Manipulation of Rumen Fermentation by Bioindustrial Products of Cashew Nut Shell (*Anacardium occidentale*) to Reduce Methane Production

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ABSTRAK

Saenab A, Wiryawan KG, Retnani Y, Wina E. 2018. Manipulasi fermentasi rumen oleh produk bioindustri cangkang biji mete (*Anacardium occidentale*) untuk menekan produksi metana. JITV 23(2): 61-70. DOI: http://dx.doi.org/10.14334/jitv.v23i2.1821

Salah satu cara menurunkan gas metana yang dihasilkan oleh ternak ruminansia adalah dengan penggunaan pakan aditif yang berasal dari tanaman. Tanaman yang cukup potensial adalah tanaman jambu mete (*Anacardium occidentale*) khususnya bagian cangkang biji. Cangkang dapat diproses menjadi 3 produk bioindustri yaitu biofat, biochar, biosmoke. Tujuan penelitian untuk mengevaluasi efektivitas 3 produk bioindustri terhadap produksi gas metana dan produk akhir fermentasi pakan dalam rumen secara in vitro. Penelitian menggunakan rancangan acak kelompok berdasarkan periode in vitro. Perlakuan terdiri dari Kontrol = Substrat (S), Biofat: S+0.25 µL/mL, S+0.5 µL/mL, S+0.75 µL/mL; Biochar: S+0.1 mg/mL, S+0.2 mg/mL, S+0.3 mg/mL; Biosmoke: S + 2.5 µL/mL, S+5.0 µL/mL, S + 7.5 µL/mL. Perlakuan diulang 2 kali dalam 4 kali in vitro. Variabel yang diukur adalah produksi gas total dan metana; pH; kecernaan bahan kering (BK); organic (BO) dan neutral detergent fibre (NDF); konsentrasi amonia (NH_3) dan konsentrasi VFA parsial dan total. Hasil menunjukkan bahwa biofat, biochar dan biosmoke dapat menurunkan produksi gas metana masing-masing 43,88%; 24,21%; 37,88% pada level tertinggi. Produksi NH_3 meningkat secara signifikan pada pemberian biochar level tertinggi. Molar proporsi asam asetat menurun dan asam propionat meningkat secara nyata ketika ditambahkan produk biofat, biochar dan biosmoke dibandingkan dengan kontrol. Nilai kecernaan BK menurun pada penambahan biofat dan biosmoke tetapi kecernaan BO tidak berbeda nyata pada penambahan biochar dibanding kontrol. Terdapat mekanisme yang berbeda antara biofat, biochar dan biosmoke dalam mempengaruhi fermentasi rumen. Disimpulkan bahwa biofat, biochar dan biosmoke dapat dimanfaatkan sebagai agen pereduksi gas metana dan meningkatkan asam propionat dalam rumen ternak ruminansia.

Kata Kunci: Cangkang Mete, Biofat, Biochar, Biosmoke, Fermentasi Rumen, Metana

ABSTRACT

Saenab A, Wiryawan KG, Retnani Y, Wina E. 2018 Manipulation of rumen fermentation by bioindustrial products of cashew nut shell (*Anacardium occidentale*) to reduce methane production. JITV 23(2): 61-70. DOI: http://dx.doi.org/10.14334/jitv.v23i2.1821

One of the strategies to reduce methane produced by ruminants is by the application of feed additive from plant materials. One of the potential plants is cashew plant especially its shell. The cashew nut shell can be processed to become 3 bioindustrial products; ie biofat, biochar, biosmoke. The aim of this research was to evaluate the effectively of biofat, biochar and biosmoke in reducing methane and other end product of rumen fermentation. The experiment was arranged in block randomize design based on time series of in vitro to evaluate 3 levels and 3 types of bioindustrial. The treatments were Control (substrate=S), Biofat: S+0.25 µL/mL, S+0.5 µL/mL, S+0.75 µL/mL; Biochar: S+0.1 mg/mL, S+0.2 mg/mL, S+0.3 mg/mL; Biosmoke: S + 2.5 µL/mL, S+5.0 µL/mL, S + 7.5 µL/mL. Each treatment was done in duplicates and the in vitro experiment was repeated 4 times. The research measured total gas production; methane production; digestibility of dry matter, organic matter and neutral detergent fibre (NDF); ammonia concentration (NH_3); partial and total volatile fatty acids (VFA) concentration. The results showed that biofat, biochar and biosmoke reduced methane production by 43.88%, 24.21%, 37.88% at the highest level of inclusion, respectively. The NH_3 concentration slightly increased by biochar and biosmoke addition. Molar proportion of acetic acid decreased, while molar proportion of propionic acid increased by addition of the three bioindustrial products. Organic matter digestibility decreased significantly with biofat and biosmoke addition. The mechanism of biofat, biochar and biosmoke in affecting rumen fermentation was different. It can be concluded that the three bioindustrial products of cashew nut shell can be utilized as feed additive to reduce methane and increase propionic acid production in the rumen.

Key Words: Cashew Nut Shell, Biofat, Biochar, Biosmoke, Rumen Fermentation, Methane

INTRODUCTION

Global warming is a major environmental problem today. Some types of gas CO_2, N_2O and CH_4 are greenhouse gases (GHG) that cause global warming. Livestock, in particular ruminants contribute methane emissions for agricultural sector. Methane from ruminant are the result of methanogenic bacteria activity in the rumen. It was reported that dairy cows, cattle, and goat produce methane 110-145, 60-70, and 8 kg/head/year, respectively (Morgavi 2008). Therefore, methane emission from total population of ruminants in the world contributes 95% of total emissions of methane produced by livestock and humans, and approximately 18% of total GHG in the atmosphere (Kreuzer & Soliva 2008). This condition becomes a constraint in ruminant production development.

One of the strategies to reduce methane emissions is by use feed additives derived from plant extracts that contain secondary compounds, such as tannins (Beauchemin et al. 2007, Jayanegara et al. 2010), saponins (Hess et al. 2003; Wina 2012), essential oils (Patra & Yu 2012). Those three secondary compounds reported have low potency and short term effect on mitigating methane (Gerber et al. 2013). Therefore, researchers are still looking for alternatives of bioactive containing plant materials that safe both for environment and animal to mitigate methane from animal.

Cashew (*Anacardium occidentale*) are planted for its nut, whereas the nut shell which is 45-50% of the whole nut is rarely used (Muljohardjo 1991).The nut shell has 20-30% of fat content, which is ussualy extracted to produce cashew nut shell liquid (CNSL). The residue after the extraction may be thrown away. These cashew nut shells was processed to become three industrial products, namely biofat, biochar and biosmoke. The method to produce these bioindustrial products have been developed (Simpen 2008; Sudrajat & Salim 1994) and described in Saenab et al. (2016). The information on the utilization of these three products for livestock production is still very limited.

Biofat product or CNSL is hexane extracted fraction of cashew nut shell which contains several bioactive compounds (Saenab et al. 2016). The major compounds are anacardic acids, which are phenolic compounds attached to fatty acids (C_{15}). It has been reported that CNSL reduced methane and suppressed certain bacteria in the rumen (Watanabe et al. 2010; Shinkai 2010; Mitsumori et al. 2014).

Biochar is a pyrolysis product from the cashew nut shell after hexane extraction. During the pyrolysis process, a mixture of liquid smoke (biosmoke) is also obtained. The BC from rice husk has been reported to have ability to suppress methane production in the rumen and increase body weight of cattle (Leng et al. 2012a). While biochar from bamboo reported increased goat's live weight (Do et al. 2006).

Liquid smoke is generally used as an anti-diarrhea for pigs (Choi 2009) due to its phenolic compounds contained (Naim et al. 2012). Moreover phenolic compound can reduce methane production in the rumen (Jayanegara et al. 2009)

Therefore, the aim of study was to investigate the effectiveness of three bioindustry products (biofat, biochar and biosmoke) from cashew nut shell as feed additives to reduce methane and improve rumen fermentation end products.

MATERIALS AND METHODS

The experiment has been approved by the Animal Welfare Commission of the Indonesian Agency for Agricultural Research and Development (Balitbangtan/Balitnak/Rm/05/2016). The experiment was conducted at the Feed Laboratory of the Indonesian Research Institute for Animal Production.

Bioindustrial products of cashew nut shell (biofat, biochar and biosmoke) were used as feed additive in the experiment. The method of preparing biofat (BF) (Simpen 2008), biochar (BC) and biosmoke (BS) (Sudrajat and Salim 1994) was described in Saenab et al. (2016). Complete feed for *in vitro* study consisted of grass, *Gliricidia sepium* leaves, yellow corn, coconut cake, molasses, rice bran, urea, salt (NaCl), limestone (CaCO3), and premix. The nutrient content of complete feed used as substrate in the *in vitro* assays is presented in Table 1. The grass (*Pennisetum purpureum*) was obtained from Balitnak farm while other feed ingredients were obtained from local trader.

Table 1. The nutrient composition of complete feed used as substrate in in vitro assays

The nutrient composition (%)	Complete feed
Cp	15.63
Cf	3.83
GE (Kcal/Kg)	3785
Ash	6.85
NDF	28.41
ADF	15.02
Ca	0.69
P	0.29
TDN	69.7

In vitro rumen fermentation

In *in vitro* method, a buffer medium consisted of bicarbonate buffer solution, macro-mineral solution, micro-mineral solution, resazurin, distilled water, reducing solution and rumen fluid as described in Makkar (2003) was used. Feed samples were incubated in the *in vitro* system according to the method modified by Theodorou et al. (1994). Cashew nut shell bioindustrial products BF, BC and BS as feed additives were mixed with feed sample as substrate for *in vitro*. A total of 750 mg of substrate was weighed into the bottle. Rumen buffer solution (75 mL) was added under CO_2 atmosphere and the rubber stopper was quickly applied on the bottle. The bottle was placed in the water bath and incubated at 39^{o}C for 48 hours. Rumen fluid was collected just before morning feeding from a rumen fistulated Friesian Holstein cow fed with commercial concentrate and elephant grass.

The total gas and methane production were measured at 3, 6, 9, 12, 24, 30, 36, 48 hours of incubation by using syringes glass followed method developed by Tjandraatmadja (1981). At 48 hours of incubation, the supernatant was separated from the substrat by filtration using centreglass. The residue was dried in the oven with temperature 105^{o}C for 24 hours and weighed. Ash content of feed and residue was determined according to AOAC method (2000) and NDF analysis was conducted following Goering and Van Soest (1970) by method without addition of amylase. The *in vitro* dry matter (DM) and organic matter (OM) of digested fractions were calculated from the DM and OM of initial sample minus those of residues. The DM or OM of digested fractions divided by the DM or OM of initial sample was calculated as DM or OM digestibility. Other residue samples of *in vitro* incubation were digested using NDS to obtain residual NDF fraction. The pH, ammonia (NH_3) and volatile fatty acid (VFA) were measured after 48 hours of incubation. The NH_3 content in the supernatant was determined using Conway microdiffusion technique. The supernatant for VFA analysis was kept in low pH by adding sulphuric acid. The VFA products from fermentation was analysed by using gas chromatography *(Bruker Scion 436 GC*) with capillary column BR-Wax fame containing WCOT fused silica with the length of column 30 m x 0.32 mm ID. The carrier gas was Nitrogen 25 ml/min and the burning gas was Hidrogen 30 mL/min. Injector temperature was 250^{o}C, while the column temperature gradient was 70 – 150^{o}C in 11 minutes. The detector used was FID with temperature of 275°C

Experimental design

The study used a randomized block design based on the time series of *in vitro* repetation to test treatments as follow Control (without feed additive = 0 BF, 0 BC and 0 BS), Biofat levels were 0.25 µL/mL (BF1), 0.5 µL/mL (BF2), 0.75 µL/mL (BF3); Biochar levels were 0.1 mg/mL (BC1), 0.2 mg/mL (BC2), 0.3 mg /mL (BC3), Biosmoke 2.5 µL/mL (BS1), 5.0 µL/mL (BS2), 7.5 µL/mL (BS3). Each treatment was done in duplicate and the *in vitro* was repeated 4 times.

Statistical analysis

The data collected from each addition of BF, BC and BS compared to control were analyzed separately by PROC GLM using SPSS Program Package 16. Further analysis using Duncan test was done for obtaining significant differences among treatments.

RESULTS AND DISCUSSION

Characteristics of rumen fermentation end products

Methane production profile in the rumen as a result of fermentation of feed added by different level of BF is presented in Figure 1. At 3 hours of incubation, the methane production was similar among the treatments. The production was different after 6 to 48 hours of incubation. The line regression shows the rate of methane production (10.01, 8.92, and 6.77 mL/hour) was decreasing in line with increasing level of BF addition (0.25, 0.5, and 0.75 µL/mL), respectively compared to control (12.57 mL/hour). At the different level of BC addition (Figure 2), the methane production profile of all the treatment was similar with control during the 48 hours of incubation. The methane production curves when BS added (Figure 3) were similar to those of BF addition. The higher level of BS addition (2.5, 5.0, 7.5 µL/mL) caused the lower rate of methane production (10.71, 9.59, 8.62 mL/hour), respectively, compared to control (12.57 mL/hour),

Total gas and methane production at 48 hours of incubation decline very significant ($P<0.01$) with the increasing level of BF (Table 2). Increasing level of BF from 0.25 µL/mL to 0.5 µL/mL and 0.75 µL/mL reduced significantly ($P<0.01$) total gas and methane production by 21.73%; 20.76%; 45.25% and by 20.83%; 29.79%; 43.88% respectively, compared to control. Addition of BC increased total gas production

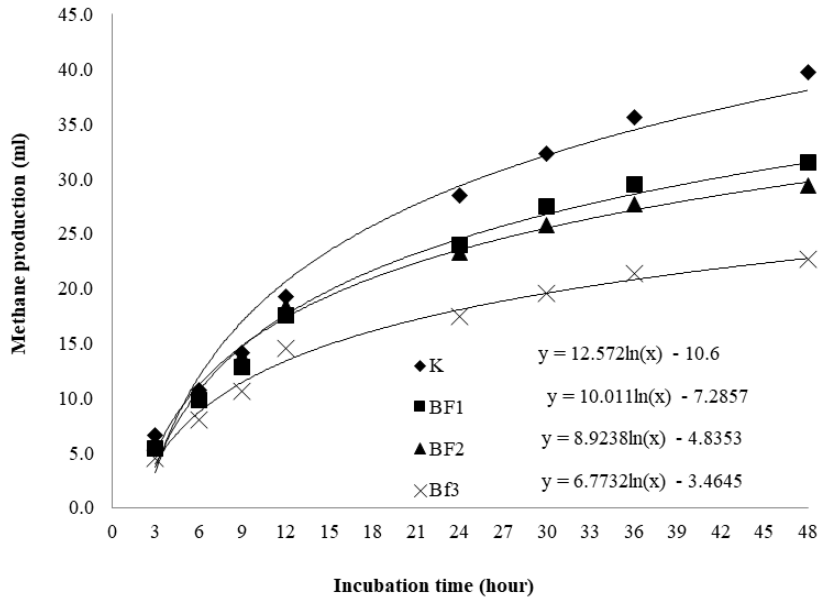

Figure 1. The profile of methane production at different level of Biofat (BF) addition during 48 hours of incubation.

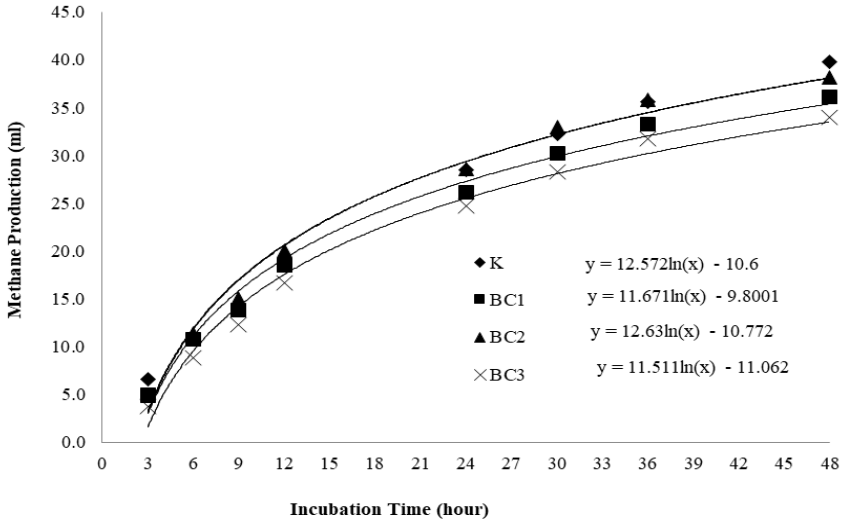

Figure 2. The profile of methane production at different level of Biochar (BC) addition during 48 hours of incubation.

by 18.76%, 14.47%, and 9.56%, but decreased methane production by 9.12%, 4.09%, and 24.21% after addition of BC 0.1 mg/mL, 0.2 mg/mL, and 0.3 mg/mL respectively, compared to control.

Addition of BS to the *in vitro* fermentation caused total gas and methane production decreased significantly (P<0.05) compared to control. Methane production was reduced by 31.55%, 30.69%, and 37.88% with addition of BS 2.5, 5.0, and 7.5 µL/mL. The NH_3 concentration and pH at 48 hours of incubation (Tabel 2) were not significantly different (P>0.05) between addition of BS and control. However, the highest level of BC increased NH_3 concentration significantly (P<0.05) compared to control. The BS additional was not significantly increased NH_3 production.

Inclusion of BF to the feed (Table 3) improved proportion of propionate significantly (P<0.05),

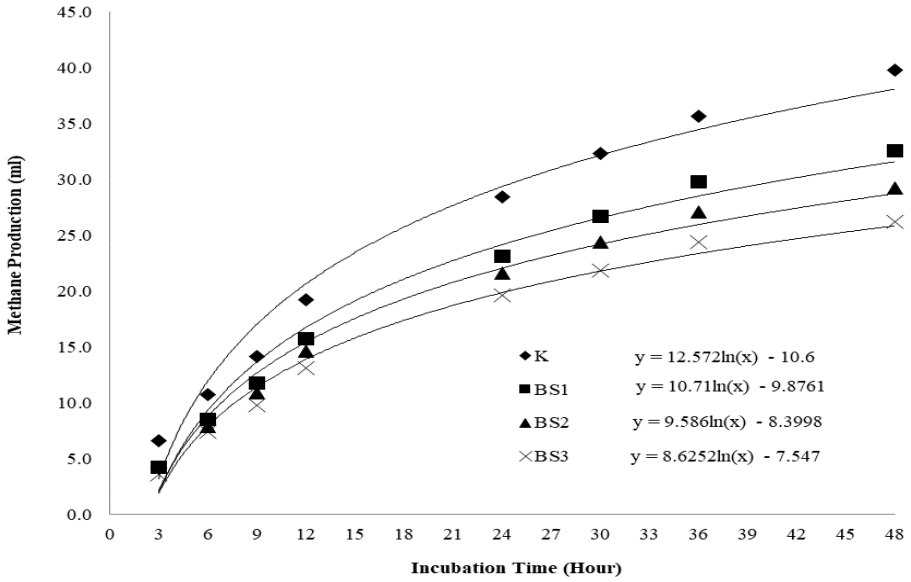

Figure 3. The profile of methane production at different level of Biosmoke (BS) addition during 48 hours of incubation.

Table 2. The effect of biofat (BF), biochar (BC) and biosmoke (BS) of cashew nut shell on total gas, methane production, NH_3 concentration and pH at 48 hours of incubation

Additive	Level	Total Gas (mL)	Methane (mL)	NH_3 (mM/g BK)	pH
BF	0	157.3^{d}	39.7^{d}	8.30	6.67
	0.25 µL/mL	143.5^{c}	31.5^{c}	8.18	6.68
	0.50 µL/mL	127.5^{b}	27.9^{b}	8.12	6.70
	0.75 µL/mL	101.4^{a}	22.3^{a}	7.53	6.71
SE		2.60	0.72	0.47	0.01
P-value		0.00	0.00	0.41	0.17
BC	0	157.3^{a}	39.7^{c}	8.30^{a}	6.67^{a}
	0.25 mg/mL	164.2^{a}	36.1^{b}	8.49^{a}	6.72^{b}
	0.50 mg/mL	164.4^{a}	38.1^{bc}	8.95^{a}	6.70^{ab}
	0.75 mg/mL	190.9^{b}	30.1^{a}	10.78^{b}	6.70^{ab}
SE		3.13	0.87	0.48	0.01
P-value		0.00	0.001	0.003	0.05
BS	0	157.3^{d}	39.7^{c}	8.30	6.67
	0.25 µL/mL	145.9^{c}	27.20^{b}	11.30	6.69
	0.50 µL/mL	121.9^{b}	27.5^{b}	10.56	6.70
	0.75 $\mu L mL^{-1}$	114.3^{a}	24.7^{a}	9.23	6.72
SE		2.98	0.76	0.89	0.01
P-value		0.00	0.00	0.14	0.11

Different letter on the same column indicates significant difference ($P<0.05$). Statistical analysis was done separately for each additive against control

Table 3. Molar proportion of acetate, propionate, butyrate, valerate, BCVFA, ratio of A/P and total VFA (mM) production in the *in vitro* rumen fermentation of feed added by biofat (BF), biochar (BC) and biosmoke (BS) of cashew nut shell after 48 hours of incubation

Additive	Level	Acetate	Propionate	Butyrate	Valerate	BCVFA	A/P	Total VFA (mM)
		----------%----------						
BF	0	60.72	23.37^{a}	10.99^{b}	1.82	3.10^{b}	2.64	87.10
	0.25µL/mL	56.83	31.86^{b}	9.10^{a}	1.42	2.54^{a}	1.98	112.69
	0.5 µL/mL	59.26	27.76^{b}	9.18^{a}	1.35	2.35^{a}	2.2	96.25
	0.75µL/mL	56.24	31.37^{b}	8.98^{a}	1.21	2.40^{a}	1.87	110.56
SE		2.543	1.568	0.442	0.172	0.106	0.191	7.546
P-Value		0.502	0.044	0.035	0.150	0.004	0.242	0.106
BC	0	60.72	23.37^{a}	10.99	1.82	3.10	2.64	87.10^{a}
	0.1 mg/mL	58.18	24.90^{b}	11.84	1.67	4.24	2.34	120.02^{ab}
	0.2 mg/mL	51.70	28.55^{b}	13.51	2.00	3.08	1.85	108.38^{ab}
	0.3 mg/mL	59.98	29.37^{b}	11.39	1.74	3.81	2.20	125.5^{b}
SE		1.143	1.419	1.080	0.169	0.444	0.092	8.393
P-Value		0.123	0.049	0.249	0.565	0.236	0.002	0.035
BS	0	60.72^{c}	23.37^{a}	10.99	1.82	3.10^{ab}	2.64^{c}	87.11
	2.5 µL/mL	49.93^{a}	36.67^{c}	9.91	1.49	3.29^{b}	1.39^{a}	107.25
	5.0 µL/mL	56.22^{b}	26.32^{b}	11.85	1.86	3.36^{b}	2.24^{c}	102.2
	7.5 µL/mL	54.20^{b}	27.28^{b}	12.64	2.04	3.24^{a}	2.07^{b}	104.37
SE		0.880	0.835	0.737	0.151	0.240	0.093	4.610
P-Value		0.000	0.000	0.110	0.130	0.026	0.000	0.043

BCVFA = Branched chain volatile fatty acid
A/P = Acetate/Propionate
Different letter on the same column shows significant difference (P<0.05)
Statistical analysis of every bioindustrial product treatment against control was conducted separately

however decreased proportion of butyrate and BCVFA significantly (P<0.05) compared with control (P<0.05). But not significantly decreased proportion of acetate and valerate and not significantly increased total VFA. The results also showed that BC addition enhanced total VFA production and proportion of propionate produced (P<0.05) (Table 3). The highest level of total VFA and proportion of propionate was obtained at the BC addition of 0.3 mg/mL. In contrast, proportion of acetate and ratio of A/P decreased significantly (P<0.05) due to BC addition compared to control.

Addition of BS increased significantly proportion of propionate and total BCVFA (P<0.01) while it reduced proportion of acetate (P<0.05) (Table3).

Effect of bioindustrial products of cashew nut shell on rumen digestibility

The DM, OM and NDF digestibility of feed significantly (P<0.05) decreased in the presence of BF (Table 4). Increasing level of BF inclusion resulting in decreasing feed digestibility. The reduction of NDF digestibility was higher than the reduction of DM and OM digestibility. The BC addition at any level did not cause any significant effect on DM, OM and NDF digestibility. Similar to BF, the BS addition significantly (P<0.05) reduced DM, OM and NDF digestibility.

The effect of Biofat (BF) on rumen fermentation and methane mitigation

The results indicated that increasing level of BF addition followed by reducing in the production of total gas and methane. Our results on decreased methane production were due to BF was in line with previous reports (Watanabe et al. 2010). The ability of BF in reducing production of methane in the rumen could be attributed by several factors (Watanabe et al. 2010; Shinkai et al. 2010; Mitsumori et al. 2014). Watanabe et al (2010) stated that anacardic acids were the active compounds in BF that might reduce methane and caused changes in fermentation process. Saenab et al. (2016) analysed the phenolic compounds in BF indicated that there were some phenolic compounds, which are binded to the long chain fatty acids (C15).

Some of these compounds were identified as 78.16% anacardic acid, 13.18% cardol, 4.66% cardanol and 3.56% methyl cardol. The long chain lipid that binded to the phenolic structure has none, one, two or three double bond in their lipid structure (Njuku et al. 2014). Plant secondary compounds including phenolic compounds possessed antimicrobial activity that may inhibit the growth of methanogenic bacteria and some other bacteria, hence, reduced methane and total gas production (Kamra et al. 2012, Jayanegara et al. 2013). Watanabe et al. (2010) also reported that all Gram positive bacteria including rumen bacteria were highly or moderately influenced by CNSL (BF), however, several Gram negative bacteria were not influenced by CNSL (BF). The exact bactericidal mechanism of BF may be due to the surfactant properties of anacardic acids that may disrupt the bacteria cell membrane.

Reducing methane by BF addition may be due to the lower population or activity of methanogenic bacteria. As reported by Shinkai et al. (2012) that CNSL or BF reduced the activity of methanogens to produce methane. It might due to the less hydrogen availability in the rumen. The growth of hydrogen and formic acid forming bacteria may be suppressed by BF (Shinkai et al. 2012). There might also be competition in hydrogen

Table 4. The DM, OM and NDF digestibility of feed in the presence of biofat (BF), biochar (BC) and biosmoke (BS) of cashew nut shell in the *in vitro* rumen fermentation after 48 hours of incubation

Additive	Level	Digestibility		
		BK (%)	BO (%)	NDF (50
BF	0	67.96^{bc}	69.20^{c}	54.03^{c}
	0.25 µLmL^{-1}	67.37^{c}	68.80^{bc}	50.88^{bc}
	0.5 µLmL^{-1}	63.15^{ab}	64.46^{ab}	40.73^{ab}
	0.75 µLmL^{-1}	59.88^{a}	60.48^{a}	36.42^{a}
SE		1.85	1.47	2.46
P-Value		0.033	0.004	0.001
BC	0	67.96	69.55	54.03
	0.1 mg/mL	66.48	70.03	52.51
	0.2 mg/mL	65.86	67.86	50.39
	0.3 mg/mL	69.17	71.11	52.39
SE		2.37	1.89	1.66
P-Value		0.762	0.683	0.505
BS	0	67.96	69.55	54.03^{c}
	2.5 µL/mL	58.54	63.18	34.28^{a}
	5.0 µL/mL	58.51	62.10	42.82^{ab}
	7.5 µL/mL	54.89	58.55	38.73^{ab}
SE		3.99	3.47	4.44
P-Value		0.178	0.216	0.047

Different letter on the same column shows significant difference (P<0.05). Statistical analysis of every bioindustrial product treatment against control was conducted separately

utilization by methanogenic bacteria, propionic acid forming bacteria, sulfate reducing bacteria and other bacteria that consume hydrogen (Mitsumori et al. 2014). Hydrogen is released from the formation of acetic acid and butyric acid during fermentation in the rumen. The hydrogen will be taken up by bacteria because hydrogen is the main component required in producing methane and propionate. There is a competitive action of bacteria producing methane and propionate in hydrogen utilisation. The results showed that methane production decreased as propionate increased with the addition of BF, might be due to more hydrogen used by propionate forming bacteria. These results are in line with observation of Watanabe et al. (2010) who reported that the population of propionate forming bacteria (*Selemonas ruminantium*, *Megasphaera elsdenii*, *Prevotella ruminicola*) increased as increasing in the amount of hydrogen taken by these bacteria. It then reduced the amount of hydrogen taken up by other hydrogen consuming bacteria.

The lipid structure in anacardic acids may also suppress methane production as it may suppress the methanogenic process as reported by Martin et al. (2010). Lipid can reduce fiber digestibility in the rumen since unsaturated fatty acid is toxic to fiber degrading bacteria (Maia et al. 2007), hence, less fiber degrading bacteria that grew in the presence of BF. Watanabe et al. (2010) found that the addition of CNSL (BF) inhibited the growth of *Ruminococcus flavefaciens* and *Ruminococcus albus* that have the ability to digest fiber. This also resulted in decreasing the DM, OM and NDF digestibility as addition of BF increased.

The BF inclusion in the diet did not affect NH_3 production (Table 2), but it decreased BCVFA significantly (Table 3). The BCVFA is the result of degradation of branched amino acids (valine and leucine) by bacteria *Megasphaera elsdenii* and *Prevotella ruminicola* (Wolin et al. 1997). Phenolic lipid compounds might specifically depress the growth of bacteria that disintegrate the bond of branched amino acids but it might not interfere the growth of ammonia-forming bacteria. The NH_3 is required for rumen microbial growth to synthesis rumen microbial protein. Sutardi (1994) stated that the production range of NH_3 for a good growth of rumen microbes is between 4-12 mM. This means that the concentration of NH_3 in this study (7.53 to 8.34 mM) were within the normal range for optimum growth of rumen microbes. .

The effect of Biochar (BC) on rumen fermentation and methane mitigation

Result of study indicated that the administration of BC at the highest level (0.3 mg/mL) suppressed the methane production quite markedly 16.75%. However the activity of BC in suppressing methane production was not similar with those of BF activity. Lower methane production in *in vitro* fermentation was also reported due to the inclusion of BC from rice husk (Leng et al. 2012b) and BC from wood (Hansen et al. 2012). The difference on the mechanism of methane reduction by BC vs BF was due to the physical property of BC, but not due to the bioactive compounds in BC. The BC from cashew nut shell has highly porous structure (Saenab et al. 2016), which might absorb methane present in the rumen.

Addition of BC was very significant increased NH_3 production ($P<0.01$). Increasing level of BC added to the *in vitro* fermentation increased NH_3 produced (Table 2). The findings were similar with those reported by Leng et al. (2012), who use BC from rice hull and coconut meal in the *in vitro* experiment. The BC treatment resulted a very significant increase on the proportion of propionate and total VFA compared to control (Table 3). The highest propionate and total VFA were obtained at the highest level of BC treatment (0.75 mg/mL). Reducing methane production might be followed by inreasing in propionate forming due to the hydrogen availability.

Different results with the BF or BS addition, the addition of BC had no effect on feed digestibility. These results were similar with finding of previous study reported by Leng et al. (2012a). Even though BC did not improve feed digestibility, it increased daily weight gain of cattle (Leng et al. 2012b) and goat (Do et al. 2006). Addition of BC might improve dietary energy utilization by the animals due to the shifted of methane to propionate production, which is required for animal's weight gain.

The effect of Biosmoke (BS) on rumen fermentation and methane mitigation

Profile of methane production at various levels of BS was similar to that of BF. The mechanism of methane reduction due to BS might be different from that of BF. The BS is a liquid fraction obtained from the pyrolysis process of cashew nut shell. It has low pH (3.69) and contained acidic compounds and many simple phenolic compounds that came from degradation of lignin, cellulose, hemicellulose (Naim et al. 2012; Saenab et al. 2016). While BF has neutral pH and contained phenolic compounds bonded with C_{15} fatty acids. So it is likely that ability of phenolic compounds contained in BF affects rumen fermentation and methane reduction in different form from that ability of BS. Simple phenolic compounds as reported by Jayanegara et al. (2009) could reduce methane production, as the phenolic compounds can react and destroy the cell wall of methanogenic bacteria, hence inhibit the bacterial growth.

Some rumen bacteria such as cellulolytic bacteria are very sensitive to low pH (acidic) condition. Low rumen pH as resulted by BS inclusion might suppressed the growth or activity of methanogenic and cellulolytic bacteria. This might cause reduction on the methane production, DM, OM and NDF digestibility, but increasing in propionate production (Table 2 and Table 4). The inclusion of BF and BS in the ruminant feed must be in appropriate amount as it is beneficially mitigates methane and increases propionate production, but in the same time also might depress feed digestibility in the rumen.

CONCLUSION

This research concludes that each bioindustrial product of cashew nut shell has beneficial effect on reducing methane but enhancing propionate. Biofat, biochar and biosmoke may have different mechanism on reducing methane and affecting rumen fermentation. Biofat has the highest effect on mitigating methane, followed by biosmoke and biochar.

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