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The *in vitro* gas Production and Ruminal Fermentation of Various Feeds using Rumen Liquor from Swamp Buffalo and Cattle

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ABSTRACT

The objective of this study was to evaluate the effects of various feeds using rumen fermentation in inoculum of buffalo and cattle by using the *in vitro* gas technique. Incubations were carried out using rumen fluid obtained from rumen-fistulated swamp buffalo and cattle during which rice straw was fed on *ad libitum* as a main feed with minimal amount of concentrate (concentrate mixture: 12% CP and 76% TDN) feeding. The fermentation kinetics from twelve feeds commonly used in ruminant was studied using *in vitro* gas production technique. Rumen fermentation parameters substrate disappearance and Volatile Fatty Acids (VFA) production were determined after 96 h of incubation. The results revealed that high potentials for gas production were obtained in concentrate, upper-leaf cassava hay, cassava hay, *Trichanthera gigantea*, lower-leaf cassava hay, Plia fran leaf (*Pluchea indica*) and were highly degradable in the rumen of both species, especially significantly higher in swamp buffalo than in cattle fluid. However, rumen NH_3, VFAs, acetate and acetate to propionate ratio in cattle were higher than in buffalo. However, the propionate production resulted higher in buffalo ($p<0.001$) than in cattle. Therefore, the results indicate that twelve feeds in total gas production and C_3 in buffalo were higher than in cattle liquor accept for rumen NH_3-N, total VFAs, C_2, C_4 and C_2: C_3 concentrations.

Key words: Rumen fluid, *in vitro* gas production technique (IVGPT), ruminal fermentation, volatile fatty acids, tropical feeds

INTRODUCTION

The comparison of rumen metabolism of buffalo and cattle are quite challenging and interesting in order to understand the rumen microbial activities of these ruminant species under the same feeding or under different conditions. Earlier, Wanapat (1989) found that buffaloes could utilize feed more efficiently, particularly with low quality roughages with the digestibility of feed 3-5% higher than in cattle. Furthermore, Wanapat *et al.* (2000) suggested that buffaloes had higher population of cellulolytic bacteria, fungal zoospores, lower protozoal population and a greater recycle nitrogen to the rumen than in cattle. Nevertheless, Franzolin *et al.* (2010) observed that no affect of energy or nitrogen sources on rumen protozoa counting in buffalo and cattle. While Misra *et al.* (2002) also observed higher dry matter intake in buffaloes than in cattle fed on sorghum stover and supplemented with urea. On the other hand, Pradhan *et al.* (1997) observed that dry matter intake per unit metabolic body size was lower in buffalo than in cattle.

Calabro *et al.* (2008) have carried out *in vitro* studies with rumen fluid incubated with common feedstuffs for ruminants; it was found that gas production was lower for inoculum derived from buffalo than for samples from the rumen of cattle. However, the amount of dry matter intake was different between the buffaloes and cattle according to feeding system (Franzolin *et al.*, 2010). Currently, some feed additives that are capable of influencing fiber fermentation and digestion in ruminants were developed (Lee and Ha, 2003; Patra, 2011). Their by-products could be economically used as potential fibrous, energy and protein sources in ruminant nutrition (Aghajanzadeh-Golshani *et al.*, 2010), while legumes are important in order to design feeding strategies for ruminant animals on low quality roughages (Kiraz, 2011; Chanthakhoun *et al.*, 2011). Moreover, Sunvold *et al.* (1995) reported that the *in vitro* gas production technique were useful for study the differences in fermentation patterns, micro-organisms between animal species inoculum.

However, the understanding and research data of the rumen fermentation used end-products of these ruminant species with different feed sources has been limited. Therefore, the aim of this study was to evaluate the effects of various feeds using rumen fermentation in inoculum of buffalo and cattle by using the *in vitro* gas technique.

MATERIALS AND METHODS

Crop residues and selected roughages as substrates: Twelve feeds were used for *in vitro* assay, namely: (Tua-mun, *Phaseolus calcaratus*), cassava chip, rice straw, sorghum, urea treat rice straw, corn cob, concentrate, upper-leaf cassava hay, cassava hay, *Trichanthera gigantea*, plia fran leaf (*Pluchea indica*), lower-leaf cassava hay. These feeds were collected from the farm at Khon Kaen university, North-East of Thailand. Duplicate fresh samples (0.5 kg replicate) were dried in a hot, dry air force oven at 65°C for 72 h and weighed. The samples were then ground to pass through a 1 mm screen for *in vitro* incubation and chemical analysis. The samples were analyzed for DM, CP and ash content by procedures of AOAC (1990). NDF and ADF were assayed using the method of Van Soest *et al.* (1991). Chemical composition is presented in Table 1.

***In vitro* gas production and fermentation technique:** Rumen fluid was collected before the morning feeding from two ruminally fistulated swamp buffalo (*Bubalus bubalis*) and cattle

Table 1: Chemical composition of local feed resources used in *in vitro* gas production incubated in rumen liquor of buffalo and cattle

		DM	CP	Ash	NDF	ADF	CT
Treatments	Feedstuffs	(%)					
T_1	Tua-mun (*Phaseolus calcaratus*)	94.8	18.9	10.1	50.6	30.6	2.5
T_2	Cassava chip	87.9	2.7	10.0	51.0	46.5	–
T_3	Rice straw	91.6	3.8	12.8	78.6	52.2	–
T_4	Sorghum	90.1	5.4	14.5	58.4	38.1	–
T_5	Urea treated rice straw	89.0	6.8	18.3	72.3	50.3	–
T_6	Corn cob	91.3	3.0	12.3	66.5	48.8	–
T_7	Concentrate	90.5	11.8	7.9	16.5	10.3	–
T_8	Upper-leaf cassava hay	90.0	24.3	7.8	52.3	28.7	2.8
T_9	Cassava hay	88.9	21.6	9.9	54.0	31.2	2.2
T_{10}	*Trichanthera gigantea*	89.2	21.1	8.2	52.3	28.9	3.1
T_{11}	Lower-leaf cassava hay	87.2	20.0	10.4	55.0	33.1	2.3
T_{12}	Plia fran leaf (*Pluchea indica*)	97.0	6.8	14.8	25.1	18.3	3.4

DM: Dry matter, OM: Organic matter, CP: Crnde protein, NDF: Neutral-detergent fiber, ADF: Acid-detergent fiber, CT: Condensed tannins

(Native×Brahman) with live weight 400±5 and 320±5 kg, respectively. The animals were offered rice straw on *ad libitum* and fed at 0.5% body weight of concentrate (12% CP and 76% TDN). The animals were fed twice daily, water and mineral licks were available *ad libitum* for 14 days. Rumen fluid was taken from the middle part of the rumen. The 1000 mL rumen liquor was obtained from each of the buffalo and cattle before the morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermos flasks. Preparation of artificial saliva was done according to Menke and Steingass (1988). Artificial saliva was prepared and rumen fluid was mixed in a 2:1 ratio to prepare fermentation solution. The serum bottles with the mixture of substrate treatments were pre-warmed in a water bath at 39°C for 1 h before filling with 30 mL of rumen inoculum's mixture. During the incubation, the gas production was recorded at 0, 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 72 and 96 h. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979) as follows:

$$y = a+b(1-e^{(-ct)})$$

where, a is the gas production from the immediately soluble fraction, b is the gas production from the insoluble fraction, c is the gas production rate constant for the insoluble fraction (b), t is incubation time (a+b) is the potential extent of gas production and y is gas produced at time "t".

Determination of fermentation parameters: The sample inoculum was collected at 0, 4, 6, 8 and 12 h post feeding and were divided into 2 portions; the first portion was centrifuged at 16,000x g for 15 min and the supernatant was stored at -20°C for VFA analysis using HPLC (Samuel *et al*., 1997) and the second portion was used for NH3-N analysis using the micro-Kjeldahl methods (AOAC, 1990).

Statistical analysis: For curve fitting, the 1 mL of gas produced per g in time profiles were fitted to the model $y = a+b(1-e^{(-ct)})$ (Groot *et al*., 1996).

The fermentation characteristics and the fitted parameters were subjected to analysis of variance to detect the inoculum of buffalo and Cattle rumen fluid and various treatment effects; in the model the treatment×inoculum interaction by SAS (1996).

RESULTS AND DISCUSSION

Chemical composition of crop residues and roughages: Chemical compositions of crop residues and roughages are presented in Table 1. The crude protein content of the crop residues and roughage ranged from 2.7 to 24.3%. Rice straw had the lowest crude protein content while the upper-leaf cassava hay had the highest crude protein content. Similar crude protein content was observed in *Trichanthera gigantea* (21.1%) and cassava hay (21.6%). However, crude protein of their substrate in this area was similar to those of Wanapat (2009).

Gas production, kinetic analysis of gas production: Cumulative gas production for each of the substrate treatments are presented as gas production and values for the kinetics of gas production models for substrates studied are given in Table 2. There were not significantly difference ($p>0.05$) in the intercept (a), the fermentation of the soluble fraction of buffalo and cattle. However, there were not significantly different among treatments and species ($p>0.05$). These results were similar to other values as reported by Blummel and Orskov (1993) while

Table 2: *In vitro* fermentation characteristics of the substrates with buffalo and cattle rumen fluid

Species	Treatments	a	b	c	a+b	Gas (96 h) mL 0.2 g^{-1}
Gas Kinetics of Swamp buffalo	T_1	-2.20	86.4	0.07	84.2	95.3
	T_2	-4.30	94.6	0.07	90.3	101.1
	T_3	-1.00	62.8	0.06	61.8	69.9
	T_4	-0.70	71.6	0.06	70.8	80.3
	T_5	-2.40	65.8	0.06	63.5	70.5
	T_6	-4.40	70.9	0.07	66.5	73.8
	T_7	-7.40	153.2	0.07	145.8	159.3
	T_8	-9.50	178.0	0.06	168.6	182.6
	T_9	-3.10	134.4	0.06	131.4	144.0
	T_{10}	-0.40	101.1	0.08	100.8	114.3
	T_{11}	6.10	119.8	0.04	125.9	137.9
	T_{12}	2.00	147.2	0.07	149.2	166.5
Gas Kinetics of cattle	T_1	-3.40	93.1	0.08	89.8	98.7
	T_2	-3.60	91.7	0.09	88.1	98.4
	T_3	-2.80	66.1	0.08	63.3	71.2
	T_4	-2.70	80.5	0.09	77.8	85.9
	T_5	-2.70	56.3	0.08	53.6	61.9
	T_6	-1.00	65.7	0.06	64.6	74.7
	T_7	-7.50	138.9	0.08	131.4	138.7
	T_8	-3.50	123.1	0.07	119.6	128.9
	T_9	-5.40	119.0	0.10	113.6	123.8
	T_{10}	-2.50	108.0	0.08	105.5	117.6
	T_{11}	-3.20	108.7	0.08	105.5	116.2
	T_{12}	-3.70	135.7	0.09	132.0	143.8
SEM		0.80	2.7	0.01	2.6	2.9
Treatment		0.11	**	NS	**	**
Species		0.48	***	NS	**	***
Species×Treatment		0.33	NS	NS	NS	NS

T_1: Tua-mun (*Phaseolus calcaratus*), T_2: Cassava chip, T_3: Rice straw, T_4: Sorghum, T_5: Urea treat rice straw, T_6: Corn cob, T_7: Concentrate, T_8: Upper-leaf cassava hay, T_9: Cassava hay, T_{10}: *Trichanthera gigantea*, T_{11}: Lower-leaf cassava hay, T_{12}: Plia fran leaf (*Pluchea indica*); MSE: Mean square error; NS: Non significant; *: $p<0.05$; ***: $p<0.001$

Khazaal *et al.* (1993) reported negative values for various substrates when using mathematical models to fit gas production kinetics. This was probably due to either a deviation from the exponential cause of fermentation or delays in the onset of fermentation due to microbial colonization. It is well known that the value for absolute a (a), described ideally, reflects the fermentation of the soluble fraction.

Whereas, gas production from the insoluble fraction (b) of buffalo and cattle ranged from 62.8 to 178.0 and 56.3 to 138.9, respectively and were significantly different among treatments and species ($p<0.05$). The potential extent of gas production (a+b) of buffalo and cattle ranged from 63.5 to 168.6 and 53.6 to 132.0, respectively and were significantly different among treatments and species ($p<0.05$). Gas production rate constants for the insoluble fraction (c) of buffalo and cattle were not significantly different among treatments ($p<0.05$). Cumulative gas production at 96 h of buffalo and cattle were significantly different ($p<0.01$) among treatments and were higher in buffalo than in cattle. However, these were no significant interaction between treatments and species ($p = 0.05$). On the other hand, Chumpawadee *et al.* (2006) reported that the kraphanghom,

Table 3: Ruminal fermentation end-products parameters measured

Species	Treatments	NH_3-N	Total VFA	C2	C3	C4	C2:C3
Volatile fatty acid mL g^{-1} of Swamp buffalo	T_1	12.9	52.5	63.5	24.0	12.6	2.6
	T_2	12.5	54.4	60.3	22.9	16.8	2.6
	T_3	11.0	44.8	61.6	23.9	14.5	2.6
	T_4	10.9	53.3	63.2	22.2	14.6	2.8
	T_5	11.7	48.9	64.3	23.0	12.7	2.8
	T_6	11.4	43.7	61.2	23.1	15.7	2.6
	T_7	13.6	55.9	57.9	23.8	18.4	2.4
	T_8	9.7	55.5	59.2	24.7	16.2	2.4
	T_9	11.8	56.8	61.9	23.2	19.9	2.4
	T_{10}	12.4	52.6	61.3	24.3	14.4	2.5
	T_{11}	11.6	50.6	62.4	23.0	14.6	2.7
	T_{12}	12.3	59.9	58.2	24.9	16.8	2.3
Volatile fatty acid mL g^{-1} of Cattle	T_1	14.1	68.8	72.7	19.7	12.2	3.7
	T_2	13.6	71.9	71.3	17.0	18.6	4.2
	T_3	12.4	60.9	73.3	17.1	13.3	4.3
	T_4	12.4	64.7	73.7	17.5	13.2	4.2
	T_5	13.5	65.2	75.4	17.2	12.1	4.4
	T_6	12.9	54.9	74.9	16.3	14.1	4.6
	T_7	14.4	69.2	72.4	17.3	15.6	4.2
	T_8	9.2	71.7	73.2	16.8	15.3	4.4
	T_9	13.5	75.2	74.2	19.9	15.7	3.5
	T_{10}	13.9	65.1	73.4	17.5	13.5	4.2
	T_{11}	13.5	66.7	67.9	14.4	21.4	4.7
	T_{12}	14.0	65.1	71.6	19.1	12.4	3.7
SEM		0.3	1.6	1.7	0.4	0.7	0.2
Treatment		**	***	NS	NS	**	NS
Species		**	**	***	***	NS	***
Species×Treatment		NS	NS	NS	NS	NS	NS

T_1: Tua-mun (*Phaseolus calcaratus*), T_2: Cassava chip, T_3: Rice straw, T_4: Sorghum, T_5: Urea treat rice straw, T_6: Corn cob, T_7: Concentrate, T_8: Upper-leaf cassava hay, T_9: Cassava hay, T_{10}: *Trichanthera gigantean*, T_{11}: Lower-leaf cassava hay, T_{12}: Plia fran leaf (*Pluchea indica*); MSE: Mean square error; NS: Non significant; *: $p<0.05$; ***: $p<0.001$

rice straw, corn stover, chinese spinach and cavalcade hay were highly digestible in the rumen while, Khazaal *et al.* (1995) reported that protein fermentation did not lead to much gas production. In addition, fibrous constituents also negatively influenced *in vitro* gas production (Melaku *et al.*, 2003). In the present study, it was found that high potentials for gas production were observed in concentrate, upper-leaf cassava hay, cassava hay, *Trichanthera gigantean*, lower-leaf cassava hay, plia fran leaf (*Pluchea indica*) and were highly digestible in buffalo than in cattle fluid.

***In vitro* fermentation products:** The effect of twelve substrates on in vitro fermentation NH_3 and VFAs were given in Table 3. There were significantly difference ($p<0.01$) in the NH_3, total volatile fatty acid and butyrate, while the proportions of NH_3, VFAs, acetate, butyrate and acetate to propionate ratio, were significant different ($p<0.01$) found in cattle than in buffalo. Therefore, this may be due to cattle were able to utilize concentrate better than buffalo. However, Beever and Mould (2000) who reported that high forage diets were higher C_2 and C_4 whike high starch diets were higher C_3 although C_2 was still the predominant VFA. Thus, under earlier experiment, cattle

and swamp buffaloes also showed differences in rumen bacterial, protozoal population and fungal zoospore counts (Wanapat *et al*., 2000). Under similar experimental condition, Malakar and Walli (1995) found that the average counts of anaerobic bacteria were significantly higher for buffalo than for cow.

CONCLUSIONS

Based on this experiment, it could be summarized that there were differences in gas production, fermentation end-products using rumen liquor from swamp buffalo and cattle. These feeds were highly degradable in the rumen of both species. The differences found could contribute to the capability of feed utilization in the two species. However, more *in vivo* digestion and feeding trials should be investigated further.

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