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Original Article

Effect of urea- and molasses-treated sugarcane bagasse on nutrient composition and *in vitro* rumen fermentation in dairy cows

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ABSTRACT

The effect of urea- and molasses-treated sugarcane bagasse was studied on the chemical composition, fermentation quality, *in vitro* gas production and digestibility in dairy cows. The experiment followed a completely randomized design in a 2 × 2 × 2 factorial arrangement of treatments with a control (sugarcane bagasse without any treatment). Factor A was before or after the sugarcane bagasse had been fermented with substrate at 21 d, factor B was the level of urea (0% or 5%), and factor C was the level of molasses (0% or 5%). The results showed that the crude protein content of the sugarcane bagasse increased ($p < 0.05$) following the urea and molasses treatment, whereas treatment with urea and molasses reduced ($p < 0.05$) the neutral detergent fiber content and the acid detergent fiber content of the sugarcane bagasse. Moreover, after fermentation, all treatments of the sugarcane bagasse had higher gas kinetics and gas production compared with before fermentation and the control; adding urea and molasses resulted in the highest ($p < 0.05$) gas production from the soluble fraction (a), from the insoluble fraction (b) and the highest gas potential extent (a+b) as well as cumulative gas production. In addition, *in vitro* dry matter and organic matter degradability increased ($p < 0.05$) with the urea and molasses treatments. In conclusion, utilization of 5% urea and 5% molasses as ingredients in sugarcane bagasse treatments would be effective in enhancing the nutritive value and *in vitro* rumen fermentation. Therefore, sugarcane bagasse treated with urea and molasses could be suitable for use as a roughage source for dairy cattle.

Introduction

During the dry season in tropical areas, fresh roughage shortage generally occurs for livestock production particularly in Thailand; in this season, most livestock farmers feed their animals with local, low quality roughage, and industrial by-products such as rice straw and other agricultural crop residues such as corn stover and sugarcane tops (Wanapat et al., 2013). Sugarcane bagasse is one of the main by-products of sugar milling factories which has potential as an alternative roughage source for ruminant feeding, especially during the long dry season. However, sugarcane bagasse is low in protein and has high fiber and lignin contents, containing 2.1–2.9% crude protein (CP) with 79.4–88.3% neutral detergent fiber (NDF), 62.2–69.8% acid detergent fiber (ADF) and 10.3–10.5% acid detergent lignin (ADL) (Gunun et al., 2017; Balgees et al., 2007; Okano et al., 2006). The main components of sugarcane bagasse are cellulose, hemicellulose and lignin and as a result of the high content of lignin, ruminal digestion is inhibited and thus the nutritive value of sugarcane bagasse is limited for ruminants (Deschamps et al., 1996; Okano et al., 2006). 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 which would allow the bagasse to be used as a source of roughages for ruminants. Many studies have been conducted to raise the nutritive value of sugarcane bagasse for ruminants through physical, chemical and biological treatments for ruminant feeding (Gunun et al., 2017; Balgees et al., 2007; Okano et al., 2006). Chemical treatment, such as urea treatment, is considered effective to improve the nutritive value and nutrient

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digestibility of sugarcane bagasse; urea is an interesting alternative nitrogen source to anhydrous ammonia in the treatment of lignocellulose feedstuff due to its low cost, easy handling, low danger in handling and being non-toxic to animals (Ahmed et al., 2013; Ahmed and Babiker, 2015). Balgees et al. (2015) reported that 5% urea and 3% ammonia treatments of sugarcane bagasse increased the CP content and *in vitro* dry matter digestibility. Ahmed et al. (2013) found that CP and NDF degradation were significantly ($p < 0.05$) increased in sugarcane bagasse treated with 10% DM of urea. Therefore, the objective of the current experiment was to determine the effects of the addition of urea or molasses or both to treat sugarcane bagasse regarding its nutritive value, fermentation quality, *in vitro* rumen fermentation and degradability using *in vitro* gas production techniques.

Materials and methods

Experimental design and treatments

This experiment was conducted using an *in vitro* gas production technique. The experiment followed a completely randomized design with a $2 \times 2 \times 2$ factorial arrangement of treatments with a control and three replicates for all treatments. The control treatment involved sugarcane bagasse without any treatment. Factor A was the nutrient composition of the sugarcane bagasse before (BF; sugarcane bagasse mixed with substrate before fermentation) or after fermentation (AF) with the substrate at 21 d, factor B was the level of urea (U; 0% or 5%), and factor C was the level of molasses (M; 0% or 5%). Therefore, the nine treatments were: T1, control (sugarcane bagasse with no treatment); T2, sugarcane bagasse mixed with 0% urea and 0% molasses before fermentation (BF:U0:M0); T3, sugarcane bagasse mixed with 0% urea and 5% molasses before fermentation (BF:U0:M5); T4, sugarcane bagasse mixed with 5% urea and 0% molasses before fermentation (BF:U5:M0); T5, sugarcane bagasse mixed with 5% urea and 5% molasses before fermentation (BF:U5:M5); T6, sugarcane bagasse fermented with 0% urea and 0% molasses (AF:U0:M0); T7, sugarcane bagasse fermented with 0% urea and 5% molasses (AF:U0:M5), T8, sugarcane bagasse fermented with 5% urea and 0% molasses (AF:U5:M0); and T9, sugarcane bagasse fermented with 5% urea and 5% molasses (AF:U5:M5).

Preparation of sugarcane bagasse treatments

Sugarcane bagasse was collected from the sugar factory of Saharuang Co., Ltd., Thailand located in Mukdahan province, Thailand. The sugarcane bagasse was separated for treatment with solutions of urea or molasses or both. Liquid solution was prepared by dissolving 0 kg or 5 kg of urea or molasses with 100 L of distilled water, then spraying and mixing well with 100 kg (DM basis) of sugarcane bagasse. Thereafter, the mixed materials were packed in plastic boxes, sealed with plastic sheeting, covered and left undisturbed at room temperature for 21 d before using in the *in vitro* study.

Samples were collected of sugarcane bagasse before (after mixing with the respective treatment solution and before fermentation) and after fermentation with the respective treatment solution on day 21 of incubation. All samples were analyzed for DM, ash, ether extract (EE) and CP using a proximate analysis procedure (AOAC, 1998). The fiber contents were determined (NDF and ADF) using a detergent analysis method (Van Soest et al., 1991). In addition, samples of sugarcane bagasse fermented on day 21 of incubation were assessed for fermentation quality. Subsamples (50 g fresh material) were macerated with 150 mL of distilled water and stored in a refrigerator at 4 °C for 12 h. Then, the extract was filtered using Whatman filter paper no.5 (Bureenok et al., 2006) and the pH of the extract was measured using a portable pH temperature meter (HI 8424 microcomputer; Hanna Instruments; Singapore). For organic acid contents, samples were stored at −20 °C prior to analysis of lactic acid, acetic acid and butyric acid using the fractional distillation method according to Chewa-isarakul and Chewa-isarakul (1982).

In vitro fermentation of substrates

The method used for *in vitro* fermentation was based on the gas production technique described by Menke et al. (1979). Two crossbred (75% Holstein Friesian) lactating dairy cows were used as rumen fluid donors. The animals were individually penned and provided with clean fresh water and mineral blocks *ad libitum*. The animals were fed with urea- and molasses-treated sugarcane bagasse as roughage *ad libitum* and the concentrate was fed at a ratio of milk yield to concentrate of 2:1 in three equal portions, at 0700 h, 1200 h and 1600 h. The animals were given the diets for 7 d before the rumen fluid was collected. The rumen fluid was obtained from each animal using a stomach tube connected with a vacuum pump, before morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermo flasks. A strict anaerobic technique was used, involving flushing with CO_2. Artificial saliva was prepared according to Menke and Steingas (1988). The artificial saliva and rumen fluid were mixed in a 2:1 ratio to make the rumen inoculum. Three bottles containing only rumen inoculation mixture were used as blanks. Mean gas production of the blank samples was subtracted from each measurement to give the net gas production. The glass bottles with 200 mg of substrate treatments were pre-warmed in a water bath at 39 °C for 1 h before filling with 30 mL of rumen inoculum. Bottles were sealed with rubber stoppers and aluminum caps and incubated at 39 °C (96 h) for the *in vitro* gas test. The volume of gas production was recorded at 0 h, 2 h, 4 h, 6 h, 8 h, 12 h, 18 h, 24 h, 36 h, 48 h, 72 h and 96 h of incubation. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) shown in Eq. (1):

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

where y is the gas produced at time t; 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 the incubation time; and a+b is the potential extent of gas production. At 24 h and 48 h post inoculation, samples were taken to determine the *in vitro* degradability, when the contents were filtered through pre-weighed Gooch crucibles and residual dry matter was estimated. The percentage 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 were subjected to analysis of variance according the general linear model of SAS (2006). Data were analyzed using the model: $Y_{ij} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + \varepsilon_{ijkl}$. Differences among means were tested using Duncan's new multiple range test (Steel and Torrie, 1980) with $p < 0.05$ accepted as representing a statistically significant difference. Orthogonal contrast was used to investigate the effect of the treatment response.

Ethics statements

This study was approved by the Ethics Committee of Ubon Ratchathani Rajabhat University (Approval no. 59001) according to

the guidelines of Ethical Principles for the Use of Animals for Scientific Purposes of the National Research Council of Thailand.

Results and discussion

Chemical composition and fermentation quality

The chemical composition of sugarcane bagasse affected by urea and molasses treatments is shown in Table 1. Untreated sugarcane bagasse, which was comparable with the control (T1), had significantly higher DM and fiber proportion (NDF and ADF) whereas the CP and EE contents were lower compared with treated sugarcane bagasse (T2–T9). Although the DM content differed between the control and all treated groups, treatments with urea or molasses or both were not significantly different. In this experiment, the CP content (3.8%) of the untreated sugarcane bagasse was higher than in Gunun et al. (2017), Balgees et al. (2007) and Ahmed et al. (2013), who reported that untreated sugarcane bagasse contained 2.1–2.9% CP. This difference may have been due to the difference between the varieties and sources of sugarcane bagasse as well as the milling process in the sugar factories. The chemical compositions of treated sugarcane bagasse affected by factor A as shown in Table 1, ash, CP, NDF and ADF were significantly lower in treatments involving urea or molasses or both after fermentation compared with before fermentation. However, the CP content in the sugarcane bagasse was highest in the urea and molasses treatments (Factor B and C); the NDF and ADF contents were lowest also in the treatment involving urea and molasses both before and after fermentation (T5 and T9). Thus, fermentation with urea and molasses could increase the nutritive value of sugarcane bagasse. This result was consistent with Ahmed et al. (2013) who reported that urea treatment raised CP from 2.2% of raw sugarcane bagasse to 10.4%, while the ash and cell wall compounds as determined using NDF, ADF, ADL contents and the hemicellulose content were significantly lower for raw bagasse than for treated bagasse. The CP contents in the urea treatments were significant different before and after fermentation being lower after fermentation possibly due to the decomposition of urea and the ammonia loss during the fermentation period (Lounglawan et al., 2011) which resulted in a lower CP content after fermentation. Johnson et al. (1967) found that urea treatment of corn silage tended to increase the formation of ammonia and reduce the content of crude protein which agreed with Catchpoole (1962) who reported that when grass was fermented, the percentage of CP decreased compared to before fermentation. The interactions before and after fermentation (A), urea (B) and molasses supplementation (C) on the CP content were significant. Although the CP content was greatly different before and after fermentation, the urea treatments improved all the values of nutritive parameters. The improvement in feeding value following urea and molasses treatment of the sugarcane bagasse could have been due to the fact that urea treatment enhanced the nitrogen content of lignocellulosic materials and consequently free ammonia was released, which reacted with the lignocellulosic materials by reducing cell wall components (NDF and ADF) and thus improved their feeding value (Table 1). The reduction in the fiber content using the molasses supplement may have been due to the increased activity of microorganisms during the fermentation process. Molasses is a readily available fermentation stimulant and is an energy source for microorganisms to hydrolysis fiber plant material, therefore adding molasses could resulting in a decrease NDF and ADF content of sugarcane bagasse. For example, Bautista-Trujillo et al. (2009) found that the addition of molasses

Table 1
Nutritive value of urea- and molasses-treated sugarcane bagasse.

Treatment (T)	DM (%)	Ash	OM	CP	EE	NDF	ADF
		(% DM)					
T1 (Control)	68.6^{a}	7.3^{bc}	92.7^{bc}	3.8^{f}	1.1^{c}	84.5^{a}	59.4^{a}
T2 (BF:U0:M0)	49.1^{b}	7.4^{bc}	92.6^{bc}	3.7^{f}	1.2^{c}	85.1^{a}	57.9^{ab}
T3 (BF:U0:M5)	48.3^{b}	7.2^{bc}	92.8^{bc}	4.5^{d}	1.3^{c}	82.1^{ab}	55.7^{c}
T4 (BF:U5:M0)	48.3^{b}	8.2^{ab}	91.8^{cd}	17.8^{a}	1.2^{c}	81.0^{bc}	55.8^{bc}
T5 (BF:U5:M5)	47.5^{b}	9.5^{a}	90.5^{d}	18.0^{a}	1.3^{bc}	80.5^{bc}	58.9^{a}
T6 (AF:U0:M0)	50.1^{b}	6.1^{bc}	93.9^{ab}	3.8^{f}	1.2^{bc}	80.0^{dc}	56.5^{bc}
T7 (AF:U0:M5)	47.2^{b}	7.7^{bc}	92.3^{cc}	4.0^{e}	1.5^{a}	77.8^{cd}	51.0^{d}
T8 (AF:U5:M0)	48.9^{b}	5.2^{d}	94.8^{a}	6.7^{c}	1.4^{ab}	75.8^{b}	48.8^{e}
T9 (AF:U5:M5)	48.3^{b}	4.9^{d}	95.1^{a}	8.1^{d}	1.3^{bc}	74.7^{d}	48.2^{e}
SEM	0.87	0.05	0.05	0.00	0.00	0.23	0.09
Comparison							
Control vs Others							
Control	68.6^{a}	7.3	92.7	3.8^{b}	1.1^{b}	84.5	59.4^{a}
Others	48.5^{b}	7.0	93.0	8.3^{a}	1.3^{a}	79.6^{b}	54.1^{b}
Before vs After fermentation (A)							
Before	48.3	8.1^{a}	91.9^{b}	11.0^{a}	1.2	82.2^{a}	57.1^{a}
After	48.6	6.0^{b}	94.0^{a}	5.7^{b}	1.4	77.1^{b}	51.1^{b}
Urea 0 vs 5 (B)							
U0	48.7	7.1	92.9	4.0^{b}	1.3	81.2^{a}	55.3^{a}
U5	48.3	6.9	93.1	12.7^{a}	1.3	78.0^{b}	52.9^{b}
Molasses 0 vs 5 (C)							
M0	49.1	6.7	93.3	8.0^{b}	1.3	80.5^{a}	54.8^{a}
M5	47.8	7.3	92.7	8.7^{a}	1.4	78.8^{b}	53.4^{b}
Interaction between factors							
A × B	ns	**	**	**	ns	ns	**
A × C	ns	ns	ns	*	ns	ns	**
B × C	ns	ns	**	*	ns	ns	**
A × B × C	ns	*	*	**	ns	ns	ns

DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; BF = before fermentation; AF = after fermentation; U = urea; M = molasses; ns = non-significant; SEM = standard error of the mean.
^{abcdef} = mean within columns with different lowercase superscript letters are significantly ($p < 0.05$) different.
* = $p < 0.05$; ** = $p < 0.01$.

significantly ($p < 0.05$) reduced the NDF content in maize silage compared to the control treatment or maize silage added with whey; this could be explained by the activity of cellulolytic microorganisms that degraded the hemicellulose and cellulose in the maize silage. Furthermore, the increase in CP during the fermentation process may have been due to the fact that proteolytic activity during fermentation produces NH3-N and this proteolytic activity is inhibited and the produced NH3 helps in maintaining aerobic stability because of its fungicidal properties (Kung et al., 2000). Another possible reason for the increase in the CP contents was the efficient fermentation, preservation and stability of silage, where different types of bacteria present in the medium may have had no opportunity to perform their activity and they become the part of the medium (silage). These bacteria contain more than 75% true protein (Yang et al., 2004).

In addition, after fermentation, the pH and organic acids content of treated sugarcane bagasse was significantly different among treatments (Table 2). The levels of pH and acetic acid were affected by the urea and molasses, with the pH being highest in the urea treatments. Ishida and Hassan (1997) reported that silage without urea addition had a lower pH value (below 4.2) and a higher content of lactic acid (above 1.5%), which suggested that bacterial activity had stopped and thus, nutrient losses decreased during the preservation time. Moreover, urea addition resulted in a higher pH due to ammonia formation, which promoted bacterial activity and produced a larger amount of acetic acid and butyric acid, which was supported by the results of the current experiment. On the other hand, the pH was lower in the molasses treatments, due to molasses providing fermentation carbohydrate for lactic acid bacteria which increased the accumulation of lactic acid which subsequently reduced the pH. Molasses is a sugar-rich material and is commonly used as an effective additive for ensiling crops that have low water-soluble carbohydrate (Oladosu et al., 2016). Using molasses as an additive could improve the fermentation quality and feeding value of silage (Yakota et al., 1992) by stimulating increased supply of fermentable carbohydrate to enhance the growth of lactic acid bacteria (Li et al., 2010). Moreover, a high concentration of acetic acid was found in the urea and molasses treatments, indicating the activity of heterofermentative lactic acid bacteria thereby increasing aerobic stability; this would also increase antifungal activity to reduce the development of undesirable spoilage organisms in the ensiled mass and improve the fermentation quality of the silage (Oladosu et al., 2016).

Table 2
Fermentative quality (pH and organic acid content) of urea- and molasses-treated sugarcane bagasse after 21 d of fermentation.

Treatment (T)	pH	Lactic acid	Acetic acid	Butyric acid
		(%)		
T6 (AF:U0:M0)	4.3^{c}	0.2	0.3^{c}	0.1
T7 (AF:U0:M5)	4.1^{c}	0.2	1.1^{b}	0.2
T8 (AF:U5:M0)	8.4^{a}	0.1	1.9^{a}	0.3
T9 (AF:U5:M5)	8.1^{b}	0.2	1.9^{a}	0.4
SEM	0.00	0.00	0.00	0.00
Comparison				
Urea 0 vs 5 (B)				
U0	4.2^{b}	0.2	0.7^{b}	0.1
U5	8.2^{a}	0.1	1.9^{a}	0.3
Molasses 0 vs 5 (C)				
M0	6.3^{a}	0.1	1.1^{b}	0.2
M5	6.1^{b}	0.2	1.5^{a}	0.3
Interaction between factors				
B × C	ns	ns	**	ns

AF = after fermentation; U = urea; M = molasses; ns = non-significant; SEM = standard error of the mean.
^{abcdef} = mean within columns with different lowercase superscript letters are significantly ($p < 0.05$) different.
* = $p < 0.05$; ** = $p < 0.01$.

In vitro rumen fermentation and digestibility

Gas kinetics, cumulative gas production and *in vitro* digestibility of each substrate treatment are presented in Table 3. Gas produced from the soluble fraction (a), gas production from the insoluble fraction (b), and the rate of gas production (c), potential extent of gas production (a+b) and cumulative gas production in the untreated sugarcane bagasse (control, T1) were significantly lower than in the treated sugarcane bagasse (T2–T9). In all treatments, before fermentation (factor A, T2–T5), no parameter of gas kinetics and gas production was affected by the urea or molasses treatments, while after fermentation, gas kinetics and gas production were enhanced by sugarcane bagasse treatment (T6–T9). Gas kinetics including a, b, c, a+b and cumulative gas production at 24 h, 48 h and 96 h were highest ($p < 0.05$) after fermentation of the sugarcane bagasse with urea and molasses added (T9) with values of 35.1 mL/200 mg DM, 50.2 mL/200 mg DM and 59.0 mL/200 mg DM, respectively. These results were in agreement with Gunun et al. (2017) who found that gas kinetics including b, a+b and cumulative gas production were increased by urea treatment of sugarcane bagasse ($p < 0.05$). The increased gas kinetics and *in vitro* gas production may have been contributed to by increased degradability of structural carbohydrates such as hemicelluloses and cellulose in 2% and 4% urea treatments (Hameed et al., 2012).

Although there were no significant interactions before and after fermentation (A), urea (B) and molasses supplementation (C) on *in vitro* DM and OM degradability (IVDMD, IVOMD), treated sugarcane bagasse with urea and molasses was significantly highest in IVDMD and IVOMD (Table 4). The increase in digestibility of sugarcane bagasse treated with urea and molasses was consistent with the observed lower fiber contents and higher gas production. Sommart et al. (2000) stated that there was a positive correlation between *in vitro* organic matter digestibility (OMD) and the volume of gas released during fermentation, which was in agreement with the results of the current experiment. Moreover, Ahmed et al. (2013) showed that *in situ* degradability of NDF increased significantly for urea-treated sugarcane bagasse among all treatments compared with raw bagasse. The effective degradability in different outflows also increased with an increase in the ensiling period of the sugarcane bagasse. This result might have been due to the partial solubilization of hemicellulose by the urea treatment which was reported to cause a partial break down of the bond between the lignin and other cell wall components that lead rumen bacteria to degrade fibrous material in the rumen, which should increase both the rate and extent of digestion, as Gunun et al. (2017) found that urea and $Ca(OH)_2$ treatment could increase DM and OM degradability, true digestibility and microbial mass of sugarcane bagasse ($p < 0.05$).

The results of the current study indicated that a combination of 5% urea and 5% molasses treatment of sugarcane bagasse could improve the nutritive value and *in vitro* fermentation with increased CP content and *in vitro* DM and OM digestibility. Based on these results, utilization of 5% urea and 5% molasses as ingredients in sugarcane bagasse treatment would make the product effective as a roughage source for ruminants. Further research should be done to investigate the effects of urea- and molasses-treated sugarcane bagasse in production trials (for both meat and milk) to assess the effect of this product on animal performance and its use under practical farm conditions.

Table 3
Effect of urea- and molasses-treated bagasse on rate of gas production (c, %/hr), extent of gas production (a+b) and gas production from *in vitro* fermentation.

Treatment (T)	Gas kinetics				Gas volume (mL/0.2 g dry matter)		
	a	b	c	a+b	24 h	48 h	96 h
T1 (Control)	0.3^{a}	36.6^{f}	0.03^{b}	36.9^{f}	21.8^{e}	30.7^{f}	35.8^{f}
T2 (BF:U0:M0)	-0.2^{ab}	40.4^{e}	0.03^{b}	40.2^{e}	20.8^{e}	30.9^{ef}	38.1^{e}
T3 (BF:U0:M5)	-0.4^{bc}	40.5^{e}	0.03^{b}	40.1^{e}	21.5^{e}	31.5^{ef}	38.3^{e}
T4 (BF:U5:M0)	-0.4^{bc}	41.2^{de}	0.03^{b}	40.8^{de}	20.8^{e}	31.1^{ef}	38.5^{e}
T5 (BF:U5:M5)	-0.4^{bc}	41.6^{de}	0.03^{b}	41.2^{de}	21.8^{e}	32.2^{e}	39.3^{e}
T6 (AF:U0:M0)	-0.1^{ab}	42.2^{d}	0.04^{a}	42.1^{d}	24.7^{d}	34.9^{d}	40.8^{d}
T7 (AF:U0:M5)	-0.4^{ab}	46.4^{c}	0.04^{a}	46.0^{c}	26.8^{c}	37.9^{c}	44.6^{d}
T8 (AF:U5:M0)	-0.9^{cd}	57.6^{b}	0.04^{a}	56.7^{b}	32.2^{b}	46.2^{b}	54.8^{b}
T9 (AF:U5:M5)	-1.3^{d}	62.1^{a}	0.04^{a}	60.8^{a}	35.1^{a}	50.2^{a}	59.0^{a}
SEM	0.01	0.06	0.16	0.06	0.04	0.06	0.06
Comparison							
Control vs Others							
Control	0.3^{a}	36.6^{b}	0.03	36.9^{b}	21.8^{b}	30.7^{b}	35.8^{b}
Others	-0.5^{b}	46.1^{a}	0.03	45.6^{a}	25.3^{a}	36.6^{a}	43.8^{a}
Before vs After fermentation (A)							
Before	-0.3^{a}	40.9^{b}	0.03^{b}	40.6^{b}	21.2^{b}	31.4^{b}	38.5^{b}
After	-0.7^{b}	52.1^{a}	0.04^{a}	51.4^{a}	29.7^{a}	42.3^{a}	49.8^{a}
Urea 0 vs 5 (B)							
U0	-0.3^{a}	42.4^{b}	0.03	42.1^{b}	23.4^{b}	33.8^{b}	40.5^{b}
U5	-0.7^{b}	50.6^{a}	0.03	49.9^{a}	27.5^{a}	39.9^{a}	47.9^{a}
Molasses 0 vs 5 (C)							
M0	−0.4	45.4^{b}	0.03	45.0^{b}	24.7^{b}	35.8^{b}	43.1^{b}
M5	−0.6	47.7^{a}	0.03	47.1^{a}	26.3^{a}	38.0^{a}	45.3^{a}
Interaction between factors							
A × B	**	ns	**	**	**	**	**
A × C	ns	ns	**	**	**	**	**
B × C	ns	ns	ns	ns	ns	ns	ns
A × B × C	ns	ns	ns	ns	ns	ns	ns

a = gas production from the immediately soluble fraction in milliliters; b = gas production from the insoluble fraction in milliliters; c = gas production rate constant for the insoluble fraction in milliliters per hour, a+b = extent of gas production; BF = before fermentation; AF = after fermentation; U = urea; M = molasses; ns = non-significant; SEM = standard error of the mean.
^{abcdef} = mean within columns with different lowercase superscript letters are significantly ($p < 0.05$) different.
** = $p < 0.01$.

Table 4
Effect of urea- and molasses-treated sugarcane bagasse on *in vitro* dry matter (IVDMD) and organic matter degradability (IVOMD) at 24 h and 48 h of incubation.

Treatment (T)	*In vitro* degradability at 24 h (%)		*In vitro* degradability at 48 h (%)	
	IVDMD	IVOMD	IVDMD	IVOMD
T1 (Control)	18.6^{e}	36.0^{b}	24.1^{c}	40.6^{c}
T2 (BF:U0:M0)	19.4^{de}	39.2^{b}	29.1^{bc}	41.3^{c}
T3 (BF:U0:M5)	23.5^{cd}	40.1^{b}	32.9^{ab}	42.2^{c}
T4 (BF:U5:M0)	25.3^{bc}	41.3^{b}	33.1^{ab}	42.3^{c}
T5 (BF:U5:M5)	26.0^{bc}	42.0^{b}	34.9^{ab}	44.8^{c}
T6 (AF:U0:M0)	25.0^{bc}	48.1^{ab}	28.4^{bc}	56.3^{b}
T7 (AF:U0:M5)	28.0^{bc}	53.9^{a}	35.8^{ab}	60.1^{b}
T8 (AF:U5:M0)	28.9^{b}	57.3^{a}	37.3^{a}	62.0^{ab}
T9 (AF:U5:M5)	34.1^{a}	58.9^{a}	40.1^{a}	68.1^{a}
SEM	1.18	1.36	5.20	1.83
Comparison				
Control vs Others				
Control	18.6^{b}	35.9^{b}	24.1^{b}	40.6^{b}
Others	26.3^{a}	47.6^{a}	33.9^{a}	52.1^{a}
Before vs After fermentation (A)				
Before	23.5^{b}	40.7^{b}	32.5	42.6^{b}
After	29.0^{a}	54.5^{a}	35.4	61.6^{a}
Urea 0 vs 5 (B)				
U0	24.0^{b}	45.3	31.5^{b}	50.0^{b}
U5	28.6^{a}	49.9	36.4^{a}	54.3^{a}
Molasses 0 vs 5 (C)				
M0	24.7^{b}	46.5	32.0^{b}	50.5
M5	27.9^{a}	48.7	35.9^{a}	53.8
Interaction between factors				
A × B	ns	ns	ns	ns
A × C	ns	ns	ns	ns
B × C	ns	ns	ns	ns
A × B × C	ns	ns	ns	ns

BF = before fermentation; AF = after fermentation; U = urea; M = molasses; ns = non-significant; SEM = standard error of the mean.
^{abcdef} = mean within columns with different lowercase superscript letters are significantly ($p < 0.05$) different.

Conflict of interest

The authors declare that there are no conflicts of interest.

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