Effect of sugarcane bagasse treatment on gas production and ruminal degradability byusing *in vitro* gas production technique

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Abstract: The experiment was conducted to investigate the effectiveness of urea with whole soybean meal and calcium hydroxide Ca $(OH)_2$ treatment of sugarcane bagasse on gas production and ruminal degradability by using *in vitro* gas technique. The experimental design was a completely randomized design (CRD) and the dietary treatments were sugarcane bagasse treated with urea (0, 2, 4 %) with whole soybean meal or calcium hydroxide (0, 2.5, 5 %). Sugarcane bagasse was taken to treat with respective treatment by dissolving in 100 ml water/g sugarcane bagasse and ensiled in a plastic box at room temperature for 14 days. Ensiled sugarcane bagasses were examined by *in vitro* gas production. Rumen fluid was collected from three ruminally fistulated native crossbred beef cattle with an average body weight of 350 kg. During the incubations, gas production was recorded at 0, 3, 6, 9, 12, 18, 24, 36, 48, 72 and 96 hrs. after incubation. The results revealed that gas production from the insoluble fraction (b), potential extent of gas production (a+b) and cumulative gas production were significantly ($P<0.05$) increased in all treatments and were highest in urea 4% and urea 4%+whole soybean meal 2.5% treatment (55.3, 53.5 and 50.1 ml/0.5 g DM substrate) respectively. *In vitro* degradability of DM and OM were influenced by urea and calcium hydroxide which is the highest in urea 4%+ calcium hydroxide 5% (59.2%)($P<0.01$). Based on this experiment, it could be concluded that urea, whole soybean meal and calcium hydroxide treatment could enhance gas fermentation and degradability of sugarcane bagasse.

Keywords: Sugarcane bagasse, In vitro gas production, Degradability

Introduction

Ruminants have the unique capacity to transform relatively low-quality dietary nitrogen (N) into high-quality animal proteins (i.e., meat and milk) (Schroeder and Titgemeyer, 2008). In the tropics and subtropics, majority of roughage source for ruminant consist of leftovers from the grain harvest, grasses and foliages growing on roadsides or waste land. Rice straw is the main crop-residue which farmers usually store for use as ruminant feed in tropical areas, especially in Asia. However, it is critical during the time of feed shortage in the dry season and also during the hectic season of cultivation where labour is limited and paddy fields are being cropped (Wanapat, 1986; Wanapat and Devendra, 1992). Alternative feed resources and crop-residues are locally available to use as roughage source for livestock production in the tropical and subtropical areas. Sugarcane bagasse is potential to use as roughage for ruminant. However, the sugarcane

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bagasse is generally with high fiber and low protein contents which, may result in poor animal performance. Therefore, sugarcane bagasse needs to improve nutritive value before use as animal feed. Various chemicals have been used to improve nutritive value of sugarcane bagasse (Natthapong et al., 2013; Khanya et al., 2012; Chullanandana, 2000). Previous work, urea treatment is a conventional technique for improving the quality of sugarcane bagasse, especially increasing the nitrogen content e (Natthapong et al., 2013; Khanya et al., 2012; Chullanandana, 2000). Although, urea-treated sugarcane bagasse has been used as a roughage during the dry season but the cost was relatively high due to increasing price of urea (Wanapat, 1994; Preston, 1995). Fadel Elseed et al. (2003) suggested that calcium hydroxide, it could improve rumen digestibility. Moreover, the use of urease with urea treated sugarcane bagasse can improve *in vitro* dry matter digestibility. However, the use of urea with urease in ground whole soybean and calcium hydroxide for improve sugarcane bagasse is still limited. Therefore, the objective of this experiment was to determine effects of various treated sugarcane bagasses on *in vitro* gas production and digestibility.

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 or lime treated sugarcane bagasse. The sugarcane bagasse was taken to treat with 0, 2, 4 (w/w) of urea and 0, 2.5, 5 (w/w) of whole soybean meal or calcium hydroxide, respectively then ensiled in plastic boxes at room temperature for 14 days before using in *in vitro* study (Wanapat, 2000). Sugarcane bagasse was collected from Rerm Udom sugar factory CO., LTD. at Nong Han, Udon Thani Province, Thailand. The samples were dried by hot air oven at 60 °C then ground to pass a 1 mm sieve and for chemical analysis for dry mater (DM), crude protein (CP), ash according to AOAC (1990); neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) according to Van Soest et al. (1991). The chemical compositions of dietary treatments used in the *in vitro* experiment are shown in **Table 1**.

Animals and preparation of rumen inoculums

Three male, rumen-fistulated beef cattle with body weight of 500±30 kg was used as rumen fluid donors. Beef cattle rumen fluid was collected from animals fed with rice straw ad libitum. The animals received the diets for 14 d before the rumen fluid was collected. On day 15, 1000 ml rumen liquor was obtained from each animal before morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermo flasks and then transported to the laboratory.

In vitro fermentation of substrates

Samples of 0.5 g of ensiled sugarcane bagasse were weighed into 50 ml serum bottles. For each treatment, three replications were prepared. Ruminal fluid from each animal was mixed with the artificial saliva solution of Menke and Steingass (1988) in a proportion 2:1 (ml/ml) at 39 °C under continuous flushing with CO_2 and 40 ml of

rumen inocula mixture were added into each bottle under CO_2 flushing. Bottles were sealed with rubber stoppers and aluminum caps and incubated at 39°C (72 h) for *in vitro* gas test.

Sample and analysis

During the incubation, data of gas production was measured immediately after incubation at 0, 3, 6, 9, 12, 18, 24, 48, 72 and 96 h by using a glass syringe. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) as follows: $y = a + b [-\backslash e^{(- ct)}]$ 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''. The *in vitro* degradability was determined after termination of incubation, when the contents were filtered through pre-weighed Gooch crucibles 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 were ashed at 550 °C for determination of *in vitro* organic matter degradability (IVOMD) (Tilley and Terry, 1963).

Statistical analysis

All data from the experiment 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.

Results and Discussions

The chemical composition of urea and whole soybean meal or calcium hydroxide treatment on sugarcane bagasse is shown in **Table 1**. The CP content of ensile sugarcane bagasse was increased due to urea and whole soybean meal addition, especially by 2 and 4% urea with 2.5 and 5% calcium hydroxide treatment, and CP ranged from 6.5 to 7.1%, respectively while calcium hydroxide influenced on fiber fractions, with decrease in NDF and ADF content. The effect was due to alkaline agents can chemically break the ester bonds between lignin and hemicellulose and cellulose in sugarcane bagasse (Fadel Elseed et al., 2003). The fermentation gas parameter characteristics are presented in **Table 2**. It was found that gas production from the immediately soluble fraction (a) ranged from -1.7 to 1.1 ml and was not affected by any treatments ($P>0.05$) while gas production from the insoluble fraction (b) and gas potential extent of gas production (a+b) were influenced by urea treatment ($P<0.05$) and ranged from 44.9 to 55.3 ml. and 44.4 to 53.5 ml., respectively. Whereas the rate of gas production (c) ranged from 0.02 to 0.04 ml/hr ($P>0.05$), which was higher than that reported by Napasirth et al. (2012). Cumulative gas production for each of the substrate treatments presented as gas production curves are shown in Figure 1. The cumulative gas production was influenced by urea treatment ($P<0.05$). While there were no influenced by calcium hydroxide treatment ($P>0.05$), under this study, this could be due to a lower dissociation constant for calcium hydroxide treatment and a longer reaction period for complete effectiveness which may be required

(Rounds et al., 1979).

The *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) at 96 hrs. after incubation ranged from 18.0 to 25.7 and 37.5-59.2%, respectively (**Table 2**). The IVDMD and IVOMD were increased when ensile sugarcane bagasse with urea and calcium hydroxide ($P<0.05$). This result was similar with Wanapat et al. (2009) who found that dry matter and organic matter digestibility of urea treated rice straw in *in vitro* trial were increased from 49.5 to 53.3%.

According to Van Soest (2006) reported that ammonia, urea and urine treatment of straw influenced on fiber and lignin fractions, with small decrease in NDF (2-4%) and increase in ADF (3% and lignin 20-50%). The effect was due to urea which was able to cleave lignin to carbohydrate ester bonds. Moreover, Fadel Elseed et al. (2003) suggested that calcium hydroxide could improve rumen degradability. The concentrated alkaline agents can chemically break the ester bonds between lignin and hemicellulose and cellulose, and physically make structural fibers swollen. These effects enable rumen microbes to attack the structural carbohydrates more easily, increasing digestibility.

Table 1 Chemical composition of treated sugarcane bagasse for all treatments

Treatment	DM	Ash	OM	CP	NDF	ADF
	%		.....% DM.....			
T1 Control	72.5^{a}	7.7	92.3	2.8^{d}	79.4^{a}	69.8^{a}
T2 (U2)	30.0^{e}	7.8	92.2	4.7^{bc}	76.4^{a}	69.6^{a}
T3 (U2 : S2.5)	30.8^{cde}	7.8	92.2	6.5^{a}	79.0^{a}	69.9^{a}
T4 (U2 : S5)	35.2^{b}	8.1	91.9	7.0^{a}	72.8^{a}	67.4^{a}
T5 (U2 : C2.5)	30.4^{de}	7.9	92.1	3.7^{cd}	67.8^{ab}	62.8^{ab}
T6 (U2 : C5)	31.3^{cde}	7.8	92.2	4.1^{cd}	62.7^{b}	56.5^{c}
T7 (U4)	32.4^{c}	8.0	92.0	6.7^{a}	76.9^{a}	67.9^{a}
T8 (U4 : S2.5)	34.7^{b}	7.9	92.1	6.8^{a}	76.9^{a}	64.1^{ab}
T9 (U4 : S5)	32.1^{cd}	8.2	91.8	7.1^{a}	76.2^{a}	69.7^{a}
T10 (U4 :C2.5)	32.3^{cd}	7.9	92.1	5.9^{ab}	66.5^{b}	61.4^{ab}
T11 (U4 : C5)	34.9^{b}	8.1	91.9	5.9^{ab}	53.3^{c}	51.4^{c}
SEM	0.40	0.17	0.17	0.30	2.00	2.20
P-value	**	ns	ns	**	*	*
U0 vs U2 U4	**	ns	ns	**	ns	ns
S0 vs S2.5 S5	**	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 whole whole soybean meal meal 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 whole whole soybean meal meal meal or calcium hydroxide treated sugarcane bagasse on *in vitro* gas production and *in vitro* digestibility

Treatment	Gas kinetics				Gas production	In vitro degradability,%	
	a	b	c	a+b	ml/0.5 g DM substrate	IVDMD	IVOMD
T1 Control	-0.5	44.9^{c}	0.03	44.4^{bc}	42.8^{b}	18.0^{b}	37.5^{d}
T2 (U2)	0.1	52.0^{ab}	0.03	52.0^{ab}	47.8^{a}	19.8^{ab}	40.6^{d}
T3 (U2 : S2.5)	-0.4	52.7^{ab}	0.02	52.3^{ab}	46.0^{a}	19.7^{ab}	39.3^{d}
T4 (U2 : S5)	0.7	48.8^{ab}	0.03	49.6^{ab}	44.7^{a}	19.7^{ab}	45.5^{bcd}
T5 (U2 : C2.5)	-1.4	51.4^{ab}	0.03	49.9^{ab}	44.9^{a}	20.8^{ab}	47.0^{bc}
T6 (U2 : C5)	-0.5	46.5^{ab}	0.02	46.0^{ab}	44.0^{a}	25.0^{a}	56.4^{ab}
T7 (U4)	-1.8	55.3^{a}	0.04	53.5^{a}	48.8^{a}	21.2^{ab}	53.2^{bc}
T8 (U4 : S2.5)	-1.4	52.6^{ab}	0.04	51.2^{ab}	50.1^{a}	24.6^{a}	55.6^{ab}
T9 (U4 : S5)	1.1	49.0^{ab}	0.02	50.1^{ab}	46.2^{a}	21.3^{ab}	50.0^{bc}
T10 (U4 :C2.5)	-0.6	53.3^{ab}	0.03	52.6^{ab}	49.5^{a}	23.2^{a}	56.7^{ab}
T11 (U4 : C5)	-1.7	55.1^{a}	0.03	53.3^{a}	49.1^{a}	25.7^{a}	59.2^{a}
SEM	0.71	1.37	0.02	1.39	1.24	1.0	2.18
P-value	ns	*	ns	*	*	*	**
U0 vs U2 U4	ns	*	ns	*	*	*	*
S0 vs S2.5 S5	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), 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.

Conclusion and recommendations

Based on this study, it could be concluded that use of whole soybean meal could be used as a urease for urea treatment to improve the quality of sugarcane bagasse in order to enhance its CP content while urea and calcium hydroxide treatment could improve *in vitro* gas production and ruminal degradability, respectively. However, further researches are required using *in vivo* study in ruminants.

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