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Effect of Non Forage High Fibrous Feedstuffs as Fiber Sources in Total Mixed Ration on Gas Production Characteristics and *in vitro* Fermentation

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Abstract: The objective of this study was to determine the effect of non forage high fibrous feedstuffs as fiber sources in a total mixed ration on gas production characteristics and *in vitro* fermentation using an *in vitro* gas production technique. The experiment was designed in CRD with five replicates per treatment. The fiber sources in the total mixed ration were rice straw (rt-TMR), tomato pomace (tp-TMR), palm meal (pm-TMR), dried brewer gain (db-TMR) and soybean hulls (sh-TMR). The results showed that kinetic gas production, *in vitro* dry matter digestibility and *in vitro* organic matter digestibility, were significantly different among treatments ($p<0.05$). The soybean hulls as a fiber source in the total mixed ration gave the highest IVDMD, IVOMD and gas production parameter. *In vitro* fermentation end-products consisting of NH_3-N, TVFA and pH were significantly different among the treatments ($p<0.05$); however, the pH values were relatively stable at 7.01-7.16. All treatment means were within the normal range. NH_3-N concentration was in the optimal range for rumen ecology microbial activity. Future research should investigate the impact of the ability of non forage high fibrous feed to replace forage in intact animal.

Key words: Non forage high fibrous feedstuffs, total mixed ration, fermentation and *In vitro*

Introduction

In recent years, feeding cattle a total mixed ration (TMR) 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. Silage, forage and hay are conventional roughages found in TMR. Long hay, however, when added to the TMR, becomes a problem for mixing machines, as such, it is recommended to reduce the particle size of long hay prior to adding it to the machine. Chopping long hay to reduce particle size is expensive and time consuming. Non forage high fibrous feed is an alternative fibrous feed for ruminant. In tropical zones, there are many varieties of non forage high fibrous feedstuffs: tomato pomace, soybean hull, palm meal, leucaena meal, coconut meal, mung bean meal and dried brewer grain are abundant. The degradation characteristics of non forage high fibrous feedstuffs are the same as forage (Chumpawadee *et al.*, 2005, 2006). The fiber source of TMR is very important because it can affect feed intake, chewing activity, digestibility and production. Soybean hulls appeared to be mixed in a total mixed ration of about 20-25% and they did not affect dry matter intake and production (Sukulthanasorn *et al.*, 2007; Grant, 1997). Additionally, tomato pomace can be fed at 100 % as replacement forage for dairy cows and beef cattle (Sanitwongnaayutaya, 2005). However, the non forage high fibrous feeds are small in particle size and the effective NDF is much lower. The NRC (1989) recommends a minimum of 25 % NDF in total dietary DM, when used as traditional forage and concentrate combinations. Grant (1997) recommends, when using non forage high fibrous feeds as a fiber source, it should be up to 7-10 % from NRC recommendations.

With respect to non forage high fibrous feeds in tropical zones, 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: Non-forage high fibrous feedstuffs and others were collected from various feed mills and organizations in the northeast of Thailand. 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 (AOAC, 1990), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (Van Soest *et al.*, 1991).

Five TMRs were formulated, to have similar total digestible nutrient (TDN), CP, NDF, ADF, but differ in fiber source (Table 2). The experiment was designed in CRD with five replicates per treatment. The fiber sources of the total mixed ration were rice straw (control), tomato pomace, palm meal, dried brewer gain and soybean hulls. Five 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 inoculum 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 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 hour 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 connected 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 y = a+b [(1-Exp(-ct)] (Orskov 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'.

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: % *In vitro* DM or OM digestibility = [(DM or OM initial - DM or OM after incubate) / DM or OM initial] x 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 minutes 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 analyses: All data obtained from the trials were subjected to the analysis of variance procedure of statistical analysis system (SAS, 1996) according to a completely randomized design. Means were separated by Duncan New's Multiple Range Test. The level of significance was determined at $P<0.05$.

Results and Discussion

Chemical composition of feedstuffs and TMRs: The chemical compositions of feed ingredients used in the experiment are shown in Table 1. The feed ingredients varied widely in terms of composition. The non-forage high fibrous feedstuffs have high NDF content, more than 49.5 %. A chemical composition analyses of the five TMRs are presented in Table 2. All five TMRs had a similar chemical composition. The ration CP, ash and NDF content were approximately 12.84%, 11.02% and 36.47%, respectively.

Gas production characteristics of TMRs: Amounts of cumulative gas volume at 2, 4, 6, 12, 24, 48 and 72 after incubations were fitted using the equation y = a+b [(1-Exp (-ct)] (Orskov and McDonald, 1979). Although there are other models available to describe the kinetics of gas production, the Orskov and McDonald (1979) model was chosen because of the compatibility of its parameters with intake, digestibility and degradation characteristics and concentrate feedstuffs has been documented (Blummel and Orskov, 1993; Khazaal *et al.,* 1993; Sommart *et al.,* 2000; Nitipot and Sommart, 2003). Gas production characteristics are presented in Table 3. The a intercept value for all TRMs ranged from -1.22 to -10.41 mL. The values for a, intercept, were negative in the incubations of all TMRs in this study. 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. 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

Table 1: Chemical analysis of feedstuffs used for feed formulation in the experiments

	%DM basis					
Feedstuffs	DM (%)	CP	Ash	NDF	ADF	ADL
Rice straw	91.6	3.4	11.0	72.2	53.2	3.5
Tomato pomace	87.3	20.2	8.4	49.5	44.4	17.4
Palm meal	92.9	7.8	5.1	58.3	47.1	8.5
Dried brewer's gain	91.1	21.0	4.6	49.7	35.1	5.8
Soybean hulls	90.6	10.0	5.0	58.5	50.0	1.8
Leucaena meal	90.9	10.3	9.7	58.0	50.8	17.0
Cassava chip	93.4	1.9	2.0	6.9	6.4	1.9
Sugar cane molasses	72.4	2.2	8.5	0.0	0.0	0.0
Rice bran	91.7	14.3	6.3	20.3	8.1	2.6

Table 2: Feed formulation and chemical composition of dietary treatments

	Dietary treatments[1]				
Ingredients	rt-TMR	tp-TMR	pm-TMR	db-TMR	sh-TMR
Rice straw	40.0	-	-	-	-
Tomato pomace	-	40.0	-	-	-
Palm meal	-	-	40.0	-	-
Dried brewer gain	-	-	-	40.0	-
Soybean hulls	-	-	-	-	40.0
Leuceana meal	3.0	21.6	18.3	24.0	12.0
Cassava chip	25.0	25.0	25.0	25.0	25.0
Sugar cane molasses	3.0	4.3	8.9	9.3	6.5
Rice bran	25.0	8.0	4.6	0.5	13.6
Salt (NaCl)	0.5	0.5	0.5	0.5	0.5
Shell flour	0.4	0.1	0.1	0.1	0.2
Urea	2.6	0.05	2.1	0.1	1.7
Mineral mixed	0.5	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0	100.0
Chemical composition					
DM, %	93.68	93.86	92.28	92.39	92.38
Ash, %	12.98	12.20	10.54	9.70	9.70
CP, %	12.06	13.51	12.19	13.36	13.08
NDF, %	36.32	35.90	37.36	38.00	34.77
ADF, %	26.29	22.67	21.15	25.42	22.62
ADL, %	7.90	10.60	8.08	7.01	5.01
Total digestible nutrient* (TDN),%	60.48	60.09	63.70	62.42	62.87
Calcium (Ca)*, %	0.40	0.43	0.54	0.54	0.49
Phosphorus (P)*, %	0.57	0.40	0.27	0.26	0.34

*Calculated value. [1]rt = Rice straw as fiber source, tp = Tomato pomace as fiber source, pf = Palm meal as fiber source, db = Dried brewer gain as fiber source, sh = Soybean hulls as fiber source

value for absolute |a|, described ideally, reflects the fermentation of the soluble fraction. In this study the |a| was highest for sh-TMR and significance difference (P<0.05) with rs-TMR, tp-TMR, pm-TMR and db-TMR. It is indicated that the soluble fraction in sh-TMR was also highest. The soluble fraction makes it easily attachable by ruminal microorganisms and leads to much gas production (Table 3). Boyle (2007) also reported the fiber in soybean hull is rapidly fermented.

The gas volume at asymptote (b) described the fermentation of the insoluble fraction. The gas volume at asymptote was significantly higher in sh-TMR than that rt-TMR, tp-TMR, pm-TMR and db-TMR (P<0.05). The gas volumes at asymptote have the advantage of predicting feed intake. Blummel and Orskov (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; Nitipot and Sommart, 2003). In this study, sh-TMR showed the highest gas volume which was also the highest *in vitro* digestibility (Table 3). However, when using soybean hull as a fiber source in TMR for intact animals, the user should be aware that fiber in soybean hull ferments rapidly and has a small particle size. Soybean hulls can be used and mixed in total mixed ration about 20-25% (Sukulthanasorn *et al.,* 2007; Grant, 1997). Increasing soybean hull in the TMR has a negative affect on digestibility and growth rate (Ludden *et al.,* 1995).

Rate of gas production (c) expressed in %/h as ranked from the fastest to the slowest were; pm-TMR, tp-TMR, db-TMR, rt-TMR and sh-TMR. Fast rates of gas production were observed in pm-TMR and tp-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 sh-TMR was the highest and significantly different (p<0.05) with rt-TMR, tp-TMR, pm-TMR and db-TMR. This implies that sh-TMR was highly fermentable in the rumen. However, the potential extent of gas production of pm-TMR and db-TMR are the same with rt-TMR (control). Remarkably, the potential of gas production for rt-TMR, tp-TMR, pm-TMR and db-TMR was slightly lower compared with sh-TM, possibly due to the influence of carbohydrate fraction in the TMR. Fibrous constituents had negatively influenced *in vitro* gas production (Melagu *et al.,* 2003). The fiber content of the soybean hull rapidly ferments compared to most fiber sourced (Grant, 1997). Therefore, much gas production was shown in the sh-TMR.

***In vitro* digestibility of dry matter and organic matter:** The *in vitro* dry mater (IVDMD) and organic matter digestibility (IVOMD) at 72 h after incubation are given in Table 3. It can be seen that IVDMD and IVOMD are similar. The IVDMD and IVODM were significantly different (p<0.05) among treatment. The sh-TMR gave the highest IVDMD and IVOMD. This result implies that the microbe in the rumen and animal have high nutrient uptake. The result agrees with Loest *et al.* (2001) who found that soybean hulls have high digestibility in the rumen. In addition, the fiber in soybean hull ferments rapidly (Boyle, 2007) leading to high gas volume (Table 3). Sommart *et al.* (2000) reported that gas volume is a good parameter to predict digestibility, fermentation end product and microbial protein synthesis of substrate by rumen microbes in the *in vitro* system. The IVDMD and

Table 3: Effect of non forage high fibrous feedstuffs as fiber source in TMRs on Gas production characteristics and in vitro digestibility

	Dietary treatments[1]					
Parameters	rt-TMR	tp-TMR	pm-TMR	db-TMR	sh-TMR	SEM
Gas production characteristics[2]						
a, mL	-2.99^{a}	-4.62^{a}	-4.37^{a}	-1.22^{a}	-10.41^{b}	0.94
b, mL	109.23^{b}	90.79^{c}	98.14^{c}	98.14^{c}	161.25^{a}	5.39
c, %/h	0.034^{c}	0.056^{a}	0.057^{a}	0.044^{b}	0.030^{c}	0.00
|a|+b, mL	112.23^{b}	95.42^{c}	102.51^{bc}	100.99^{bc}	171.66^{a}	5.97
In vitro digestibility of dry matter and organic matter at 72 h (%)						
IVDMD	77.58^{c}	76.61^{c}	80.93^{b}	80.07^{b}	93.37^{a}	1.19
IVOMD	79.15^{c}	78.27^{c}	82.27^{b}	81.36^{b}	93.27^{a}	1.14

[a,b,c,d] Means within a row different superscripts differ (p<0.05), [1]rt = Rice straw as fiber source, tp = Tomato pomace as fiber source, pf = Palm meal as fiber source, db = Dried brewer gain as fiber source, sh = Soybean hulls 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)

Table 4: Effect of non forage high fibrous feedstuffs as fiber source in TMRs on pH, ammonia nitrogen (NH_3N) and total volatile fatty acid (TVFA) in *in vitro*

	Dietary treatments[1]					
Parameters	rt-TMR	tp-TMR	pm-TMR	db-TMR	sh-TMR	SEM
pH						
0 h	7.07^{b}	7.11^{ab}	7.14^{a}	7.08^{b}	7.16^{a}	0.01
3 h	7.09	7.08	7.09	7.07	7.09	0.00
6 h	7.07^{ab}	7.07^{ab}	7.06^{ab}	7.04^{b}	7.09^{a}	0.01
9 h	7.08^{a}	7.06^{b}	7.05^{b}	7.07^{ab}	7.07^{ab}	0.00
12 h	7.07^{a}	7.02^{b}	7.03^{b}	7.01^{b}	7.02^{b}	0.01
		--------- Ammonia nitrogen (NH_3N), mg% ---------				
0 h	19.70^{b}	14.97^{c}	21.84^{a}	18.83^{b}	21.81^{a}	0.55
3 h	24.74^{b}	17.20^{c}	29.34^{a}	18.05^{c}	22.69^{b}	0.98
6 h	20.77^{a}	14.09^{b}	19.19^{a}	13.42^{b}	21.70^{a}	0.78
9 h	22.45^{a}	14.86^{c}	20.72^{b}	13.47^{d}	18.22^{c}	0.71
12 h	24.82^{a}	15.18^{c}	20.99^{b}	14.86^{c}	21.36^{b}	0.84
		--------- Total volatile fatty acids, mM ---------				
0 h	36.80^{b}	38.40^{ab}	36.18^{b}	47.26^{a}	30.28^{b}	1.70
3 h	43.08	47.75	39.88	43.57	39.75	1.38
6 h	57.85^{c}	60.31^{c}	71.38^{a}	64.25^{b}	45.79^{d}	1.80
9 h	78.03^{a}	80.74^{a}	52.18^{c}	74.10^{b}	78.77^{a}	2.25
12 h	79.26^{a}	76.31^{a}	58.46^{ab}	48.49^{b}	71.39^{a}	3.68

[a,b,c,d] Means within a row different superscripts differ (p<0.05). [1]rt = Rice straw as fiber source, tp = Tomato pomace as fiber source, pf = Palm meal as fiber source, db = Dried brewer gain as fiber source, sh = Soybean hulls as fiber source.

IVOMD of rt-TMR and tp-TMR lower than pt-TMR, db-TMR and sh-TMR. The reason for that is possibly that the fiber fractions of rt-TMR and tp-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; Hindrichsen *et al.,* 2001). The higher fiber content (Table 1) of rice straw and tomato pomace 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 significantly affected by fiber source in TMRs. The pm-TMR and sh-TMR had higher pH (7.14 and 7.16) than those rt-TMR, tp-TMR and db-TMR. When monitoring pH pattern at 0, 3, 6, 9 and 12 hr after incubation, the pH values were relatively stable at 7.01-7.16 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). 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. However, non forage fiber sources should be considered when feeding intact animals because the physical form of the fiber affects their chewing activity. Less mastication, may reduce their saliva excretion leading to less buffering capacity in the rumen (Grant, 1997). Beauchemin *et al.* (2003) found that a reduction of mean particle size of alfalfa hay from 5.7 to 2.2 mm decreased ruminal pH. Generally, non forage high fibrous feedstuffs have small particle size. It was expected that they negatively affect chewing activity, rumen condition and digestion.

Ammonia nitrogen concentration was significantly different ($p<0.05$) among treatments at each hour 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 0 to 3 hours after incubation pm-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 tp-TMR and db-TMR were low at all time of sampling. It may have been that the urea level in both TMRs was lower than others. In addition, the protein in tomato pomace and dried brewer grain had low degradability (Chumpawadee *et al.,* 2006). Although, NH_3-N concentration of all TMRs was different with rt-TMR (control), it was in the normal range. Therefore, it can be used as non forage high fibrous replacement dietary forage. Future research should investigate the impact of the ability of non forage high fibrous 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, accepting 3 hours after incubation. Remarkably, TVFA concentrations in the *in vitro* medium, from 0 to 12 hours 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 tp-TMR and sh-TMR are the same with rt-TMR and difference from pm-TMR and db-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). Keadly 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.

Conclusion: In *in vitro* study, non forage high fibrous feed stuffs had an affect on gas production characteristic, *in vitro* digestibility and *in vitro* fermentation. The soybean hull as a fiber source in TMR gave the highest parameters of gas production characteristic and *in vitro* digestibility. Concentration of NH_3-N, TVFA and pH were different when TMRs contained a different non forage fiber. Future research should investigate the impact of a non forage high fibrous feed replacement of forage for intact animal.

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