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Effect of Burma Padauk (*Plerocarpus indicus*), Rain Tree (*Samanea saman* (Jacg.) Merr.) and Siamese Rough Bush (*Streblus asper*) Leaves as Fiber Sources in Total Mixed Ration on *in vitro* Fermentation

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Abstract: The objective of this study was emphasized on effect of leaves as fiber sources in total mixed ration on *in vitro* fermentation using *in vitro* gas production technique. The experimental was designed in CRD with five replicates per treatment. The fiber sources in total mixed ration were corn cob (control group), Burma padauk leaves, rain tree leaves and Siamese rough bush leaves. The results showed that the kinetic of gas production and digestibility were statistical significantly differences among treatment ($p<0.05$). The corn cop as fiber source in total mixed ration gave the highest potential of extent of gas production. However, highest rate of gas production and digestibility were observed in the Siamese rough bush leaves as fiber source. Ruminal fermentation end-products consisted of ammonia nitrogen and volatile fatty acid were significantly differences among treatments ($p<0.05$). All treatment means were within the normal range. The pH values were relatively stable at 7.0-7.3. The results demonstrated that Burma padauk leaves, rain tree leaves and Siamese rough bush leaves can be used as fiber sources in total mixed ration. Importantly, leaves are abundant and available for feeding the ruminants in dry season.

Key words: Leaves, total mixed ration, digestibility, gas production, fermentation

INTRODUCTION

In recent years, feeding a total mixed ration (TMR) for cattle has become widely accepted. The benefits of a TMR include increased milk production, enhanced use of low cost alternative feed ingredients, ability to control the forage concentrate ratio, lower incidence of metabolic and digestive disorders and reduced labor input for feeding. Fiber source of TMR is very importance, because it can be affected feed intake, chewing activity, digestibility and production (Chumpawadee and Pimpa, 2008). Generally, silage, forage, rice straw corn cop and hay are conventional roughages found in TMR. Due to the dry season have shortage fiber source for mixed TMR. Therefore, non-conventional roughage such as fodder tree is needed for fiber source in TMR. Although, they have the crucial parameters affecting fodder utilization, such as tannins saponin and non protein amino acids, which are toxic to rumen microbes or to the animal (Lowry *et al*., 1996). However, leaves of fodder trees should be used as fiber sources in TMR. Because of their feed are high content of protein, minerals and vitamins (Baloyi *et al*., 1997) and availability in the dry season. In addition, the toxic substance in leaves can be reducing by sun dry.

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With respect to leave of fodder trees, limited information is available on its use as a fiber source of TMR. The aim of this study was to investigate the *in vitro* fermentation using TMR from different fiber sources.

MATERIALS AND METHODS

Preparation of TMRs

The Burma padauk (*Plerocarpus indicus*), rain tree (*Samanea saman* (Jacg. Merr.) and Siamese rough bush (*Streblus asper*) leaves and corn cop (control) were used in this study. They were collected from the Mahasarakham province area in the North-East of Thailand. Fresh samples (1 kg) were hand harvested from three site specimens. Duplicate fresh samples (0.5 kg/replicate) were dried in a hot, dry air force oven at 65°C for 72 h and weighed. All feed samples (Table 1) were ground to pass through a 1 mm screen for chemical analysis. The feedstuff samples were analyzed for Dry Matter (DM), Crude Protein (CP) and ash, neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) (Van Soest *et al*., 1991).

Four TMRs were formulated, to have similar total digestible nutrient (TDN), CP, NDF, ADF, but differ in fiber source (Table 2). The experiment was done at division of animal science, Faculty of veterinary medicine and animal science, Mahasara Kham University, Thailand, from August 25, 2007 to June 5, 2008. The experiment was desigued in CRD with five replicates per treatment. The fiber sources of the total mixed ration were corn cop (control), Burma padauk leaf, rain tree leaf and Siamese rough bush leaf. Four TMRs for the gas production test were ground to pass through a 1 mm screen in a hammer mill.

In vitro Gas Production Test

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 L glass flask and transferred into two pre-warmed 1 L thermos flasks which were then transported to the laboratory. The medium preparation was as described by Makkar *et al*. (1995). Mixed rumen fluid inoculums were obtained from two fistulated Brahman-Thai native crossbred steers (weighing about 250±15 kg). The animals were offered rice straw on *ad libitum* and fed 0.5% body weight of concentrate (concentrate mixture: 49.80% cassava chip, 17.5% rice bran, 14.60% palm meal, 7.0% soybean meal, 1.40% urea, 0.4% salt, 1.0 % mineral mix and 8.30% sugarcane molasses). The animals were fed twice daily; water and a mineral lick were available *ad libitum* for 14 days.

The feed sample of approximately 500 mg on a fresh weight basis was transferred into a 50 mL serum bottle (Sommart *et al*., 2000). The bottles were pre-warmed in a hot air oven at 39°C for about 1 h prior to injection of 40 mL of rumen fluid medium (using a 60 mL syringe) to each bottle. The bottles were stoppered with rubber stoppers, crimp sealed and incubated in a hot air oven set at 39°C.

The rate of gas production was measured by reading and recording the amount of gas volume after incubation using a 20 mL glass syringe counected to the incubation bottle with a 23 gauge, 1.5 inch needle. Readings of gas production were recorded from 1 to 72 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 49-72 h) after incubation periods. Amounts of cumulative gas volume at 2, 4, 6, 12, 24, 48 and 72 after incubations were fitted using the equation (Ørskov and McDonald, 1979):

$$y = a+b\ [(1-Exp(-ct)]$$

Where:

a = The intercept, which ideally reflects the fermentation of the soluble fraction
b = The fermentation of the insoluble fraction
c = Rate of gas production, (a+b) = potential extent of gas production
y = Gas production at time t

In vitro digestibility of dry matter and organic matter was measured at 72 h after incubation. The residues of the TMRs were removed by filtering through a glass filtering crucible, residue was washed with 250 mL boiled distilled water and the amount of DM and OM in the residue was estimated. Calculation of *in vitro* DM and OM digestibility as a percent of total DM and OM followed the equation:

$$\textit{In vitro}\ \text{DM or OM digestibility (\%)} = \frac{\text{DM or OM initial-DM or OM after incubate}}{\text{DM or OM initial}} \times 100$$

In vitro Fermentation Measurement

The bottles were sampling at 0, 3, 6, 9 and 12 h after incubation. Rumen fluid medium pH was measured immediately after sampling using a portable pH meter. The rumen fluid medium was acidified with 5 mL 6 N HCl and centrifuged at 3000 rpm for 15 min and the clear supernatant was stored in plastic tubes at -20°C until analyzed for ammonia nitrogen (Bremner and Keeney, 1965) and total volatile fatty acid concentration (Briggs *et al*., 1957).

Statistical Analysis

All data obtained from the trials were subjected to the analysis of variance procedure of statistical analysis system according to a completely randomized design. Means were separated by Duncan New's Multiple Range Test. The level of significance was determined at $p<0.05$.

RESULTS AND DISCUSSION

Chemical Composition of Feedstuffs and TMRs

The feed ingredients varied widely in terms of composition. The leaves have high NDF content, more than 40.5% (Table 1). All TMRs had a similar chemical composition. The ration CP, ash and NDF content were approximately 11.8, 7.8 and 41.7 %, respectively (Table 2).

Gas Production Characteristics of TMRs

A comparison of the gas production characteristics of different treatments indicated significant differences between treatment ($p<0.05$). The a intercept value for all TRMs ranged from -4.2 to -8.7 mL. The values for a, intercept, were negative in the incubations of all TMRs in this study (Table 3). These data suggested that a lag phase due to a delay in microbial colonization of the substrate may occur in the early state of incubation. Blummel and Becker (1997) have also reported negative values with various substrates when using mathematical models to fit gas production kinetics.

Table 1: Chemical analysis of feedstuffs used for feed formulation in the experiments (% of DM)

Feedstuffs	DM	CP	Ash	NDF	ADF	ADL
Corn cob	91.3	1.4	2.3	86.3	51.3	10.2
Burma padauk	40.5	13.9	6.6	51.7	35.8	14.4
Rain tree	30.0	21.9	4.6	51.5	34.8	15.1
Siamese rough bush	31.0	14.2	17.4	40.5	35.8	4.4
Cassava chip	93.4	1.9	2.0	6.9	6.4	1.9
Sugar cane Molasses	72.4	2.2	8.5	-	-	-
Rice pollard	90.5	5.9	14.1	61.2	45.9	11.9

DM = Dry Matter, OM = Organic Matter, CP = Crude Protein, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, ADL = Acid Detergent Lignin

Table 2: Feed formulation and chemical composition of dietary treatments

	Dietary treatments[1]			
Ingredients	C-TMR	B-TMR	R-TMR	S-TMR
Corn cob	30.0	-	-	-
Burma padauk	-	40.0	-	-
Rain tree	-	-	40.0	-
Siamese rough bush	-	-	-	40.0
Leuceana meal	10.0	10.0	15.0	4.5
Cassava chip	40.5	27.5	24.5	21.0
Sugar cane Molasses	8.0	5.0	7.0	6.0
Rice pollard	7.0	15.0	12.3	25.8
Salt (NaCl)	0.5	0.5	0.5	0.5
Urea	3.0	1.4	0.1	1.3
Mineral mixed	0.5	0.5	0.5	0.2
Di-calcium phosphate	0.5	0.1	0.1	0.7
Total	100.0	100.0	100.0	100.0
Chemical composition (% of [2]DM)				
DM	94.1	92.0	92.6	92.0
Ash	7.8	6.5	6.2	10.7
CP	11.4	11.9	12.1	12.0
NDF	41.3	42.0	42.0	41.4
ADF	21.8	26.4	28.0	30.7
ADL	7.2	10.0	10.3	6.5
Total digestible nutrient* (TDN)	63.5	60.4	63.2	60.1
Calcium (Ca)*	0.4	0.4	0.5	0.4
Phosphorus (P)*	0.2	0.2	0.2	0.3

*Calculated value, [1]C-TMR = Corn cob as fiber source, B-TMR = Burma padauk leaf as fiber source, R-TMR = Rain tree leaf as fiber source, S-TMR = Siamese rough bush leaf as fiber source, [2]DM = Dry matter, OM = Organic matter, CP = Crude protein, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin

Table 3: Gas production characteristics and *in vitro* digestibility of dietary treatments using *in vitro* gas production technique

	Dietary treatments[1]				
Parameters	C-TMR	B-TMR	R-TMR	S-TMR	SEM
Gas production characteristics[2]					
a (mL)	-8.70^{c}	-7.40^{b}	-4.20^{a}	-8.20^{bc}	0.4
b (mL)	142.90^{a}	119.50^{c}	104.80^{d}	132.60^{b}	3.4
c, %/h	0.05^{b}	0.06^{a}	0.05^{b}	0.06^{a}	0.0
|a|+b (mL)	151.60^{a}	126.90^{c}	109.00^{d}	140.80^{b}	3.0
***In vitro* digestibility of dry matter and organic matter at 72 h (%)**					
IVDMD	79.60^{b}	77.30^{b}	77.90^{b}	85.40^{a}	0.8
IVOMD	79.80^{b}	77.20^{b}	77.70^{b}	85.80^{a}	0.8

$^{a, b, c, d}$ Means within a row different superscripts differ ($p<0.05$), [1]C-TMR = Corn cob as fiber source, B-TMR = Burma Padauk leaf as fiber source, R-TMR = Rain Tree leaf as fiber source, S-TMR = Siamese Rough Bush leaf as fiber source, [2]a = the intercept (mL), which ideally reflects the fermentation of the soluble fraction, b = The fermentation of the insoluble fraction (asymptote) (mL), c = Rate of gas production (%/h), |a|+b = Potential extent of gas production (mL)

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|, described ideally, reflects the fermentation of the soluble fraction. In this study the |a| was highest for C-TMR and significance difference ($p<0.05$) with B-TMR and R-TMR. It is indicated that the soluble fraction in C-TMR was also highest. The soluble fraction makes it easily attachable by ruminal microorganisms and leads to much gas production (Table 3). The soluble fraction of corn cop was higher than leaves. It's possibly structure and solubility characteristics of carbohydrate and protein in corn cop which easily attach with microorganisms in the rumen. While fodder trees had high fibrous content it is difficult to attach by microorganisms. Generally, leaves have a large proportion of lignified cell walls (Table 1) with low fermentation rates and digestibility. Therefore, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) content in fodder tree are the crucial parameters affecting fodder utilization.

The gas volume at asymptote (b) described the fermentation of the insoluble fraction. The gas volume at asymptote was significantly higher in C-TMR than that B-TMR, R-TMR and S-TMR ($p<0.05$). The gas volumes at asymptote have the advantage of predicting feed intake. Blummel and Ørskov (1993) found that the gas volume at asymptote could account for 88% of variance in intake. Sommart *et al*. (2000) suggested that gas volume is a good parameter from which to predict digestibility, fermentation end-product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Additionally, *in vitro* dry matter and organic matter digestibility were shown to have high correlation with gas volume (Sommart *et al*., 2000). In this study, C-TMR showed the highest gas volume (Table 3). Low gas volumes at asymptote were observed in B-TMR and R-TMR. It's possibly reflected by phenolic compounds, which are toxic to rumen microbes (D'Mello, 1992). Getachew *et al*. (2000) reported that the concentration of phenolic compounds (particularly tannin) in tree leaves is generally high. Lowry *et al*. (1996) have also reported that many of the multi propose tree are problematic as feed supplements because they often contain anti-nutritional compounds, such as tannin, saponin and non-protein amino acid, which are either toxic to rumen microbe or to the animal, or their metabolic products are toxic.

Rate of gas production (c) expressed in %/h as ranked from the fastest to the slowest were; B-TMR, S-TMR, C-TMR and R-TMR. Fast rates of gas production were observed in B-TMR and S-TMR. This result might have been influenced by the carbohydrate fraction that was readily available to the microbial population.

The potential extent of gas production (|a|+b) of C-TMR was the highest and significantly different ($p<0.05$) with B-TMR, R-TMR and S-TMR. This implies that C-TMR was highly fermentable in the rumen. Remarkably, the potential of gas production for B-TMR and R-TMR was slightly lower compared with C-TMR and S-TMR, possibly due to the influence of carbohydrate fraction in the TMR. Fibrous constituents had negatively influenced *in vitro* gas production (Melaku *et al*., 2003). The fiber content of the B-TMR and R-TMR were high, when compared with C-TMR and S-TMR. The high fibrous content is difficult to attach by microorganisms. Therefore, less gas production was shown in the B-TMR and R-TMR.

In vitro Digestibility of Dry Matter and Organic Matter

It can be seen that IVDMD and IVOMD are similar. The IVDMD and IVODM were significantly different ($p<0.05$) among treatment. The S-TMR gave the highest IVDMD and IVOMD. This result implies that the microbe in the rumen and animal have high nutrient uptake (Table 3). The IVDMD and IVOMD of C-TMR, B-TMR and R-TMR lower than S-TMR. The reason for that is possibly that the fiber fractions of C-TMR, B-TMR and R-TMR have a large proportion of lignified cell walls leading to attachment difficulty by microorganism, with low fermentation rates, low digestibility rate and limited intake (Ibrahim *et al*., 1995). The higher fiber content (Table 1) of Burma Padauk leaf and rain tree leaf probably resulted in lower *in vitro* dry matter and organic matter digestibility since high NDF and ADL content in feedstuffs result in lower fiber degradation (Van Soest, 1988).

In vitro Fermentation Pattern

Concentrations of NH_3-N, TVFA and pH in the *in vitro* fluid were used to monitor the *in vitro* fermentation pattern (Table 4). The pH was not affected by fiber source in TMRs. When monitoring pH pattern at 0, 3, 6, 9 and 12 h after incubation, the pH values were relatively stable at 7.0-7.3 and all treatment means were within the normal range that has been reported as optimal pH (6.0-7.0) for microbial digestion. The buffer in the rumen fluid medium is the reason for pH remaining stable at all times of fermentation. The buffer is a factor that should be considered when using the gas production technique. The exhaustion of the buffer would lead to a lowering of the pH (Getachew *et al*., 1998).

Table 4: Effect of Burma Padauk (*Plerocarpus Indicus*), Rain Tree (*Samanea Saman* (Jacg.) Merr.) and Siamese Rough Bush (*Streblus Asper*) Leaves as Fiber Sources in Total Mixed Ration on pH, ammonia nitrogen (NH_3N) and total volatile fatty acid (TVFA) in *in vitro*

	Dietary treatments[1]				
Parameters	C-TMR	B-TMR	R-TMR	S-TMR	SEM
pH					
0 h	7.3	7.2	7.2	7.2	0.02
3 h	7.1	7.2	7.1	7.2	0.01
6 h	7.1	7.1	7.1	7.1	0.01
9 h	7.1	7.1	7.1	7.1	0.01
12 h	7.0	7.0	7.0	7.0	0.01
Ammonia nitrogen (NH_3N) (mg %)					
0 h	20.4	20.7	20.0	20.0	0.37
3 h	19.7^{ab}	16.2^{bc}	15.7^{c}	20.4^{a}	0.75
6 h	17.1^{a}	14.5^{b}	12.8^{c}	17.7^{a}	0.51
9 h	17.9^{a}	12.7^{b}	10.9^{b}	12.9^{b}	0.86
12 h	11.9	11.3	10.4	10.3	0.32
Total volatile fatty acids (mM)					
0 h	22.7	22.4	22.4	22.7	0.20
3 h	33.2^{b}	35.9^{a}	31.8^{b}	36.2^{a}	0.55
6 h	39.9^{b}	40.1^{b}	36.4^{c}	44.3^{a}	0.74
9 h	51.9^{b}	57.9^{a}	48.3^{c}	56.9^{a}	1.02
12 h	55.1^{c}	62.0^{a}	57.6^{bc}	60.8^{ab}	0.85

$^{a, b, c, d}$Means within a row different superscripts differ ($p<0.05$), [1]C-TMR = Corn cob as fiber source, B-TMR = Burma Padauk leaf as fiber source, R-TMR = Rain tree leaf as fiber source, S-TMR = Siamese rough bush leaf as fiber source

At a lower pH, the celluolytic bacteria becomes less active (Russell and Dombrowski, 1980). In this study the buffer was not exhausted. Therefore, this condition was optimal for microbial activity.

Ammonia nitrogen concentration was significantly different ($p<0.05$) among treatments at each hour of sampling, excepted at 0 and 12 h of sampling. The difference in NH_3-N concentrations among treatments may have been related directly to urea and degradability of protein in the TMRs. Although, nitrogen recycling in the rumen and *in vitro* is different, NH_3-N concentration was in the optimal range for rumen ecology, microbial activity (Perdok and Leng, 1990; Wanapat and Pimpa, 1999). At 3 to 9 h after incubation C-TMR had the highest NH_3-N, when compared with other TMRs. When ammonium nitrogen is high it indicates that the soluble fraction of protein is also high. Remarkably, NH_3-N concentration of B-TMR and R-TMR were low at all time of sampling. It may have been that the urea level in both TMRs was lower than C-TMR. In addition, the protein in Burma Padauk leaf and rain tree leaf had low degradability (Chumpawadee *et al*., 2007). Although, NH_3-N concentration of all TMRs was different with C-TMR (control), it was in the normal range. Therefore, it can be used as fiber sources in the TMRs. Future research should investigate the impact of the ability of leaves feed to replace forage in intact animals.

Total volatile fatty acid concentrations were significantly different ($p<0.05$) among treatments at all times of sampling, excepting 0 h after incubation. Remarkably, TVFA concentrations in the *in vitro* medium, from 0 to 12 h after incubation, tend to be increased. The reason for that is possibly VFAs accumulated in the medium. The VFA can not absorb via the *in vitro*, but most of the VFA can be absorbed into rumen wall. Although, VFA increased in the medium, pH did not change because the buffer in the medium was not exhausted. This is the advantages of the gas production technique. The VFA production of B-TMR are the same with S-TMR and difference from C-TMR and R-TMR, this result might have been influenced by carbohydrate fraction in TMRs. The rate and extent of carbohydrates degradation are influenced by the condition of rumen fermentation and rate and extent of VFAs production (Cheng *et al*., 1991). Keady and Mayne (2001) also suggest that VFAs concentration is similar when the animal fed diets contained a similar carbohydrate composition. In this study, have difference source of fiber in TMRs, thus VFA concentration was also different.

CONCLUSIONS

In *in vitro* study, leaves as fiber source in TMRs had an affect on gas production characteristic, *in vitro* digestibility and *in vitro* fermentation. The corn cop as a fiber source in TMR gave the highest parameters of gas production characteristic. The Siamese rough bush as fiber source in TMR gave the highest *in vitro* digestibility. Concentration of NH_3-N, TVFA and pH were different when TMRs contained a different fiber sources, but they are in the normal range. Therefore, leaves can be used as fiber source in TMRs. Future research should investigate the impact of a leaves feed replacement of forage for intact animal.

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