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Nutritional Evaluation of Protein Feed Sources for Ruminant Using *in vitro* Gas Production Technique

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ABSTRACT

Six protein feed sources were used to evaluate nutritive value using the *in vitro* gas production technique. The rumen mixed microbe inoculums were taken from fistulated Brahman-Thai native crossbred steers. The treatments were 1) kapok seed meal, 2) soybean meal, 3) coconut meal, 4) peanut meal 5) whole cotton seed and 6) fish meal and were assigned to completely randomize design. The results indicated that soluble gas fraction (a; -2.44, -17.08, -22.87, -4.12, 0.60 and -2.88 ml, respectively), fermentation of insoluble fraction (b; 37.73, 117.01, 122.87, 89.53, 53.09 and 36.30 ml, respectively), rate of gas production (c; 0.089, 0.078, 0.056, 0.066, 0.079 and 0.022%/h, respectively) and potential of extent of gas production ($|a|+b$; 40.14, 134.02, 145.74, 93.65, 37.24 and 39.19 ml, respectively) were significantly different ($P<0.01$) among protein feed sources. The cumulative gas volumes at 24, 48 and 96 h after incubation were significantly different ($P<0.01$). These results suggest that coconut meal, soybean meal and peanut meal are exhibited high fermentation in the rumen.

Keywords: protein, *in vitro* gas production, nutritive value

INTRODUCTION

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

With respect to protein feed sources in Thailand, limited information is available on kinetics of gas production. Therefore, the aim of this study was to evaluated nutritive values of protein feed sources in ruminants using the *in vitro* gas production technique.

MATERIALS AND METHODS

Feedstuffs preparation and analysis

The feedstuffs{kapok seed meal, soybean meal, coconut meal (solv-extd), peanut meal, whole cotton seed and fish meal} were collected from various feed mills and organizations (Kantharavichai dairy cooperation, Khonkaen dairy cooperation, Maha Sarakham University feed mill, Khon Kaen University feed mill, Numhenghoad feed suppliers, Chareon Esan commercial feed mill, Songserm Kankaset feed supplier) in the Northeast of Thailand. All samples were ground to pass through a 1 mm screen for the *in vitro* gas production technique incubation and chemical analysis. The samples were analyzed to determine dry matter (DM), crude protein (CP) and ash content (AOAC, 1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were assayed using the method proposed by van Soest et al. (1991).

Experimental design

The experimental design was completely randomized (four replicates per treatment). Strict anaerobic techniques were used in all steps during the rumen fluid transfer and incubation period. Rumen fluid inoculums was removed before the morning feeding under vacuum pressure via the rumen fistula into a 2 liter glass flask and transferred into two pre-warmed 1 liter thermos flasks which were then transported to the laboratory. The medium preparation was as described by Sommart et al. (2000). Mixed rumen fluid inoculums were obtained from two fistulated Brahman-Thai native crossbred steers (weighed 250±15 kg). The animals were offered rice straw on *ad libitum* and 0.5% body weight of concentrate (concentrate mixture: 49.80% cassava chip, 17.5% rice bran, 14.60% palm meal, 7.0% soybean meal, 1.40% urea, 0.4% salt, 1.0% mineral mix and 8.30% sugarcane molasses). The animals were fed twice daily, water and mineral licks were available *ad libitum* for 14 days.

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

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

Statistical analyses

All data obtained from the trials were subjected to the analysis of variance procedure of statistical analysis system (SAS, 1996) according to a completely randomized design. Means were tested using Duncan New’s Multiple Range Test. The level of significance was determined at $P<0.05$.

RESULTS AND DISCUSSION

Chemical composition of protein feed sources

Chemical compositions of protein feed sources are presented in Table 1. Generally, wide variations existed in the chemical composition of the investigated feedstuffs. The CP content of soybean meal was higher than that reported by Department of Livestock Development (2004); Promkot and Wanapat (2004) and Danesh Mesgaran and Stern (2005), but lower than that report by Woods et al. (2003). It was similar to reports by NRC (2001). The NDF and ADF content were lower than those reported by NRC (2001) and Department of Livestock Development (2004), but were similar to reports by Woods et al. (2003); Promkot and Wanapat (2004) and Danesh Mesgaran and Stern (2005).

The CP content of coconut meal (solv-extd) was higher than that reported by Ibrahim et al. (1995) and Department of Livestock Development (2004); Krishnamoothy et al. (1995) and Woods et al. (2003). The NDF and ADF content higher than those reported by Ibrahim et al. (1995); Krishnamoothy et al. (1995) and Department of Livestock Development (2004). The ADF content was similar to that reported by Woods et al. (2003), but lower than that reported by Krishnamoothy et al. (1995).

The CP content of peanut meal was lower than that reported by NRC (2001) and Department of Livestock Development (2004). The NDF content was higher those reported by Department of Livestock Development (2004). The ADF content of peanut meal was in agreement with that reported by the Department of Livestock Development (2004).

The CP content of whole cotton seed was higher than that reported by Department of Livestock Development (2004), but similar to reports by NRC (2001). The NDF, ADF and ADL contents of whole cotton seed were similar to reports by NRC (2001).

The CP content of fish meal was lower than that reported by Harstad and Prestlokken (2001), but similar to reports by NRC (2001) and Department of Livestock Development (2004).

This study indicates that fish meal was highest crude protein content as compared to other protein feed source. Whole cotton seed was shown to have the lowest crude protein content. Coconut meal (Solv-extd) showed highest NDF and ADF content as compared to other protein feed sources. Soybean meal showed lowest NDF, ADF and ADL content. Many factors affect chemical composition such as oil extraction process (Mara *et al*., 1999), stage of growth, (Promkot and Wanapat, 2004) maturity, species or variety (von Keyserlingk *et al*., 1996; Agbagla-Dohnani *et al*., 2001), drying method, growth environment (Mupangwa *et al*., 1997) and soil types (Thu and Preston, 1999). These factors may partially explain differences in chemical composition between our study and others.

Table 1 Chemical composition of various protein feed source (means±SD).

Feedstuffs[1]	DM (%)	CP	Ash	NDF	ADF	ADL
		………...……………….% DM basis…...…………..…………….				
KM	91.01±0.11	28.09±0.06	8.91±0.07	42.50+0.07	29.49±0.55	16.34±0.01
SM	91.31±0.03	47.24±0.33	7.12±0.01	12.84±1.15	8.26±0.16	0.10±0.002
CMS	86.01±0.01	24.69±0.90	8.59±0.10	80.80±2.17	43.45±0.83	7.94±1.28
PM	92.24±0.07	40.79±0.04	8.72±0.02	28.22±1.24	13.25±0.72	4.95±0.13
WCS	92.64±0.23	21.75±0.02	3.86±0.04	52.28±0.82	37.80±0.27	11.67±0.39
FM	90.01±0.10	61.89±0.52	27.84±0.01	-	-	-

[1]KM = kapok seed meal, SM = soybean meal, CMS =coconut meal (solv-extd), PM = peanut meal, WCS = whole cotton seed and FM = fish meal

Gas production characteristics of protein feed sources

Gas production from the fermentation of protein feed sources were measured at 2, 4, 6, 12, 24, 48, 72 and 96 h *in vitro* using gas tests adapted to describe the kinetics of fermentation base on the modified exponential model $y = a+b\ [(1\text{-Exp}(-ct)]$ (Ørskov and McDonald, 1979). Although there are other models available to describe the kinetics of gas production, the Ørskov and McDonald (1979) model was chosen because the relationship of its parameters with intake, digestibility and degradation characteristic of forages and concentrate feedstuffs had been documented (Blummel and Ørskov, 1993; Khazaal *et al*., 1993; Sommart *et al*., 2000; Nitipot and Sommart, 2003).

Gas production characteristics are presented in Table 2 and Figure 1. A comparison of gas production characteristics of different treatments indicated significant differences between them ($P<0.01$). The value for *a* intercept for all feeds ranged from -22.87 to 0.60 ml. Coconut meal had the lowest value for *a*, intercept, while whole cotton seed had the highest value for *a*, intercept value. The values for *a* intercept were negative in the incubations in this study, with the exception of whole cotton seed. These data suggested that a lag phase due to delay in microbial colonization of the substrate may occur in the early stage of incubation. Several authors (Khazaal *et al*., 1993; Blummel and Becker, 1997) have also reported negative values with various substrates when using mathematical models to fit gas production kinetics. This is due to either a deviation from the exponential cause of fermentation or delays in the onset of fermentation due to the microbial colonization. It is well known that the value for absolute *a* (|*a*|), intercept described ideally reflect the fermentation of the soluble fraction. In this study the |*a*| was highest for coconut meal. The soluble fraction in coconut meal was also found to be highest. High |*a*| was also observed in soybean meal and could be due to structural and solubility characteristics of the protein found in soybean meal which make it easily attachable by ruminal microorganisms (Mahadevan *et al*., 1980).

The gas volume at asymptote (*b*) described the fermentation of the insoluble fraction. The gas volumes at asymptote of kapok seed meal, soybean meal, coconut meal, peanut meal, whole cotton seed and fish meal were: 37.73, 117.01, 122.87, 89.53, 35.09 and 36.30 ml, repetitively. It can be seen that gas production at asymptote of kapok seed meal, whole cotton seed meal and fish meal were very low when compared to other feeds, possibly a reflection of a high level of lignin (Table 1). In addition, fish meal has a high value of undegraded protein, leading to difficulties attachment by microorganisms (NRC, 2001).

Rates of gas production (*c*) expressed in %/h as ranked from the highest to the lowest were: kapok seed meal, whole cotton seed, soybean meal, peanut meal, coconut meal and fish meal, respectively. High rates of gas production were observed in kapok seed meal, whole cotton seed and soybean meal, possibly influenced by the carbohydrate fraction's ready availability to the microbial population. Slowest gas production was observed in fish meal, indicating that fish meal less readily available to the microbes in the rumen.

Potential extent of gas production (|*a*|+*b*) expressed in ml as ranked from highest to lowest were: coconut meal (solv-extd), soybean meal, peanut meal, kapok seed meal, fish meal and whole cotton seed. Remarkably, the potential of gas production for protein feed sources was, by comparison, lower than that of carbonaceous concentrates feedstuffs. The results agree with Gatachew et al. (1998), who suggested that gas production is basically the result of the fermentation of carbohydrates into acetate, propionate and butyrate. Khazaal et al. (1995) also reported that protein fermentation does not lead to extensive gas production. In this study, high potential extents of gas production were observed in coconut meal (solv-extd), soybean meal, peanut meal, while, potential extent of gas production in kapok seed meal, fish meal and whole cotton seed was low. This implies that coconut meal (solv-extd), soybean meal and peanut meal were highly fermentabillity in the rumen.

Gas production profiles are more difficult to interpret than feed disappearance studies using nylon bag techniques because gas is generated from a wide range of different substrates with both soluble carbohydrate and fiber components (Schofield *et al*., 1994). However, measurement of gas production can be providing valuable quantitative information on the kinetics of rumen digestion (Menke *et al*., 1979; Menke and Steingass, 1988).

Gas volume of protein feed sources

The cumulative gas volumes at 24, 48 and 96 h after incubation are shown in Table 2. The results indicate that cumulative gas volumes at 24, 48 and 96 h after incubation were significantly different ($P<0.01$) between treatments. Based on these observations of protein feed sources, the gas volumes ranked from highest to lowest were: soybean meal, coconut meal (solv-extd), peanut meal, kapok meal, whole cotton seed and fish meal, respectively. Curves of cumulative gas production for each treatment are presented in Figure 1. It can be seen that gas production reached a plateau after 48 h fermentation. Cumulative gas volume at each sampling time was affected by a variety of protein feed sources. These findings indicate that the fraction of substrate and degradability of protein feed sources are different. Gas produced is directly proportional to the rate at which the substrate degraded (Dhanoa *et al*., 2000). Additionally, kinetics of gas production is dependent on the relative proportions of soluble, insoluble but degraded, and undegradable particles of the feed (Getachew *et al*., 1998). Menke et al. (1979) suggested that gas volume at 24 h after incubation has a direct relationship with metabolizable energy level in feedstuffs. Sommart et al. (2000) reported that gas volume is a good parameter with which to predict digestibility, volatile fatty acids production and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Additionally, *in vitro* dry matter and organic matter digestibility were shown to have a high correlation with gas volume (Nitipot and Sommart, 2003). Gas volume also has a correlation with feed intake (Blummel and Becker, 1997) and growth rate (Blummel and ∅rskov, 1993).

Table 2 Gas production characteristics and gas volume of protein feed source using *in vitro* gas production technique.

Parameters	Treatment[1]						SEM
	KM	SM	CMS	PM	WCS	FM	
Gas production characteristic parameters[2]							
a (ml)	-2.44^{ab}	-17.08^{c}	-22.87^{d}	-4.12^{b}	0.60^{a}	-2.88^{ab}	1.87
b (ml)	37.73^{c}	117.01^{a}	122.87^{a}	89.53^{b}	35.09^{c}	36.30^{c}	8.01
c (%/h)	0.089^{a}	0.078^{abc}	0.056^{c}	0.066^{bc}	0.079^{ab}	0.022^{d}	0.01
$\|a\|+b$ (ml)	40.14^{d}	134.02^{b}	145.74^{a}	93.65^{c}	37.24^{d}	39.19^{d}	9.59
Gas production (ml/0.5g DM substrate)							
24 h	30.25^{c}	80.37^{a}	70.73^{ab}	66.0^{b}	29.37^{c}	12.12^{d}	5.60
48 h	32.75^{d}	118.12^{a}	102.37^{b}	78.25^{c}	32.12^{d}	22.12^{e}	7.87
96 h	37.25^{d}	130.87^{a}	114.37^{b}	88.75^{c}	38.25^{d}	28.75^{d}	8.50

[a, b, c, d] Means within a row different superscripts differ ($P<0.01$) , [1]KM = kapok seed meal, SM = soybean meal, CMS = coconut meal (solv-extd), PM = peanut meal, WCS = whole cotton seed and FM = fish meal, [2]*a* = the intercept(ml), which described ideally reflects the fermentation of the soluble fraction, *b* = the fermentation of the insoluble fraction (asymptote) (ml), *c* = rate of gas production (%/h), (|*a*|+*b*) = potential extent of gas production (ml)

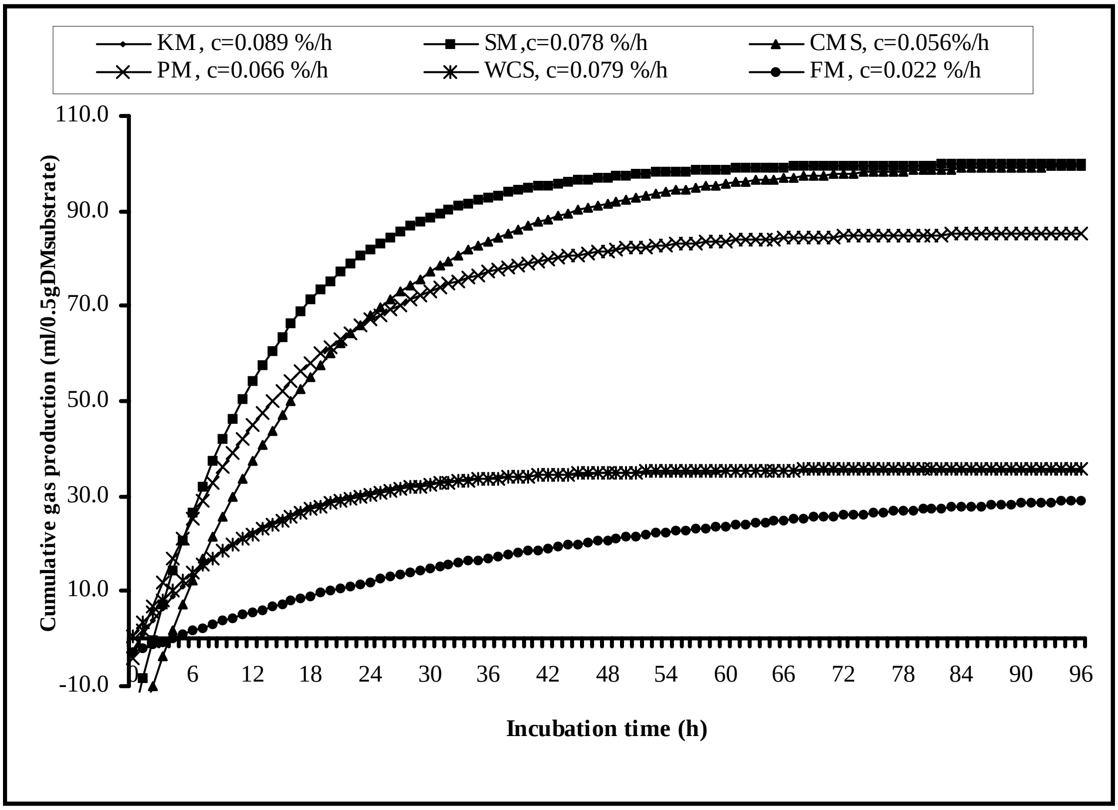

Figure 1 Cumulative gas volume estimated by y = a+b [(1-Exp(-ct)] (ml/0.5 g DM substrate) throughout 96 h. (KM = kapok seed meal, SM = soybean meal, CMS = coconut meal (solv-extd), WCS = whole cotton seed, PM = peanut meal and FM = fish meal)

CONCLUSIONS

The protein feed sources showed a great variation in chemical composition. The results of this study demonstrates that kinetics of gas production of protein feed sources differed among feed. Based on this study, high fermentability for protein feed source use in ruminant ranked from the highest to the lowest were; coconut meal (solv-extd), soybean meal, peanut meal, kapok seed meal, fish meal and whole cotton seed meal.

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