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REGULAR ARTICLES

Effect of treating sugarcane bagasse with urea and calcium hydroxide on feed intake, digestibility, and rumen fermentation in beef cattle

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Abstract Four beef cattle with initial body weight of 283 ± 14 kg were randomly allocated according to a 4 × 4 Latin square design to study on the effect of feeding sugarcane bagasse (SB) treated with urea and/or calcium hydroxide ($Ca(OH)_2$) on feed intake, digestibility, and rumen fermentation. The treatments were as follows: rice straw (RS), untreated SB (SB), 4 % urea-treated SB (SBU), and 2 % urea + 2 % $Ca(OH)_2$-treated SB (SBUC), respectively. The results revealed that cattle fed with SBU and SBUC had higher feed intake and apparent digestibility. Ammonia nitrogen and blood urea nitrogen were increased in cattle fed with SB as roughage source ($P < 0.05$). Feeding SBU and SBUC to cattle resulted in higher propionic acid and lower acetic acid, acetic to propionic ratio, and methane production ($P < 0.05$). Moreover, the number of fungi was increased in SBU- and SBUC-fed groups while protozoa population was unchanged. This study concluded that the nutritive value of SB was improved by urea and/or $Ca(OH)_2$ treatment, and feeding treated SB could increase feed intake, digestibility, and rumen fermentation. This study suggested that SB treated with 2 % urea + 2 % $Ca(OH)_2$ could be used as an alternative roughage source for ruminant feeding.

Keywords Sugarcane bagasse · Urea · Calcium hydroxide · Digestibility · Rumen fermentation

Introduction

Sugarcane bagasse (SB) is one of a fibrous residue remaining after extraction of juice from cane stem and could be used as roughage source for ruminants (Ahmed et al. 2013). However, SB has been reported containing low protein (<3 % DM), high cellulose (>40 % DM), hemicellulose (>35 % DM), and lignin (>15 % DM) (Costa et al. 2015), and low digestibility (20–30 % DM); thus, resulting in poor animal performances. A potential use of SB as a ruminant feed may be realized through the development of physical, chemical, and biological treatments to disrupt the lingo-cellulose complex (Balgees et al. 2007; Okano et al. 2006). Balgees et al. (2015) revealed that urea-treated SB increased nutritive value in terms of protein content and ruminal digestibility. These could be due to the effect of enzyme urease release ammonia from urea and the effect of ammonia on the cell wall of bagasse. In addition, Ahmed et al. (2013) revealed that urea-treated SB could reduce fiber content and increase fiber degradation by in situ study.

Moreover, alkali treatments are potential chemical treatments for improving quality of roughages; thus a reduction of treatment cost was obtained. The concentration of alkaline agents can chemically break the ester bonds between lignin and hemicellulose and cellulose, and physically make structural fibers swollen (Wanapat et al. 2009) which enable rumen microbes to attack the structural carbohydrates more easily (Castañón-Rodríguez et al. 2015). Moreover, Carvalho et al.

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(2013) reported that feeding alkali-treated SB increased feed intake and body weight (BW) of ruminant. However, there are still a limitation data on the use of calcium hydroxide ($Ca(OH)_2$) and/or combination with urea for SB treatment on nutritive quality and the use as roughage source for ruminant feeding. Therefore, the objective of this study was to determine the effects of feeding SB treated with urea and/or ($Ca(OH)_2$ on feed intake, digestibility, and rumen fermentation in beef cattle.

Materials and methods

Animals, diets, and experimental design

Four crossbred (Brahman × Thai native) beef cattle with 283 ± 14 kg of BW were randomly assigned according to a 4 × 4 Latin square design to receive four dietary treatments and were as follows: rice straw (RS), untreated SB (SB), 4 % urea-treated SB (SBU), and 2 % urea + 2 % $Ca(OH)_2$-treated SB (SBUC). The SB was collected from Rerm Udom sugar factory Co., Ltd. at Udon Thani Province, Thailand. Dietary treatments were prepared by adding urea and $Ca(OH)_2$ (as hydrated lime) according to the respective ratio using 100 l of water to 100 kg air-dry SB. Bagasse was sprayed with the solution and then covered with a plastic sheet for a minimum of 14 days before feeding directly to the animals (Wanapat and Pimpa 1999; Wanapat et al. 2009). The concentrate was formulated to contain 16.0 % crude protein (CP) and 71.5 % total digestible nutrient (TDN) (Table 1). Animals were fed concentrate at 0.7 % BW, and respective roughage sources were fed ad libitum.

Data collection and chemical analysis

The experiment was run for four periods and each period lasted for 21 days, with the first 14 days was for feed adaptation and intake measurements whist the last 7 days was for samples collection. Feed, refusal, and fecal samples were dried at 60 °C and ground (1 mm screen using the Cyclotech Mill, Tecator, Sweden) and analyzed for DM, ash, and CP content using the standard methods of AOAC (1997) and neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) according to Van Soest et al. (1991).

At the end of each period, approximately 200 ml of rumen fluid were collected at 0 and 4 h post-feeding by a stomach tube connected to a vacuum pump. Rumen fluid samples were then strained through four layers of cheesecloth and divided into two parts. The first 45 ml of rumen fluid was collected and kept in a plastic bottle to which 5 ml of 1 M H_2SO_4 was added and then centrifuged at 3000*g* for 10 min. Approximately 20–30 ml of supernatant was collected and froze at −20 °C for analysis of volatile fatty acid (VFA) according to Samuel et al. (1997) and NH_3–N by Kjeltech Auto 1030 Analyzer (AOAC 1997). The second portion of 1 ml rumen fluid was collected and kept in a plastic bottle to which 9 ml of 10 % formalin solution (1:9 *v*/*v*, rumen fluid: 10 % formalin) was added and stored at 4 °C for measuring fungal zoospores and protozoa according to the method of Galyean (1989). Blood samples (approximately 10 ml) were collected from the jugular vein into tubes at the same time as rumen fluid sampling. Serum was used for analysis of blood urea nitrogen (BUN) according to Crocker (1967). Calculation of ruminal methane (CH_4) production was based on VFA proportions according to Moss et al. (2000) as follows:

$$CH_4 \text{ production} = 0.45(\text{acetate}) - 0.275(\text{propionate}) + 0.4(\text{butyrate})$$

Table 1 Ingredients and chemical compositions of the diet used in the experiment

Item	Concentrate	RS	SB	SBU	SBUC
Ingredient, %					
Cassava chip	56.1				
Rice bran	17.2				
Whole cottonseed	11.0				
Soybean meal	8.6				
Urea	2.1				
Molasses	3.0				
Mineral premix	1.0				
Salt	0.5				
Sulfur	0.5				
Chemical composition					
Dry matter, %	92.9	92.5	91.5	34.0	34.8
	% DM				
Organic matter	93.7	89.8	93.2	95.8	92.0
Crude protein	16.0	2.8	2.7	6.5	4.5
Neutral detergent fiber	31.0	77.0	73.9	71.7	67.2
Acid detergent fiber	23.9	58.5	69.9	65.8	60.8
Acid detergent lignin	–	9.8	10.3	7.5	6.9
Total digestible nutrients[a]	71.5	–	–	–	–
Price ($US/kg)	0.27	0.06	0.01	0.04	0.03

RS rice straw, *SB* sugarcane bagasse, *SBU* 4 % urea-treated sugarcane bagasse, *SBUC* 2 % urea + 2 % $Ca(OH)_2$-treated sugarcane bagasse

[a] Calculated value

Statistical analysis

The data were analyzed in a 4 × 4 Latin square design by analysis of variance run in the GLM Procedure (SAS 1998). Differences between treatment means were determined by Duncan's new multiple range test (Steel and Torrie 1980).

Group of treatment response was performed by orthogonal contrast, and significant effects were identified at $P<0.05$.

Results and discussions

Chemical composition of feeds

The CP content of SB was increased by urea treatment, and the values were 6.5 and 4.5 % for SBU and SBUC, respectively (Table 1). Moreover, SB contained higher NDF, ADF, and ADL content compared to SBU and SBUC. These results were in agreement with Hameed et al. (2012) who reported that CP content of SB increased with the increasing levels of urea treatment at 2, 4, or 6 % for 2, 4, or 6 weeks treating. In addition, Polyorach and Wanapat (2015) also reported that fiber content of feed was reduced when urea and $Ca(OH)_2$ were used in treatment as it could reduce strong odor of free ammonium or ammonium carbonate (Recktenwald and Van Ambrugh 2006). The alkali in the lignocellulosic material induces the swelling of the material, increasing the internal surface, and reducing the polymerization degree and crystallinity, which results in the rupture of the lignin (Castañón-Rodríguez et al. 2015). Moreover, Beukes and Pletschke (2011) indicated that alkali alters the structure of lignin, therefore could be solubilized and be removed from the lignocellulose. Rezende et al. (2011) showed that lignin removal lead to the un-structuring of the sugarcane cell wall that occurred in two levels; as one refers to the loss of cohesion between neighboring cell walls and the second one corresponds to the degradation inside the wall itself caused by peeling off and hole formation. This could explain lower fiber content of SBUC compared to SB and SBU in the present study. Moreover, using SBUC (0.030$US/kg) as roughage source costed less than SBU (0.037 $US/kg).

The effect on feed intake and digestibility

The effect of dietary treatments on feed intake and digestibility are presented in Table 2. The results showed that cattle fed with SBU and SBUC as roughage sources had higher roughage and total DM intake compared to RS and SB feeding ($P<0.05$), and this could be due to high fiber content in RS and SB which affected on intake of animals. This means that urea or urea plus $Ca(OH)_2$ treatment of SB could improve the nutritive value and potentially use as high quality roughage source for cattle. Apparent digestibility of OM and CP in cattle fed with SBU was the highest ($P<0.05$) among groups while NDF and ADF digestibility was most improved in cattle consumed SBUC as roughage source, followed by SBU, RS, and SB, respectively. According to Khejornsart et al. (2011) and Carvalho et al. (2013), alkaline treatment could improve digestibility and feed intake of ruminants because it alters the structure of lignin removal from the lignocellulose.

Table 2 The effect of the rice straw and sugarcane bagasse diets on feed intake and apparent digestibility

Items	RS	SB	SBU	SBUC	SEM	RS vs. SB	SBU vs. SBUC	SB vs. SBU, SBUC
Roughage DM intake								
kg/d	3.6^{a}	2.1^{b}	3.0^{a}	3.2^{a}	0.20	**	**	**
%BW	1.5^{a}	0.8^{b}	1.3^{ab}	1.2^{ab}	0.14	*	ns	ns
g/kg $BW^{0.75}$ daily	59.0^{a}	30.6^{b}	50.0^{a}	50.0^{a}	5.04	**	ns	**
Concentrate DM intake								
kg/d	2.1	2.0	2.1	2.0	0.06	ns	ns	ns
%BW	0.7	0.7	0.7	0.7	0.04	ns	ns	ns
g/kg $BW^{0.75}$ daily	33.8	29.1	33.1	28.1	1.40	ns	ns	ns
Total DM intake								
kg/d	5.8^{a}	4.1^{b}	5.2^{a}	5.1^{a}	0.30	*	ns	*
%BW	2.2^{a}	1.5^{b}	2.0^{ab}	2.0^{ab}	0.18	*	ns	ns
g/kg $BW^{0.75}$ daily	92.9^{a}	60.0^{b}	82.7^{a}	77.8^{ab}	6.30	*	ns	*
Apparent digestibility, %								
DM	59.2	53.6	61.9	60.9	1.24	ns	ns	ns
OM	62.2^{ab}	55.8^{b}	66.5^{a}	63.9^{ab}	1.27	ns	ns	*
CP	53.4^{b}	54.6^{b}	73.2^{a}	62.8^{ab}	1.78	ns	ns	*
NDF	48.5^{b}	47.1^{b}	57.4^{a}	65.6^{a}	1.23	ns	ns	**
ADF	48.3^{b}	47.8^{b}	59.3^{a}	59.1^{a}	0.66	ns	ns	**

Values on the same row with different superscripts differed ($P<0.05$)

ns not significant

*$P<0.05$, **$P<0.01$

Table 3 Effect of the rice straw and sugarcane bagasse diets on ruminal pH, NH_3–N, and BUN concentration

Items	RS	SB	SBU	SBUC	SEM	RS vs. SB	SBU vs. SBUC	SB vs. SBU SBUC
NH_3–N, mg/dl								
0 h post feeding	18.2	25.0	25.0	26.6	1.39	ns	ns	ns
4	19.3^{c}	24.2^{ab}	27.5^{a}	23.3^{b}	0.51	**	**	**
Mean	18.7^{b}	24.6^{a}	26.3^{a}	24.9^{a}	0.78	*	ns	ns
BUN, mg/dl								
0 h post feeding	5.8^{b}	11.0^{a}	14.4^{a}	14.0^{a}	1.38	*	ns	ns
4	6.8^{b}	11.5^{a}	16.3^{a}	15.5^{a}	1.14	*	ns	ns
Mean	6.3^{b}	11.3^{a}	15.3^{a}	15.0^{a}	1.25	*	ns	ns

Values on the same row with different superscripts differed ($P < 0.05$)

ns not significant

*$P < 0.05$, **$P < 0.01$

Removing lignin from the inner parts of the cell wall consequently resulted in a damage and porous morphology by separation of cell bundles, forming long cellular structures, which are well connected in the longitudinal direction (Rezende et al. 2011). In addition, alkali treatment caused the swelling of the cellulose fibers which could increase the internal surface area and would have allowed cellulases to have major contact with the substrate inducing a greater level of hydrolysis in the amorphous region and subsequently increasing the proportion of crystalline cellulose (Castañón-Rodríguez et al. 2015). These effects enable rumen microbes to attack the structural carbohydrates more easily; hence, higher degradability and intake could be obtained (Wanapat and Cherdthong 2009).

Characteristics of ruminal fermentation and blood metabolites

Ruminal NH_3–N and BUN concentration were found higher ($P < 0.05$) in cattle received SBU and SBUC followed by SB- and RS-fed groups (Table 3). This increase was partially due

Table 4 Effect of the rice straw and sugarcane bagasse diets on volatile fatty acid and methane production

Items	RS	SB	SBU	SBUC	SEM	RS vs. SB	SBU vs. SBUC	SB vs. SBU SBUC
Total VFA, mM								
0 h post feeding	114	111	112	116	3.45	ns	ns	ns
4	115	112	116	112	1.09	ns	ns	ns
Mean	120	112	114	114	1.00	ns	ns	ns
	mol/100 mol							
C2								
0 h post feeding	68.7	69.9	67.7	68.2	1.01	ns	ns	ns
4	73.8	72.9	69.5	69.1	1.18	ns	ns	ns
Mean	71.3^{a}	71.0^{a}	68.6^{b}	68.6^{b}	0.31	ns	ns	*
C3								
0 h post feeding	18.4	19.0	19.9	20.4	1.09	ns	ns	ns
4	15.4^{a}	16.3^{b}	19.7^{b}	19.2^{b}	0.68	*	ns	ns
Mean	16.9^{a}	17.6^{a}	19.8^{b}	19.8^{b}	0.30	ns	ns	*
C4								
0 h post feeding	11.9	12.8	12.4	11.4	1.18	ns	ns	ns
4	10.8	11.2	10.8	117	0.89	ns	ns	ns
Mean	11.4	12.2	11.6	11.6	0.90	ns	ns	ns
C2:C3								
0 h post feeding	3.4	3.4	3.5	3.6	0.51	ns	ns	ns
4	5.3^{a}	4.5^{a}	3.5^{b}	3.6^{b}	0.25	ns	ns	*
Mean	4.4^{a}	4.2^{a}	3.5^{b}	3.6^{b}	0.15	ns	ns	*
CH_4, mol/100 mol								
0 h post feeding	30.6	30.8	30.0	29.6	0.72	ns	ns	ns
4	33.5^{a}	33.7^{a}	30.3^{b}	30.4^{b}	0.51	ns	ns	*
Mean	32.4	32.3	30.1	30.1	0.68	ns	ns	ns

Values on the same row with different superscripts differed ($P < 0.05$)

ns not significant

*$P < 0.05$

Table 5 Effect of the rice straw and sugarcane bagasse diets on microbial population in the rumen

Items	RS	SB	SBU	SBUC	SEM	RS vs. SB	SBU vs. SBUC	SB vs. SBU SBUC
Direct count, cell/ml								
Protozoa, $\times 10^5$								
0 h post feeding	2.8	3.2	3.2	3.1	0.44	ns	ns	ns
4	4.1	3.8	4.6	4.7	0.25	ns	ns	ns
Mean	3.4	3.6	3.9	3.9	0.29	ns	ns	ns
Fungi, $\times 10^6$								
0 h post feeding	3.1^b	3.6^b	4.8^{ab}	6.1^a	0.45	ns	ns	*
4	3.0^c	4.0^{bc}	5.6^a	4.6^b	0.29	ns	*	*
Mean	3.1^b	3.8^b	5.2^a	4.8^{ab}	0.36	ns	ns	*

Values on the same row with different superscripts differed ($P < 0.05$)

ns not significant

*$P < 0.05$

to urea treatment enhanced its nitrogen content of SB which contributed to the addition of nitrogenous substrate (Ahmed et al. 2013). The CP content of SB was significantly increased by urea treatment and this could be attributed to the increased solubilization leading to higher NH_3–N retention. Ruminal NH_3–N ranging from 15 to 30 mg/dl was reported an optimal range for the improvement of fermentation, microbial growth, and feed intake in ruminants fed urea-treated rice straw (Wanapat and Pimpa 1999). Furthermore, BUN of cattle consumed treated SB ranged from 14.0 to 16.3 mg/dl, which was reported in the normal range. Wanapat and Pimpa (1999) reported that ruminant consumed diets with P/E balance had BUN concentrations at approximately 15 mg/dl. In addition, feeding SBU and SBUC increased propionic acid and reduced acetic acid, acetic to propionic ratio and CH_4 production (Table 4; $P < 0.05$). The increase of VFA profile strongly corresponded to the increasing of microbial population and this was reported by Vinh et al. (2011) that buffalo fed urea-treated rice straw increased *F. succinogenes* number which mainly produces primarily succinate as the major precursor of propionate in the rumen. Moreover, Ørskov et al. (1999) also indicated that ruminants fed with high-fiber diets had high acetic acid while high in propionic acid when fed with low-fiber diets.

Rumen microorganism population

Fungi zoospores counts were enhanced ($P < 0.05$) in cattle fed with SBU and SBUC as main roughage source (Table 5). The alkali in the lignocellulosic material induces the swelling of the material, increasing the internal surface, and reducing the polymerization degree and crystallinity, which resulted in the rupture of the lignin (Castañón-Rodríguez et al. 2015). This effect avails the rumen microbes to attack the structural carbohydrates more easily and improved digestibility, as well as the palatability of treated straw (Bod'a 1990). Vinh et al. (2011) showed that when feeding 2 % urea + 2 % $Ca(OH)_2$-treated straw could enhance fungal population ($P < 0.05$). In addition, Chen et al. (2008) also reported that chemical treatments enhanced the nutritive value of roughage through increasing the number of accessible sites of microbial attachment on the surface of the particles and increasing fibrolytic microbe quantity, hence fibrolytic enzyme activities and the overall rumen fermentation characteristics. In addition, the population density of protozoa in the rumen appears to be influenced by dietary treatments particularly by the fiber content. According to Vinh et al. (2011), the lower number of protozoa count was found as buffaloes fed with urea-lime-treated rice straw. Polyorach and Wanapat (2015) also reported that addition of alkali to rice straw significantly decreased protozoal population in beef cattle. However, Wanapat et al. (2009) reported that there was no effect of alkali on protozoa which was in consistency to the recent result which found that feeding urea or/and $Ca(OH)_2$-treated SB failed to reduce protozoal population in beef cattle.

Conclusions

In conclusion, treatment of SB with urea and/or $Ca(OH)_2$ could improve the nutritive value and feeding-treated SB could increasing feed intake, digestibility, and rumen fermentation end-product mainly NH_3–N and propionic acid. This study suggested that SB treated with 2 % urea + 2 % $Ca(OH)_2$ could be used as an alternative roughage source for ruminant feeding. However, further researches on feeding trail of treated SB are recommended to investigate its effects on animal performances and production such as meat and milk.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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