The Journal of Animal & Plant Sciences, 27(6): 2017, Page: 1758-1765
ISSN: 1018-7081

IMPROVING THE QUALITY OF SUGARCANE BAGASSE BY UREA AND CALCIUM HYDROXIDE ON GAS PRODUCTION, DEGRADABILITY AND RUMEN FERMENTATION CHARACTERISTICS

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ABSTRACT

The objective of this study was to determine the influence of urea and whole soybean meal or calcium hydroxide [$Ca(OH)_2$] treatment on sugarcane bagasse nutritive values and *in vitro* fermentation. The experimental design was a completely randomized design (CRD) and the dietary treatments were sugarcane bagasse treated with urea (0, 20, 40g/kg) and whole soybean meal or $Ca(OH)_2$ (0, 25, 50g/kg). It was found that the protein content of sugarcane bagasse was significantly increased by whole soybean meal and urea treatment, and the values ranged from 65-71 g/kg DM. On the other hand, treatment of $Ca(OH)_2$ 50 g/kg with urea 40 g/kg could reduce ($p<0.05$) fiber content of sugarcane bagasse and the values were 533, 514 and 104 g/kg DM for NDF, ADF and ADL, respectively. Gas production from the insoluble fraction (b), potential extent of gas production (a+b) and cumulative gas production were significantly increased ($p<0.05$) in sugarcane bagasse treated with urea 40 g/kg and urea 40 g/kg + whole soybean meal 25 g/kg (55.3, 53.5 and 50.1 ml), respectively. Urea and $Ca(OH)_2$ treatment could increase DM and OM degradability, true digestibility and microbial mass of sugarcane bagasse($p<0.05$). Furthermore, the concentration of C3, C2 and C2:C3 were improved by urea and whole soybean meal or $Ca(OH)_2$ treatment. In addition, methane production was decreased in sugarcane bagasse treated with urea 40 g/kg in the present study. Based on this experiment, it could be concluded that treatment of urea and $Ca(OH)_2$ was an alternative method to improve the nutritive value of sugarcane bagasse, gas production, digestibility and ruminal fermentation characteristics.

Keywords: Sugarcane bagasse, Urea, Calcium hydroxide, Digestibility, Rumen fermentation.

INTRODUCTION

In the tropics, majority of roughage source for ruminant consist of leftovers from plant harvest, grasses and foliage growing on roadsides or waste land. However, it is critical during dry season and hectic season of cultivation (Wanapat, 1986; Wanapat and Devendra, 1992) when it is a limitation of availability of feed. Alternative feed resources and industrial by-product are potential to use as roughage source for livestock production. Sugarcane bagasse is sugarcane industrial by-product practically used by farmers for ruminant feeding especially during the long dry season (Costa *et al*., 2015; Pessoa *et al*., 2009; Leme *et al*., 2003) . However, sugarcane bagasse is low in nutritive value as very low level of protein (<30 g/kg), high fibre and lignin content (NDF >800 g/kg, ADF > 600 g/kg and ADL>100 g/kg) (Balgess *et al*., 2007; Okano *et al*., 2006), and low digestibility (200-300 g/kg); thus, resulted in poor animal performance (Jayasuiry, 2007). Therefore, a potential use of sugarcane bagasse as a ruminant feed may be realized through the development of physical, chemical and biological treatments to disrupt the lingo-cellulose complex.

Various methods have been used to improve the nutritive value of sugarcane bagasse including physical, chemical, biological treatments for ruminants feeding (Balgees *et al*., 2007; Okano *et al*., 2006). Ahme *et al*. (2013) revealed that nutritive values of sugarcane bagasse were improved by urea treatment. In addition, Balgees *et al*. (2015) reported that 50 g/kg urea and 30 g/kg ammonia treatments of sugarcane bagasse increased the CP content and *in vitro* dry matter digestibility. However, due to the cost of urea was remarkably expensive, it resulted in a higher cost of production. Fadel Elseed *et al*. (2003) suggested that calcium hydroxide [$Ca(OH)_2$] could improve rumen digestibility. The concentrated

alkaline agents can chemically break the ester bonds between lignin and hemicellulose and cellulose, and physically make structural fibres swollen (Wanapat *et al*., 2009). These effects enable rumen microbes to attack the structural carbohydrates more easily (Polyorach and Wanapat, 2014) . Moreover, calcium residue which remained in the treated straw causes no serious problems to the animal or environment and can be a calcium supplement to the animals (Wanapat *et al*., 2009) . Moreover, Sarmento (2001) presented that digestibility of ground whole soybean had trend to improve the utilization of sugarcane bagasse.The soybean treatment is based on adequate ureolysis due to the effect of enzyme urease on the cell wall of sugarcane bagasse. However, the use of urea with urease in ground whole soybean and calcium hydroxide for improve sugarcane bagasse are still limited. Therefore, the objective of this experiment was to determine the effects of urea plus ground whole soybean or $Ca(OH)_2$ treatment on sugarcane bagasse nutritive value, gas production, degradability and rumen fermentation characteristics in *in vitro* gas techniques.

MATERIALS AND METHODS

Experimental design and dietary treatments: The experimental design was a completely randomized design (CRD) and the dietary treatments were various levels of urea and whole soybean meal or calcium hydroxide [$Ca(OH)_2$] treated sugarcane bagasse. The sugarcane bagasse was collected from Rerm Udom sugar factory CO., LTD. at Nong Han, Udon Thani Province, Thailand. Sugarcane bagasse was treated with urea at 0, 20, 40 g/kg and whole soybean meal or $Ca(OH)_2$ at 0, 25, 50 g/kg, respectively, ensiled in plastic boxes at room temperature for 14 days before analysis of nutritive value and use in *in vitro* study (Wanapat, 2000). The samples were dried by hot air oven at 60 °C then ground to pass a 1 mm sieve and analyzed for dry matter (DM), crude protein (CP) and ash (AOAC, 1990) , neutral detergent fiber (NDF) and acid detergent fibre (ADF) (Van Soest et al., 1991). The chemical compositions of each treated sugarcane bagasse are shown in Table 1.

Animals and inocula: Two male, rumen-fistulated beef cattle with body weight of 500±30 kg were used as rumen fluid donors. Animals were fed rice straw*ad libitum* and were housed in individual pens and had free access to fresh water and vitamin/mineral block for at least 14 days. On day 15, about 1,000 ml of rumen liquor was taken through the cannula of each animal before the morning feeding and strained through four layers of cheesecloth into an Erlenmeyer flask and then transported to the laboratory.

***In vitro* fermentation of substrates:** The ensiled sugarcane bagasse (500 mg) was weighed into 50 ml serum bottles. Ruminal fluid from each animal was mixed with the artificial saliva solution prepared according to Menke and Steingass (1988) in a proportion 2:1 (ml/ml) at 39 °C under continuous flushing with CO^2. Forty millilitres of rumen inoculum's mixture were added into each bottle under CO^2 flushing. Bottles were sealed with rubber stoppers and aluminium caps and incubated at 39 °C.

Sample collection and analysis: During the incubation, the gas production was measured at 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 96 h by using a pressure transducer and a calibrated syringe. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) as follows:

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

where a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), t = incubation time, (a+b) = the potential extent of gas production. y = gas produced at time "t". Inoculum ruminal fluid was sampled at 12 and 24 h post inoculations and pH were measured immediately using a portable pH temperature meter (Hanna Instruments HI 8424 microcomputer, Singapore). The fluid samples were then filtered through four layers of cheesecloth. Samples was centrifuged at 16,000×g for 15 min, and the supernatant was stored at −20 °C before NH_3–N analysis using the micro-Kjeldahl methods AOAC (1990) and VFA analysisusing High Pressure Liquid Chromatography (HPLC, Instruments by Water and Novapak model 600E; water mode l484 UV detector; column novapak C18; column size 3.9 mm × 300 mm; mobile phase10 mM H_2PO_4 [pH 2.5]) according to Samuel *et al.* (1997). *In vitro* degradability was determined after termination of incubation at 96 h. The contents were filtered through pre-weighed Gooch crucibles (40 mm of porosity) and residual dry matter was estimated. The percent loss in weight was determined and presented as *in vitro* dry matter degradability (IVDMD). The dried feed sample and residue left above was ashed at 550°C for determination of *in vitro* organic matter degradability (IVOMD) (Tilley and Terry, 1963). At 48 h post inoculation, two bottles of each sample was determined *in vitro* true digestibility according to Van Soest *et al*. (1991). The true digestibility was used to calculate microbial mass according to the method of Blümmel *et al*. (1997). Calculation of ruminal methane (CH_4) production using VFA proportions according to Moss *et al*. (2000) as follows:

CH_4 production = 0.45(acetate) −0.275 (propionate) +0.4(butyrate)

Statistical analysis: All data were analyzed as a completely randomized design using the GLM procedure of SAS (1998). Differences between treatment means were determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980) and differences among means with $p<0.05$ were accepted as representing statistically significant differences. Group of treatments respond was performed by orthogonal contrast.

RESULTS

Chemical composition of feeds: Table 1 shows chemical composition of sugarcane bagasse affected by urea and whole soybean meal or $Ca(OH)_2$ treatment. Crude protein of sugarcane bagasse was increased by urea and whole soybean meal treatment especially at40 g/kg urea and 50 g/kg whole soybean meal treatment (71 g/kg CP). Moreover, treatment with urea and $Ca(OH)_2$ could decrease the NDF, ADF and ADL content in the sugarcane bagasse and the lowest was in group of 40 g/kg urea and 50 g/kg $Ca(OH)_2$ treatment.

***In vitro* gas production and digestibility:** Gas kinetics, cumulative gas production and *in vitro* digestibility of each substrate treatments are presented in Table 2. Gas production from the insoluble fraction (b), gas potential extent of gas production (a+b) and cumulative gas production were influenced by increasing urea treatment level ($p<0.05$); but neither by whole soybean meal nor $Ca(OH)_2$ treatment ($p>0.05$). The IVDMD and IVOMD at 96 hr after incubation were increased in sugarcane bagasse treated with urea and $Ca(OH)_2$ ($p<0.05$). True digestibility of sugarcane bagasse treated with 40 g/kg urea and 50 g/kg $Ca(OH)_2$ was the highest ($p<0.05$) among treatment and followed by the 40 g/kg urea + 25 g/kg $Ca(OH)_2$ group, respectively. Moreover, urea and $Ca(OH)_2$ treatment of bagasse can significantly ($p<0.05$) enhance microbial mass.

Characteristics of ruminal fermentation: The effect of sugarcane bagasse treated with urea and whole soybean meal or $Ca(OH)_2$ on *in vitro* fermentation parameters are presented in Table 3 and 4. Ruminal pH and NH_3-N ranged from 6.6-6.7 and 16.4-22.3 mg/dl which were not alter by any treatment. Moreover, total VFA concentrations and proportion of butyric acid were not different among treatments. Propionic acid in the treated group was increased ($p<0.05$) while acetic acid (C2) and acetic to propionic ratio (C2:C3) was decreased. The proportion of propionic acid (C3) in the 40 g/kg urea and 50 g/kg $Ca(OH)_2$ treated sugarcane bagasse was the highest (24.7%). The C2:C3 ratio and methane production (CH_4) in the 40 g/kg urea and 50 g/kg $Ca(OH)_2$ treated sugarcane bagasse was the lowest ($p<0.05$) and followed by the 40 g/kg urea and 25 g/kg $Ca(OH)_2$ treated sugarcane bagasse, respectively (Table 3).

Table 1. Effect of urea and whole soybean meal or calcium hydroxide treatment on nutritive value of sugarcane bagasse.

Treatment	DM	Ash	OM	CP	NDF	ADF	ADL
	g/kg		.....g/kg DM.....				
T1 Control	725^{a}	77	923	28^{d}	794^{a}	698^{a}	147^{a}
T2 (U2)	300^{e}	78	922	47^{bc}	764^{a}	696^{a}	141^{a}
T3 (U2 : S2.5)	308^{cde}	78	922	65^{a}	790^{a}	699^{a}	143^{a}
T4 (U2 : S5)	352^{b}	81	919	70^{a}	728^{a}	674^{a}	141^{a}
T5 (U2 : C2.5)	304de	79	921	37^{cd}	678^{ab}	628^{ab}	124^{b}
T6 (U2 : C5)	313^{cde}	78	922	41^{cd}	627^{b}	565^{c}	108^{c}
T7 (U4)	324^{c}	80	920	67^{a}	769^{a}	679^{a}	139^{a}
T8 (U4 : S2.5)	347^{b}	79	921	68^{a}	769^{a}	641^{ab}	138^{a}
T9 (U4 : S5)	321^{cd}	82	918	71^{a}	762^{a}	697^{a}	137^{a}
T10 (U4 :C2.5)	323^{cd}	79	921	59^{ab}	665^{b}	614^{ab}	109^{c}
T11 (U4 : C5)	349^{b}	81	919	59^{ab}	533^{c}	514^{c}	104^{c}
SEM	0.40	0.17	0.17	0.30	2.00	2.20	0.39
P-value	**	ns	ns	**	*	*	*
U0 vs U2 U4	**	ns	ns	**	Ns	ns	ns
S0 vs S2.5 S5	**	ns	ns	**	Ns	ns	ns
C0 vs C2.5 C5	**	ns	ns	ns	*	*	*

^{abcde}= Mean within columns with different superscript letters differ ($P<0.05$); U=urea, S=whole soybean meal, C=calcium hydroxide, DM=dry matter, OM=organic matter, CP=crude protein, NDF=neutral detergent fiber, ADF=acid detergent fiber, *$p<0.05$, **$p< 0.01$, ns = non-significant, SEM= standard error of the mean.

Table 2. Effect of urea and whole soybean meal or calcium hydroxide treated sugarcane bagasse on *in vitro* gas production, *in vitro* digestibility and microbial mass.

Treatment	Gas kinetics				Gas production ml/0.5 g DM substrate	*In vitro* degradability,%		True digest	Microbial mass
	a	b	c	a+b		IVDMD	IVOMD		
T1 Control	-0.5	44.9^{c}	0.03	44.4^{bc}	42.8^{b}	18.0^{b}	37.5^{d}	32.0^{ef}	4.7^{d}
T2 (U2)	0.1	52.0^{ab}	0.03	52.0^{ab}	47.8^{a}	19.8^{ab}	40.6^{d}	31.4^{f}	5.4^{cd}
T3 (U2 : S2.5)	-0.4	52.7^{ab}	0.02	52.3^{ab}	46.0^{a}	19.7^{ab}	39.3^{d}	36.6^{def}	8.5^{bc}
T4 (U2 : S5)	0.7	48.8^{ab}	0.03	49.6^{ab}	44.7^{a}	19.7^{ab}	45.5^{bcd}	37.7^{cdef}	8.9^{bc}
T5 (U2 : C2.5)	-1.4	51.4^{ab}	0.03	49.9^{ab}	44.9^{a}	20.8^{ab}	47.0^{bc}	32.1^{ef}	6.7^{cd}
T6 (U2 : C5)	-0.5	46.5^{ab}	0.02	46.0^{ab}	44.0^{a}	25.0^{a}	56.4^{ab}	39.3^{bcde}	13.4^{a}
T7 (U4)	-1.8	55.3^{a}	0.04	53.5^{a}	48.8^{a}	21.2^{ab}	53.2^{bc}	45.7^{ab}	12.7^{a}
T8 (U4 : S2.5)	-1.4	52.6^{ab}	0.04	51.2^{ab}	50.1^{a}	24.6^{a}	55.6^{ab}	45.0^{ab}	12.7^{a}
T9 (U4 : S5)	1.1	49.0^{ab}	0.02	50.1^{ab}	46.2^{a}	21.3^{ab}	50.0^{bc}	44.5^{abc}	12.7^{a}
T10 (U4 :C2.5)	-0.6	53.3^{ab}	0.03	52.6^{ab}	49.5^{a}	23.2^{a}	56.7^{ab}	47.4^{a}	12.8^{a}
T11 (U4 : C5)	-1.7	55.1^{a}	0.03	53.3^{a}	49.1^{a}	25.7^{a}	59.2^{a}	47.8^{a}	12.9^{a}
SEM	0.71	1.37	0.02	1.39	1.24	1.0	2.18	1.14	0.56
P-value	ns	*	ns	*	*	*	**	**	**
U0 vs U2 U4	ns	*	ns	*	*	*	*	*	*
S0 vs S2.5 S5	ns	ns	ns	ns	ns	Ns	Ns	ns	ns
C0 vs C2.5 C5	ns	ns	ns	ns	ns	*	*	*	*

^{abc}= Mean within columns with different superscript letters differ (P<0.05), U=urea, S=whole soybean meal, C=calcium hydroxide, a= the gas production from the immediately soluble fraction (ml), b= the gas production from the insoluble fraction (ml), c= the gas production rate constant for the insoluble fraction (ml/hr), IVDMD=*in vitro* dry matter digestibility (%), IVDMD=*in vitro* organic matter digestibility (%), *p<0.05, **p< 0.01, ns = non-significant, SEM= standard error of the mean.

Table 3. Effect of urea and whole soybean meal or calcium hydroxide treated sugarcane bagasse on volatile fatty acids.

Treatment	TVFA, (mmol)			% C2			% C3			% C4			C2:C3 ratio		
	12h	24h	mean	12h	24h	mean	12h	24h	mean	12h	24h	mean	12h	24h	mean
T1 Control	12.9	15.9	14.4	71.5^{a}	63.7	68.1	22.2^{bc}	19.3	20.8^{d}	6.3	8.9	7.9	3.1^{ab}	3.4	3.3^{a}
T2 (U2)	17.7	18.5	18.1	68.6^{abc}	68.8	68.7	22.7^{bc}	21.2	21.9^{bcd}	8.8	9.9	9.4	3.1^{ab}	3.3	3.1^{abc}
T3 (U2 : S2.5)	15.4	19.7	17.5	67.9^{abc}	68.1	68.0	25.2^{ab}	19.9	22.6^{abcd}	6.8	12.0	9.4	2.7^{bc}	3.4	3.0^{abcd}
T4 (U2 : S5)	12.6	20.0	16.3	69.5^{ab}	67.7	68.6	22.9^{bc}	20.0	21.4^{cd}	7.6	12.4	9.9	3.0^{ab}	3.4	3.2^{ab}
T5 (U2 : C2.5)	8.9	18.9	15.4	67.9^{abc}	64.6	65.8	22.5^{bc}	21.2	22.6^{abcd}	6.5	14.3	10.4	3.0^{ab}	3.1	3.0^{abcd}
T6 (U2 : C5)	13.2	18.0	15.6	67.9^{abc}	63.7	65.8	24.0^{abc}	20.1	22.4^{abcd}	8.1	15.6	11.8	2.8^{bc}	3.1	3.0^{abcd}
T7 (U4)	14.8	19.0	16.9	65.9^{bc}	67.2	66.6	26.4^{ab}	21.3	23.8^{abc}	7.8	11.5	9.6	2.5^{bc}	3.2	2.8^{bcd}
T8 (U4 : S2.5)	13.8	18.5	16.1	66.2^{bc}	69.1	67.7	26.5^{ab}	21.7	24.1^{ab}	7.3	9.2	8.2	2.5^{bc}	3.2	2.8^{bcd}
T9 (U4 : S5)	10.7	20.0	16.9	68.0^{abc}	68.2	68.1	25.0^{ab}	21.0	23.0^{abcd}	7.1	10.8	8.9	2.8^{bc}	3.3	2.9^{abcd}

T10 (U4 :C2.5)	11.4	18.8	15.1	67.3^{bc}	70.0	68.7	26.2^{ab}	21.9	24.0^{ab}	6.5	8.0	7.3	2.6^{bc}	3.2	2.8^{bcd}
T11 (U4 : C5)	13.7	10.5	12.1	67.5^{bc}	68.1	67.8	27.2^{a}	22.2	24.7^{a}	5.3	9.7	7.5	2.5^{bc}	3.1	2.7^{cd}
SEM	2.57	1.71	1.67	1.00	1.33	0.91	0.81	0.50	0.49	0.69	1.27	0.72	0.14	0.10	0.09
P-value	ns	ns	ns	**	ns	ns	*	ns	*	ns	ns	ns	*	ns	*
U0 vs U2 U4	ns	ns	ns	**	ns	ns	**	ns	**	ns	ns	ns	**	ns	**
S0 vs S2.5 S5	ns	ns	ns	**	ns	ns	**	ns	**	ns	ns	ns	**	ns	**
C0 vs C2.5 C5	ns	ns	ns	**	ns	ns	**	ns	**	ns	ns	ns	**	ns	**

^{abcd}= Mean within columns with different superscript letters differ (P<0.05); U=urea, S=whole soybean meal, C=calcium hydroxide, *p<0.05, **p< 0.01, ns = non-significant, SEM= standard error of the mean.

Table 4. Effect of urea and whole soybean meal or calcium hydroxide treated sugarcane bagasse on ruminal pH, ammonia nitrogen and methane production.

Treatment	Ruminal pH	NH_3-N,(mg/ dL)			CH_4 production[1], mM/L		
		12h	24h	mean	12h	24h	mean
T1 Control	6.6	23.8	16.8	20.3	28.6^{a}	30.9	28.1a
T2 (U2)	6.7	24.5	18.2	21.4	28.2^{a}	30.1	28.7^{a}
T3 (U2 : S2.5)	6.7	16.1	24.8	20.5	26.4^{c}	30.0	28.1^{a}
T4 (U2 : S5)	6.7	19.9	21.3	20.7	28.0^{ab}	29.9	28.9^{a}
T5 (U2 : C2.5)	6.7	19.9	18.9	19.4	27.0^{b}	29.0	27.6^{a}
T6 (U2 : C5)	6.7	23.5	17.8	20.7	27.2^{b}	29.4	28.2^{a}
T7 (U4)	6.7	19.3	18.6	18.9	25.5^{c}	29.0	27.3^{b}
T8 (U4 : S2.5)	6.7	25.6	19.1	22.3	25.4^{c}	28.8	27.1^{b}
T9 (U4 : S5)	6.7	20.7	19.6	20.1	26.6^{ab}	29.2	27.9^{a}
T10 (U4 :C2.5)	6.7	25.6	19.1	16.4	25.7^{bc}	28.7	27.2^{b}
T11 (U4 : C5)	6.7	22.4	18.9	20.7	25.0^{c}	28.4	26.7^{b}
SEM	0.70	1.46	1.76	0.94	0.15	0.65	0.25
P-value	ns	ns	ns	ns	*	ns	*
U0 vs U2 U4	ns	ns	ns	ns	**	ns	*
S0 vs S2.5 S5	ns	ns	ns	ns	**	ns	*
C0 vs C2.5 C5	ns	ns	ns	ns	**	ns	*

^{abc}= Mean within columns with different superscript letters differ (P<0.05); U=urea, S=whole soybean meal, C=calcium hydroxide, *p<0.05, **p< 0.01, ns = non-significant, SEM= standard error of the mean. [1]Calculated according to Moss et al. (2000) CH_4 production=0.45(acetate)−0.275(propionate)+0.4(butyrate).

DISCUSSIONS

Chemical composition of feeds: Nutritive value of sugarcane bagasse was improved by urea and whole soybean meal or $Ca(OH)_2$ treatment. The urease from whole soybean meal is the responsible enzyme for hydrolysis of urea into ammonia (Sarmento *et al*., 2001). The use of a urease source for the treatment of sugarcane bagasse with urea can improve the urea decomposition process into ammonia, enhancing the nitrogen content of lingo-cellulosic materials (Sarmento *et al*., 2001; Mohammed *et al*., 2013). Hameed *et al*. (2012) reported that the quality of feed was increased with the increasing of urea addition (20, 40, or 60 g/kg) and ensiled period (2, 4 or 6 weeks).The cell wall components (NDF, ADF and ADL) was decreased by $Ca(OH)_2$ treatment. Theander and Aman (1984) stated that saponification of ester linkages between acetic acid and phenolic acids and polysaccharides and/ or lignin as well as such linkages between uronic acids residues of xylans in hemicelluloses and lignin would be expected to occur during the alkaline treatment of sugarcane bagasse. High temperature and alkaline condition causes cleavages in the lignin and formation of other linkages between phenyle propane units and free phenolic groups. As a result of the

accompanying decrease in the molecular weight and cleavages of linkages to the hemicellulose, an increased solubility of lignin in the alkaline solution will occur (Theander and Aman, 1984).

***In vitro* gas production and digestibility:** Gas kinetic and *in vitro* gas production were no influenced by whole soybean meal and $Ca(OH)_2$ treatment ($p>0.05$).This could be due to a lower dissociation constant for whole soybean meal and $Ca(OH)_2$ treatment and a longer reaction period for complete effectiveness may be required (Rounds *et al*., 1979). Meanwhile, gas production from the insoluble fraction (b), gas potential extent of gas production (a+b) and cumulative gas production were improved by urea treatment of sugarcane bagasse ($p<0.05$). The increased gas kinetic and *in vitro* gas production may be contributed by an increase of degradability. An increased degradable fraction may have been attributed to an increased degradability of structural carbohydrates such as hemicelluloses and cellulose for 20 and 40 g/kg urea treatment (Hameed *et al*., 2012). Moreover, $Ca(OH)_2$ treatment also enhanced the*in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD). The concentrated alkaline agents (urea and $Ca(OH)_2$) can chemically change structural fibre swollen and easily digested by rumen microbes (Wanapat *et al*., 2009). This effect avails the rumen microbes to attack the structural carbohydrates more easily, improve digestibility, as well as the palatability of treated straw (Bod'a, 1990). Consistently, Wanapat and Cherdthong (2009) found that increasing CP content in rice straw indicated in significant increase in degradability and feed intake in ruminants. Moreover, increasing DM and OM digestibility in this study may also affect on *in vitro* true digestibility. This could be related to the considerable amounts of semidigested material and/or to unknown factors that enhanced rumen microorganisms (Ørskov, 1994). In addition, higher *in vitro* true digestibility reflects higher microbial biomass (MB) (Infascelli *et al*., 2005; Zicarelli *et al*., 2011) and result was also found in this experiment for urea plus $Ca(OH)_2$ treated sugarcane bagasse. The MB produced in the rumen by micro-organisms is the major source of protein for the ruminants and the prediction of efficiency of MB production is very important in ruminant nutrition (Karabulut*et al*., 2007; Kongmun*et al*., 2010).

Characteristics of ruminal fermentation: Under this study, ruminal pH and NH_3-Nconcentration ranged from 6.6-6.7 and 16.1-25.6 mg/dl and was similar to the values reported by Wanapat and Pimpa (1999). The ruminal NH_3-N result obtained under this study was closer to15–30 mg/dl (Wanapat and Pimpa, 1999). These concentrations could improve rumen fermentation and feed intake in swamp buffaloes offered urea-treated rice straw. Ørskov *et al*. (1999) stated that, when high fiber diets were offered, VFAs in ruminal fermentation shifted from 65:25:10 to 70:20:10 (C2: C3: C4, in molar percentage) ratios. The effects of various treated sugarcane bagasse on VFA concentrations (Table 3) especially those of C2 were decreased ($p<0.01$) and those of C3 were increased ($p<0.01$), thus C2:C3 was subsequently lowered ($p<0.05$) in 40 g/kg urea + 50 g/kg $Ca(OH)_2$ treated sugarcane bagasse and 40 g/kg urea + 25 g/kg $Ca(OH)_2$ treated sugarcane bagasse, respectively. This could be explained by the effect of high *in vitro* digestibilities of OM and DM and these results also agreed with Cherdthong and Wanapat (2013). Volatile fatty acids concentration in this experiment were similar to those reported by Polyorach and Wanapat (2014) who reported the increasing of VFAconcentrations especially C3 by various treated rice straw; thus, C2:C3 was subsequently lowered. Moreover, chemical treatments enhanced the nutritive value of sugarcane bagasse through increasing the number of accessible sites of microbial attachment on the surface of the particles, increasing microbe quantity and improving rumen fermentation characteristics (Chen *et al*., 2008) especially those of C2 and C2:C3 ratio were decreased and those of C3 were increased. In addition, higher C3 and lower C2 and C2:C3 ratio reflects lower methane production (Anantasook and Wanapat, 2012; Anantasook *et al*., 2014) and result was also found in this experiment for urea plus whole soybean meal and $Ca(OH)_2$ treated sugarcane bagasse.

Conclusions: Based on this study, it could be concluded that treatment of urea and $Ca(OH)_2$ could improve the nutritive value of sugarcane bagasse,*in vitro* digestibility, microbial mass, ruminal volatile fatty acids while decreased methane production. The results under this study offer additional and practical data on the use of low quality roughage such as sugarcane bagasse and applicability for use under practical farm conditions. Furthermore, *in vivo* study (metabolism trail and feeding trail) are recommended to investigate its effects on palatability, rumen characteristics and animal production such as meat and milk.

Acknowledgements: The authors would like to express their sincere thanks to the Thailand Research Fund (TRF) and the research and development institute, Udon Thani Rajabhat University through the Research Grant for New Scholar (Contract no. TRG5780118) for their financial support. Special thank to the Program in Animal Production and Technology, Faculty of Technology, Udon Thani Rajabhat University for providing laboratory and the use of research facilities. Thank to Rerm Udom sugar factory CO., LTD. for providing sugarcane bagasse.

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