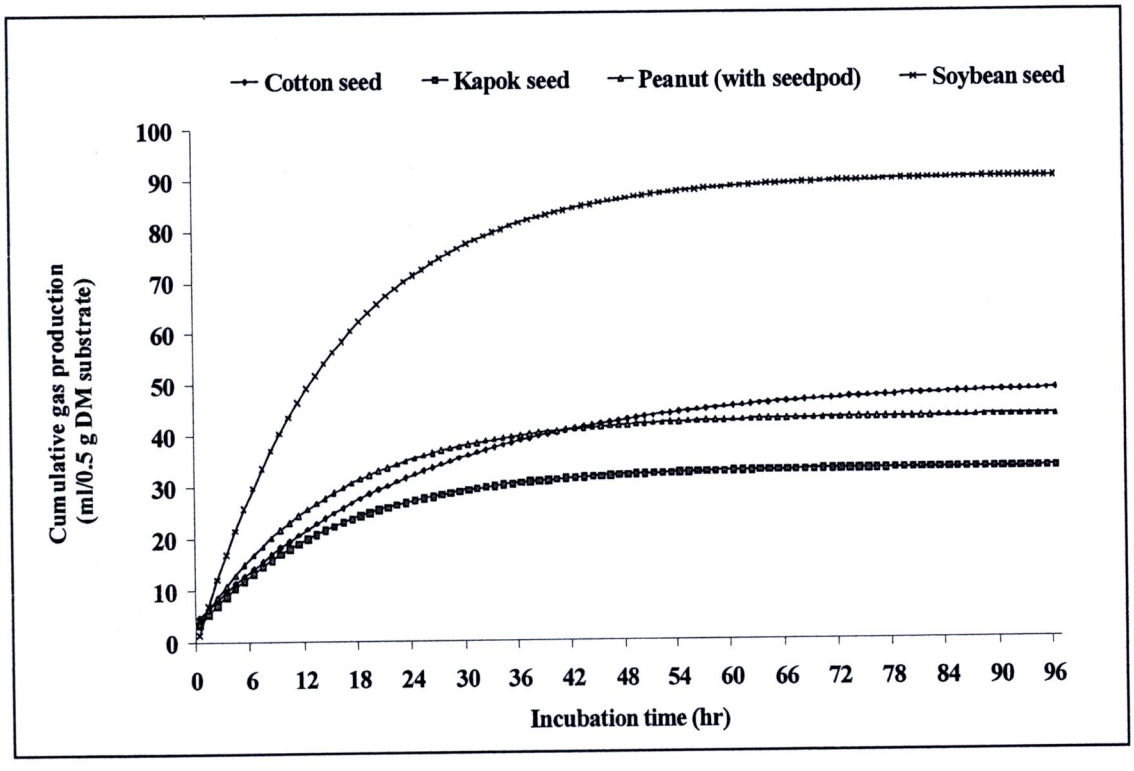


Figure 3.5.1 Cumulative gas volume estimated by $y = a + b\ (1\text{-}e^{-ct})$ (ml/0.5g DM substrate) throughout 96 h of incubation of selected oilseeds.

The gas volume at asymptote (*b*) describes the fermentation of the insoluble fraction. The *b* value of soybean seed (88.77 ml/0.5g DM substrate) was greater ($P<0.01$) than another oilseeds while peanut seed with husk was the lowest. The *b* value of soybean seed and kapok seed in this study were slightly lower than the findings of Chumpawadee et al. (2007b) (98.48 and 33.88 ml/0.5g DM substrate, respectively). A similar pattern can be seen in the potential extent of gas production ($|a|+b$), which ideally reflects the fermentation of the soluble fraction plus insoluble fraction.

Data suggested that even though gas production volume of soybean seed was highest in these selected oilseeds, all of them produced lower gas volume than forages feedstuffs. The high fat content in these feedstuffs may act to obstruct microbial accesses to utilize nutrients resulting in the lower gas volume.

Rate of gas production (*c*, %/h) of selected oilseeds was lowest ($P<0.01$) in cottonseed while kapok seed, peanut seed with husk and soybean seed were not significantly different from each other ($P>0.05$).

3.5.3.4 Calculated metabolizable energy

The calculated metabolizable energy of oilseed feedstuffs is presented in Table 3.5.2. Estimation of ME content was calculated according to the equations of Menke et al. (1979); ME (MJ/kg DM) = 2.20+(0.136×Gv)+(0.057×CP) (Eq1) and Menke and Steingass (1988); ME (MJ/kg DM) = 2.20+(0.1357×Gv) + (0.0057×CP) + (0.0002859×EE^2) (Eq2) by using gas volume produced at 24 h of incubation (Gv, ml), crude protein (CP, %) and ether extract (EE, %) as factors of prediction. From the equations, therefore, Gv at 24 h of incubation, CP and EE content have a positive effect on estimated ME.

Soybean seed had the highest calculated metabolizable energy in selected oilseeds while kapok seed was the lowest. Calculated metabolizable energy content of soybean seed and kapok seed were slightly higher than that found by Chumpawadee et al. (2007b) (7.10 and 5.08 MJ/kg DM, respectively). These variations might be caused by the difference in CP content and gas production at 24 h of incubation which are the factors affecting the estimated metabolizable energy value.

3.5.4 Conclusion

The selected oilseeds showed variation in chemical compositions. Soybean seed showed the greatest CP content while EE content was highest in peanut with husk. Fiber content (NDF, ADF and ADL) was lowest in soybean seed. The selected oilseed feedstuffs showed great variation in digestibility. The IVDMD and IVOMD were highest in soybean seed, and were lowest in whole cotton seed and kapok seed. The IVNDFD showed great variation, being highest in soybean seed and lowest in kapok seed and peanut seed with husk. Data suggest that potential extent of gas production of soybean seed is the greatest. Soybean seed demonstrates the highest estimated metabolizable energy. Based on these data, even though these feedstuffs have high fiber content, they are potential sources of energy and protein feed.

3.6 EXPERIMENT 1.6: NUTRITIVE EVALUATION OF HIGH FIBER BY-PRODUCT FEEDSTUFFS

3.6.1 Introduction

In an agricultural country such as Thailand, there are many agricultural industrial products. These products are processed through factories and produce a lot of by-product that can be used as animal feed, and categorized as energy or protein source feedstuffs according to Harris et al. (1982) and WTSR (2008). Some feedstuffs can not be classified to either feed sources, because they are low protein and high fiber content (Chumpawadee et al., 2005b). However, those feedstuffs are usually used as ruminant feeds. Chemical composition, nutritive value, digestibility and energy content of feedstuffs are essential data for farmers to use for diet formulation to meet animal requirements. Chemical composition data, such as proximate analysis (AOAC, 1990); and fiber (Van Soest, 1991) are available in many sources such as the NRC (2000), NRC (2001) and WTSR (2008) tables. However, in Thailand, there is meagre digestibility data and very limited energy content information, especially metabolizable energy (ME).

Since there are several agro-industrial by-products, determining their digestibility and energy content, requires considerable investment of time, labor and finance. *In vitro* gas production technique, nowadays, is widely used to evaluate feed digestibility and kinetic of gas production. It is a useful technique to use as a screening test when searching for potential feedstuffs for ruminants, being less expensive, easy and suitable for use in developing countries (Chumpawadee et al., 2005a). Moreover, this technique can be used to determine ME content by prediction equation using chemical composition and gas production (Menke et al., 1979; Menke and Steingass, 1988).

The objectives of this experiment, therefore, were to determine chemical composition, nutritive values and to compare digestibility, kinetics of gas production and metabolizable energy of selected high fiber by-product feedstuffs using *in vitro gas* production procedures.

3.6.2 Materials and Methods

3.6.2.1 Experimental design and treatment

The experiment was arranged into randomized complete block design. There were 4 replicates for kinetics of gas production determination, 8 replicates for *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) evaluation and 4 replicates for *in vitro* neutral detergent fiber digestibility (IVNDFD) evaluation.

Five high fiber by-product feedstuffs in the experiment were collected from Nam Pong Dairy Co-operative Limited, Khon Kaen Province and Laboratory of Khon Kaen Animal Nutrition Research and Development Center, Khon Kaen. They were coconut meal, coconut milk residue, palm kernel meal, mung bean bran and soybean hull. The high fiber by-products were classified by their crude protein (CP) content which is below 20 % and cell wall (neutral detergent fiber; NDF) content which is above 35 % following the guideline of Harris et al. (1982) and WTSR (2008).

3.6.2.2 Animals

Three male crossbred Holstein Friesians of body weight approximately 200 kg were used as the source of rumen inoculum. The animals were maintained on dried chopped sweet sorghum *(Sorghum bicolor)* as a roughage source at a Department of Animal Science's experimental station, Faculty of Agriculture, Khon Kaen University. Rumen fluid was removed under vacuum pressure via stomach tube into a 2 liter suction flask and transferred into two pre-warmed 1.8 liter vacuum flasks which were then transported to the laboratory for preparation of rumen inoculum.

3.6.2.3 Feedstuff samples and chemical analysis

All test feedstuff samples were dried at 60°C in hot air oven for 48 h and ground through a 1 mm screen for chemical analysis and *in vitro* gas production technique. The feedstuff 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 determined using the method proposed by Van Soest et al. (1991).

Feed samples of 500 mg (fresh weight basis) were transferred into 50 ml serum bottles (Sommart et al., 2000). The bottles were stoppered with rubber stopper, crimp sealed and incubated in a hot air oven with water bath set at 39°C. Bottles

were pre-warmed for 1 hour at 39°C and were injected with 40 ml of rumen inoculum prepared as following Sommart et al. (2000).

3.6.2.4 Data collections

1) Gas volume (Gv)

The rate of gas production was measured by reading and recording the 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. Cumulative gas volumes after incubations were fitted using the equation $y = a + b\ (1\text{-}e^{-ct})$ (Ørskov and McDonald, 1979), where y = gas production at time 't', a = the intercept, which ideally reflects the fermentation of the soluble fraction, b= the fermentation of the insoluble fraction, c = rate of gas production, and $(|a|+b)$ = potential extent of gas production.

2) Digestibility

After a 24 hour of incubation, a 12 bottle sample of each treatment was taken and kept in a freezer to inhibit microbial activity. Subsequently, 8 bottles of each treatment were subjected to *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) determination and 4 bottles were subjected to neutral detergent fiber digestibility (IVNDFD) determination.

To determine the IVDMD, residues of incubated samples in bottles were filtered through a groose crucible and dried at 105°C in a hot air oven for DM determination. Thereafter, the DM residues were subjected to incineration in a muffle furnace at 550°C for ash determination in order to evaluate the IVOMD. To determine the IVNDFD, residues of incubated samples in bottles were filtered through a groose crucible. Then, the residues were analyzed for NDF and lastly for ash. The obtained residue DM, ash and NDF data were used for calculation of digestibility according to the following equations:

THE NATIONAL RESEARCH COUNCIL OF THAILAND
RESEARCH LIBRARY

$$\text{IVDMD (\%)} = \frac{[\text{DM initial incubation (g)} - \text{DM after incubation (g)}] \times 100}{\text{DM initial incubation (g)}}$$

$$\text{IVOMD (\%)} = \frac{[\text{OM initial incubation (g)} - \text{OM after incubation (g)}] \times 100}{\text{OM initial incubation (g)}}$$

$$\text{IVNDFD (\%)} = \frac{[\text{NDF initial incubation (g)} - \text{NDF after incubation (g)}] \times 100}{\text{NDF initial incubation (g)}}$$

3) Estimation of metabolizable energy (ME)

Estimation of ME content was calculated following the equations of Menke et al. (1979); ME (MJ/kg DM) = 2.20+(0.136×Gv)+(0.057×CP) (Eq1) and Menke and Steingass (1988); ME (MJ/kg DM) = 2.20+(0.1357×Gv) + (0.0057×CP) + (0.0002859×EE^2) (Eq2) by using gas volume produced at 24 h of incubation (Gv, ml), crude protein (CP, %) and ether extract (EE, %) as factors of prediction.

Since gas production, in this study, was collected from incubation of a 0.5 g of DM, the gas volume was adjusted to be equal to 0.2 g of DM for ME calculation in the above equations. The equation for adjusting is shown as follows:

Gv produced from 0.2 g DM = (0.2× Gv produced from 0.5 g DM)/0.5

3.6.2.5 Statistical analysis

All data obtained from the trials were subjected to the analysis of variance procedure of statistical analysis system (SAS, 1996) according to a Randomized Complete Block Design (RCBD). Means were compared by using the LSMEANS and STDERR statement in PROC GLM. Mean separation were determined using the PDIFF statement in PROC GLM with a $P < 0.05$ significance level. The original model includes the treatment and block effects as follows:

$$\mathbf{Yij = \mu + \tau i + \beta j + \varepsilon ij}$$

Where, **Yij** = observation value in feedstuff sample i, batch of injected rumen inoculum j

μ = over all mean

τi = effect of feedstuff sample i when i = 1, 2,.., 5

ßj = effect of batch of injected rumen inoculum j when j = 1, 2, 3 and 4

εij = residual error

3.6.3 Results and Discussion

3.6.3.1 Chemical composition

Chemical compositions of high fiber by-product feedstuffs are presented in Table 3.6.1. Generally, wide variations existed in the chemical composition of the investigated feedstuffs. The CP content was below 20 % following the guideline of Harris et al. (1982) and WTSR (2008). The CP content of coconut meal was the highest (16.49 %) while coconut milk residue was the lowest (6.59 %). The CP content of both coconut meal and coconut milk residue were similar to that reported in WTSR (2008) (17.1 and 6.5 %, respectively). The CP content of coconut meal was higher than reported by Chumpawadee et al. (2005b) (10.93 %) but it was lower than coconut oil cake (25.2 %) in the report of Ramachandran et al. (2007). Palm meal had CP content similar to solvent extracted palm meal (16.68%) but higher than mechanical extracted palm meal (11.31 %) found by Chumpawadee et al. (2005b). Rice pollard had CP content higher than WTSR (2008) but slightly lower than Chumpawadee et al. (2005c) (5.70 and 8.46 %, respectively). Soybean hull in this study had CP content (11.38 %) which agrees with the report of Miron et al. (2001), Engel et al. (2008), Araujo et al. (2009) and Mielenz et al. (2009) (10.1, 12.0, 13.9 and 10.7 %, respectively).

Table 3.6.1 Chemical composition of high fiber by-product feedstuffs

Feedstuff	Chemical composition						
	DM[1],%	CP	EE	Ash	NDF	ADF	ADL
		% of DM					
Coconut meal	91.73	16.49	9.28	5.49	61.90	33.15	6.83
Coconut milk residue	91.95	6.59	27.11	0.96	69.72	40.81	6.90
Mung bean bran	94.23	13.07	1.84	30.13	36.25	29.50	8.42
Palm meal	92.43	15.21	6.26	4.05	74.47	44.00	13.74
Rice pollard	89.10	7.43	1.79	18.38	72.72	59.44	23.25
Soybean hull	90.12	11.38	0.47	5.06	76.03	55.82	1.46

[1]DM=dry matter, CP= crude protein, EE = ether extract, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin

The EE of coconut milk residue (27.11 %) was similar to WTSR (2008) (26.8 %). It was higher than that of coconut meal (9.28 %). This is because of the different production process where coconut meal is processed through high pressure mechanical means or is solvent extracted, while coconut milk residue is processed by

manual or low pressure machine. Soybean hull in this study had the lowest EE content (0.47 %) which is lower than that reported by Araujo et al. (2009) (1.60 %).

The NDF content of selected high fiber by-product feedstuffs was above 35 % as classified following the guideline of Harris et al. (1982) and WTSR (2008). Most of them had NDF content ranged between 60 to 80 %, except for mung bean bran, with NDF content (36.25 %) lower than the others. Coconut meal had NDF content (61.90 %) which is higher than the study of Krishnamoorthy et al. (1995) and in WTSR (2008) tables (44.58 and 47.4 %, respectively) but lower than reported by Chumpawadee et al. (2005b) (67.3 %). Palm meal had NDF content (74.47 %) lower than both mechanical and solvent extracted palm meal in the report of Chumpawadee et al. (2005b) (82.47 and 82.70 %, respectively). Soybean hull had NDF content (55.82 %) higher than the previous report of Engel et al. (2008) and Mielenz et al. (2009) (47.0 and 41.2 %, respectively) but similar to Miron et al. (2001) (52.7 %).

The ADF and ADL content were highest in rice pollard (59.44 and 23.25 %, respectively) while ADL content of soybean hull was the lowest (1.46 %). The ADF and ADL component of rice pollard was highest when compared to other energy feed sources, possibly because it was contaminated with rice hull. This agrees with Chumpawadee et al. (2005a). Palm meal had ADF content (44.0 %) lower than both mechanical and solvent extracted palm meal in report of Chumpawadee et al. (2005b) (57.23 and 51.41 %, respectively). Coconut meal had ADF content lower than that of Krishnamoorthy et al. (1995) and Chumpawadee et al. (2005b) (44.5 and 42.7 %, respectively).

Based on this study and reviewed data, the chemical composition of commercially available meals differ greatly according to the botanical origin and the processing method of oil extraction, temperature and duration of heating. Furthermore, nutritive values of meals can differ significantly from the mean value given in tables of feedstuff composition (Fevier et al., 2001). These factors may partially explain differences in chemical composition between this study and others.

3.6.3.2 *In vitro* digestibility

The digestibility at 24 h of incubation of high fiber by-product feedstuffs is shown in Table 3.6.2. It can be seen that IVDMD of each feedstuff follows the same pattern as IVOMD. This result agrees well with Chumpawadee et al. (2007a,b).

Coconut meal showed the highest (P<0.01) IVDMD, IVOMD and IVNDFD in this group (66.76, 67.03 and 64.99 %, respectively). By contrast, IVDMD, IVOMD and IVNDFD of coconut milk residue (21.26, 21.83 and 6.87 %, respectively) and rice pollard (19.45, 15.74 and 13.54 %, respectively) were lowest (P<0.01). Data indicates that coconut meal had the highest potential of fiber digestibility than other feedstuffs in this group while coconut milk residue and rice pollard had the least potential of fiber digestibility.

The IVDMD and IVOMD of palm meal, soybean hull, and coconut meal in the present study were higher than that reported by Chumpawadee et al. (2007b). By contrast, coconut milk residue and rice pollard had similar value of IVDMD and IVOMD to Chumpawadee et al. (2007a,b). Nevertheless, Chumpawadee et al. (2007b) found that the IVDMD and IVOMD of coconut meal (25.59 and 27.76 %, respectively) were not different from that of coconut milk pressed residue (18.05 and 20.80 %, respectively).

Table 3.6.2 *In vitro* digestibility, gas production kinetics and calculated ME of high fiber by-product feedstuffs

Feedstuffs	Digestibility, %[2]			Kinetic of gas production				ME [3] (MJ/kg DM)	
	IVDMD	IVOMD	IVNDFD	*a*, ml	*b*, ml	|*a*|+*b*, ml	*c*, %/h	Eq1	Eq2
Coconut meal	66.76^{a}	67.03^{a}	64.99^{a}	-6.08^{d}	107.13^{b}	113.22^{b}	0.112^{a}	8.09^{a}	7.26^{a}
Coconut milk residue	21.26^{d}	21.83^{d}	6.87^{e}	4.65^{a}	18.08^{e}	22.73^{e}	0.061^{b}	3.50^{d}	3.37^{e}
Mung bean bran	44.93^{c}	46.64^{c}	11.07^{d}	3.19^{a}	92.33^{c}	95.51^{c}	0.032^{c}	5.77^{c}	5.10^{d}
Palm meal	50.77^{b}	50.69^{b}	47.29^{b}	-4.22^{c}	111.36^{b}	115.58^{b}	0.064^{b}	7.81^{a}	6.83^{b}
Rice pollard	19.45^{d}	15.74^{d}	13.54^{d}	-0.56^{b}	32.79^{d}	34.71^{d}	0.016^{c}	3.07^{d}	2.70^{f}
Soybean hull	50.33^{b}	49.70^{b}	39.98^{c}	-13.78^{e}	219.79^{a}	233.56^{a}	0.023^{c}	6.81^{b}	6.22^{c}
P value	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
SEM	1.3566	1.2544	1.2035	0.5359	2.6729	2.8122	0.0048	0.1479	0.1280

[1]IVDMD, IVOMD and IVNDFD= *in vitro* DM, OM and NDF digestibility, respectively,
[2]calculated ME follow equation of Menke et al. (1979) (Eq1) and Menke and Steingass (1988) (Eq2),
a, b, c, d, e, f, g, h, I, j, k, l means within column with different superscripts differ significantly (P<0.05)

This result reflects the influence of feedstuff chemical composition on their digestibility. Coconut milk residue had the high EE content which could inhibit microbes attaching and digesting feed particles while rice pollard had the high fiber content, especially ADL content (23.25 %) which is considered an indigestible fraction. Chumpawadee et al. (2007b) suggested that 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.

3.6.3.3 Kinetics of gas production

The kinetics of gas production of selected high fiber by-product feedstuffs are presented in Table 3.6.2 and Figure 3.6.1. The *in vitro* gas production technique is adapted to describe kinetics of fermentation based on the modified exponential model $y = a + b\ (1\text{-}e^{-ct})$ (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 compatibility of its parameters with intake, digestibility and degradation characteristic of feedstuffs had been documented (Blummel and Ørskov, 1993; Khazaal et al., 1993; Sommart et al., 2000; Nitipot and Sommart, 2003).

The value *a*, intercept, of selected high fiber by-product feedstuffs varied and ranged from -13.78 to 4.65 ml/0.5g DM substrate. 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 a lag in microbial colonization (Chumpawadee, 2006). It is well known that the value for absolute *a* ($|a|$), ideally reflects the fermentation of the soluble fraction. Therefore, the $|a|$ value of soybean hull was highest ($P<0.01$) reflecting the highest fermentation of the soluble fraction while coconut milk residue and mung bean bran were the lowest.

The gas volume at asymptote (*b*) describes the fermentation of the insoluble fraction. Soybean hull exhibited the highest ($P<0.01$) *b* value (219.79 ml/0.5g DM substrate) while coconut milk residue and rice pollard had very low gas volume at asymptote (18.08 and 32.79 ml/0.5g DM substrate, respectively). The *b* value of soybean hull in the present study was higher than result of Chumpawadee et al. (2007b) (160.65 ml/0.5g DM substrate). However, the *b* value of soybean hull in study of Chumpawadee et al. (2007b) higher than the other high fiber feedstuffs such as coconut meal, palm meal, dried brewer's grain, coconut milk residue and kapok seed. This result reflects their chemical composition and digestibility. Deaville and Givens (2001) reported that the kinetics of gas production could be affected by the carbohydrate fraction. However,

although soybean hull had the lower digestibility than coconut meal it exhibited the greater gas volume at asymptote. It might be due to the lower ADL content of soybean hull.

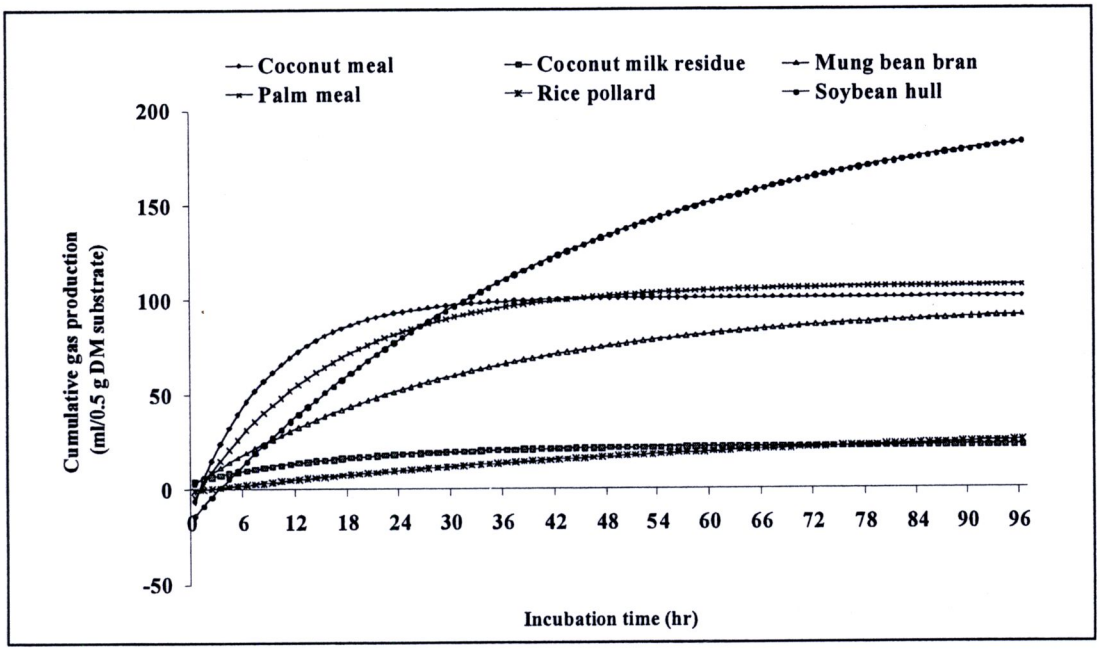


Figure 3.6.1 Cumulative gas volume estimated by $y = a + b\ (1\text{-}e^{-ct})$ (ml/0.5g DM substrate) throughout 96 h of incubation of high fiber by-product feedstuffs.

Potential extent of gas production ($|a|+b$) of selected high fiber by-product feedstuff is represented in summation of the fermentation of the soluble and insoluble fraction, resulting in the same direction as their *b* value.

Rate of gas production (*c*, %/h), is possibly influenced by the carbohydrate fraction's ready availability to the microbial population. The highest ($p<0.01$) rate of gas production was observed in coconut meal while palm meal and coconut milk residue were at the moderate and mung bean bran, coconut milk residue, soybean hull and rice pollard were at the lowest.

3.6.3.6 Calculated metabolizable energy

The calculated metabolizable energy of oilseed feedstuffs is presented in Table 3.6.2. Estimation of ME content was calculated following equations of Menke et al. (1979); ME (MJ/kg DM) = 2.20+(0.136×Gv)+(0.057×CP) (Eq1) and Menke and Steingass (1988); ME (MJ/kg DM) =2.20+(0.1357×Gv)+(0.0057×CP)+(0.0002859×EE^2)

(Eq2) by using gas produced at 24 h of incubation (Gv, ml), crude protein (CP, %) and ether extract (EE, %) as factors of prediction. From the equations, therefore, Gv at 24 h of incubation, CP and EE content have a positive effect on estimated ME.

The highest calculated metabolizable energy was observed in coconut meal following by palm meal, soybean hull and mung bean bran. It was very low in coconut milk residue and rice pollard.

Compared with reports of previous experiments which used the same equation of Menke et al. (1979), the calculated metabolizable energy of palm meal and coconut meal of Chumpawadee et al. (2007b) (4.69 and 5.91 MJ/kg DM, respectively) was lower than that of the present study. In contrast, the calculated metabolizable energy of rice pollard in experiment of Chumpawadee et al. (2007a) (4.46 MJ/kg DM) was higher than in the present study. However, the calculated metabolizable energy of some feedstuffs agrees with previous reports such as coconut milk residue (Chumpawadee et al., 2007b) (3.51 MJ/kg DM), mung bean brand and soybean hull (Chumpawadee et al., 2007a) (5.24 and 6.18 MJ/kg DM, respectively). The variation of calculated metabolizable energy may be due to the different chemical compositions of feedstuffs affecting gas production, and differences among laboratories.

3.6.4 Conclusion

The investigated high fiber by-product feedstuffs exhibited wide variations in chemical composition. The results of this study demonstrate that coconut meal had highest potential of fiber digestibility while coconut milk residue and rice pollard have the least potential of fiber digestibility. Data indicate that kinetics of gas production of high fiber by-product feedstuff differs among feeds. Soybean hull exhibits highest potential extent of gas production while coconut milk residue and rice pollard showed the lowest. Based on this study, calculated metabolizable energy ranked from the highest to the lowest are coconut meal, palm meal, soybean hull, mung bean bran, coconut milk residue and rice pollard.