Synergistic Effect of Biofat and Biochar of Cashew Nutshell on Mitigate Methane in the Rumen

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ABSTRAK

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Salah satu cara untuk mengurangi emisi metana adalah dengan menggunakan aditif pakan yang berasal dari ekstrak tumbuhan yang mengandung senyawa metabolik sekunder. Penelitian ini bertujuan untuk mengevaluasi kombinasi biofat (BF) dengan biochar (BC) hasil pengolahan cangkang buah mete sebagai pakan aditif untuk menekan produksi CH_4 dan meningkatkan kinerja rumen secara *in vitro*. Penelitian ini menggunakan rancangan acak blok yang terdiri dari 6 perlakuan dan 4 ulangan. Perlakuan terdiri dari kombinasi biofat (BF) dengan biochar (BC) dalam rasio yang berbeda dan ditambahkan ke substrat sebagai berikut: Kontrol = substrat; BFBC1 = 0%BF: 100%BC; BFBC2 = 25%BF:75%BC; BFBC3 = 50%BF:50%BC; BFBC4 = 75%BF:25%BC; BFBC5 = 100%BF: 0%BC. Variabel yang diukur: produksi gas total dan CH_4, degradasi bahan kering (BK); bahan organic (BO) dan *neutral detergent fiber* (NDF), NH_3 dan konsentrasi *volatile fatty acid* (VFA) parsial. Hasil analisis menunjukkan penambahan kombinasi berbagai level BF dan BC menyebabkan penurunan secara sangat signifikan ($P<0,01$) terhadap produksi CH_4 di dalam rumen. Dibandingkan kontrol, produksi CH_4 turun pada BFB1 sebesar 11,50% BFBC2 36,85%, BFBC3 38,50% , BFBC4 41,84% dan BFBC5 26,07%. Kombinasi sampai pada level BFBC4 tidak meningkatkan kadar NH_3 secara nyata dibanding kontrol, tapi terjadi peningkatan produksi propionat dan total *volatile fatty acid* (VFA) secara signifikan di dalam rumen ($P<0.05$) pada penambahan kombinasi biofat dan biochar dibanding kontrol. Nilai degradasi BK dan BO sama dengan kontrol ($P>0,05$). Dapat disimpulkan bahwa efek sinergitas dari pakan aditif dalam menekan CH_4 dan meningkatkan produk fermentasi rumen adalah kombinasi BF dengan BC dengan rasio 75%BF:25%BC.

Kata Kunci: Cangkang Biji Mete, Biofat, Biochar, Kombinasi, *In Vitro*, Rumen

ABSTRACT

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One way to reduce methane emissions is by using feed additives derived from plant extracts containing secondary metabolic compounds. This study aimed to evaluate the effectiveness of combinations of biofat and biochar (bioindustrial products of cashew nut shells) as feed additive in reducing methane production and improving *in vitro* rumen fermentation. In this experiment, a randomized block design with 6 treatments and 4 replications was applied. The treatments were different combination of biofat (BF) and biochar (BC) as follows: Control= substrate only without addition of biofat or biochar; BFBC1 = 0%BF: 100%BC; BFBC2 = 25%BF:75%BC; BFBC3 = 50%BF:50%BC; BFBC4 = 75%BF:25%BC; BFBC5 = 100%BF: 0%BC. The measured variables were: total gas and CH_4 productions, dry matter (DM); organic matter (OM); and neutral detergent fiber (NDF) ruminal degradabilities, NH_3 and partial volatile fatty acid (VFA) concentrations. Result showed that the addition of combinations of biofat and biochar into the substrates resulted in significant decrease ($P<0.01$) of CH_4 production in the ruminal fluid. Compared to control, CH_4 production was lower by 11.50% (BFBC1), 36.85% (BFBC2), 38.50% (BFBC3), 41.84% (BFBC4) and 26.07% (BFBC5). All combinations except BFBC5 produced similar NH_3 concentration but significantly higher propionate and total VFA concentration in the *in vitro* rumen than control, dry matter degradability and organic matter degradability in the presence of combination of biofat and biochar at different ratios were similar to the control ($P>0.05$). In conclusion, the best combination in producing a synergistic effect as a feed additive to reduce methane, and enhance rumen fermentation products *in vitro* is BFBC4: biofat 75% and biochar 25%.

Key Words: Cashew Nut Shell, Biofat, Biochar, Combination, *In Vitro*, Rumen

INTRODUCTION

Methane is the second largest contributor after CO_2 to greenhouse gases in the atmosphere layer, and it has the capability of heat retention 23 times greater than CO_2. Livestock, especially ruminants, is one of the contributors to the accumulation of anthropogenic methane (about 28%). This is due to the process of methane formation or methanogenesis by archaea methanogen residing in the rumen through the reaction of CO_2 and H_2 to CH_4 (US EPA 2005; Cottle et al. 2011). Based on this, it is necessary to mitigate the emission of methane from ruminant livestock, which is not only related to the aspect of environmental conservation but also as an effort to optimize the productivity of ruminant livestock.

Nutrition strategies that have proven to be effective in methane emission mitigation are through direct inhibition of archea methanogen using ionophore compounds such as monensin (Gerber et al. 2013). However, the use of monensin or other types of antibiotics is constrained by the prohibition on the use of antibiotics as a feed additive in the diet. This led to the exploration of various natural compounds to reduce methane emissions (Jayanegara et al. 2013).

One way to reduce methane emissions is by using feed additives derived from plant extracts containing secondary metabolic compounds such as tannins (Bhatta et al. 2013), saponins (Wina 2012; Yuliana et al. 2014), essential oil (Patra & Yu 2012), and Cashew Nut Shell Liquid (CNSL) (Watanabe et al. 2010). Cashew plants (*Anacardium occidentale* Linn) are explored for their nuts, whereas the nut shells which are 45-50% of cashew fruits have not been utilized well. There are three bioindustrial products that have been developed from processing of cashew nut shell i.e CNSL or biofat, biochar and biosmoke. CNSL or biofat has been produced and used by industry **(**Rodrigues et al 2011), but biochar and biosmoke from cashew nut shell have not yet been reported. CNSL or biofat contain anacardic acid and its derivatives which are phenolic compounds to fatty acids (C_{15} = pentadecanoic acid) and exert antimicrobial activity **(**Gandhi et al. 2012). Biochar and biosmoke are products from pyrolysis process of the remaining shell after the biofat has been extracted.

In the previous experiments, these three products showed their potential activity to reduce methane production in the *in vitro* rumen fermentation (Saenab et al. 2018). However, the result showed that each product especially biofat and biosmoke reduced methane and followed by reduction of total gas and degradability of substrate in the rumen at addition of higher doses. Watanabe et al. (2010) concluded that CNSL was a methane reducing agent, but its effect on other parameters in the rumen has not shown significant results. Therefore, another substance may be used together with biofat to produce synergistic effects on rumen fermentation. Previous results (Saenab et al. 2018) concluded that addition of biofat (0.25 µl/ml), biochar (0.3 mg/ml) each showed reduced methane production without affecting feed degradability. The objective of the study was to evaluate the effect of different combinations of biofat with biochar as feed additive on reducing methane production and on improving rumen fermentation.

MATERIALS AND METHODS

Experimental procedures

The experiment was conducted from January to April 2016, at the Feed Laboratory of the Research Institute of Animal Production (IRIAP) in Bogor. 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 cashew nut shells were obtained from farmers in Pati Regency, Central Java Province.

Bioindustrial products of cashew nut shell (biofat and biochar) were used as feed additive in this experiment. The method of preparing biofat (BF) and biochar (BC).

Cashew nut shells

Shells of cashew nut that have been separated from the nuts were dried under the sun. The dry shells were grinded into smaller particles with a blender and screened to obtain small sized particles (2 mm diameter). Then, the small sized cashew nut shell would be processed into bioindustrial products namely biofat and biochar.

Extraction to obtain biofat product

The prepared cashew nut shells were weighed (100 g) and then put into an erlenmeyer flask and added with 400 ml of hexane. Once submerged, the mixture (sample and solvent) were stirred well and then left on the table for 24 hours. The filtrate was then separated and 200 ml of new hexane was added to the residue. The filtrate was mixed together and was evaporated with a rotary evaporator at 40°C until the remained was dark brown thick oil and called Biofat.

Pyrolysis process to obtain biochar

The shell residue after biofat extraction was air-dried. It was then put into an activation tank (pyrolysis

tank) and tightly closed. Then, the pyrolysis furnace was started. The pyrolysis reaction took place at the pyrolysis reactor worked at 300^{o}C for 8 hours. Pyrolysis tank was connected with a long pipe. The furnace was turned off after 8 hours and left for cooling. Black residue inside the tank called charcoal or Biochar A complete feed for cattle consisted of Grass, *Gliricidia sepium* leaves, yellow corn, coconut cake, molasses, bran, urea, salt (NaCl), limestone ($CaCO_3$), and premix used as a substrate in the *in vitro* rumen fermentation. The CP and TDN of this complete feed was 15.63% and 69.7%, respectively (Saenab et al. 2018).

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).

In vitro rumen fermentation

Treatments were different combinations of biofat (BF, 0.25 µL/mL) and biochar (BC, 0.3 mg/mL) as follows: a) Control (Substrate without any addition of Biofat or Biochar), b) Substrate + BFBC1= 0%BF:100%BC; c) Substrate + BFBC2= 25%BF:75%BC; d) Substrate + BFBC3= 50%BF:50%BC; e) Substrate + BFBC4= 75%BF:25%BC; f) Substrate + BFBC5= 100%BF: 0%BC.

Different combinations biofat and biochar were each added to the substrate. A total of 750 mg of substrate was weighed into the bottle. Rumen buffer solution (75 mL) was added 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 rumen of a fistulated Holstein Friesian cow fed with commercial concentrate and elephant grass. The total gas and methane production were recorded at 3, 6, 9, 12, 24, 30, 36, 48 hours of incubation. At the end of incubation, the supernatant was separated by filtration to obtain residue and supernatant. The residue was dried in the oven 105^{o}C for 24 hours and weighed. Ash content of feed and residue was determined according to method of AOAC (2005) and neutral detergent fiber (NDF) analysis was conducted following Van Soest et al. (1991) method without addition of amylase. The *in vitro* dry matter (DM) and organic matter (OM) of digested fractions were calculated from the dry matter and organic matter of initial sample minus those of residue. The DM or OM of digested fractions divided by the DM or OM of initial sample was calculated as *in vitro* dry matter or organic matter degradabilities. Other residue samples of *in vitro* incubation were digested using Neutral detergent solution to obtain residual NDF fraction. The NDF of digested fractions divided by the NDF of initial sample was calculated as *in vitro* NDF degradability. pH, NH_3 and VFA were measured after 4 hours of incubation. Ammonia content in the supernatant was determined using Conway microdiffusion technique. The supernatant for volatile fatty acid (VFA) analysis was kept in low pH by adding sulphuric acid. Volatile fatty acid products from fermentation was analysed by GC using gas chromatography *(Bruker Scion 436 GC*) with capillary column BR-Wax fame containing wall-coated open tubular (WCOT) used silica with the length of column 30 m x 0.32 mm imange diagnosis (ID). The carrier gas was Nitrogen 25 ml/min and the burning gas was hidrogen 30 ml/min. Injector temperature was 250ºC, while the column temperature gradient was 70 – 150^{o}C in 11 minutes. The detector used was Fingernail Imange Diagnosis (FID) with temperature of 275^{o}C.

Statistical Analysis

This study was done based on a randomized block design (RBD) with 6 treatments and 4 replications. The experimental data from different combinations of biofat and biochar and control (substrate without any addition of biofat or biochar) 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

Methane, total gas and ammonia productions, pH from *in vitro* rumen fermentation with the addition of biofat with biochar combination at different ratios are presented in Table 1.

Compared to control, the addition of biofat and biochar mixtures at different ratios caused a significant decrease ($P<0.01$) in the production of methane (Table 1). The production of methane was reduced by 11.50% (BFBC1), 36.85% (BFBC2), 38.50% (BFBC3), 41.84% (BFBC4) and 26.07% (BFBC5) compared to the control. The higher the level of biofat in the mixed of biofat and biochar, the less is methane produced, but when reaching 100% biofat (BFBC5), methane production significantly increased compared to BFBC 2,3,4. It seemed that there was a quadratic effect which indicates a synergistic effect of combination of biofat with biochar on methane reduction. The lowest methane production was observed at the biofat:biochar composition of 75:25%. Combination of two bioactive compounds to reduce methane production both *in vitro* or *in vivo* systems have been reported (El-Zaiat et al. 2014; Yogianto et al. 2014). El-Zaiat et al. (2014) reported that combination of Cashew Nut Shell Liquid (CNSL) and nitrate decreased methane production. It may be possible that CNSL and nitrate may have each different mechanism in reducing methane production so it synergistically depressed the process production of

Tabel 1. The effect addition of biofat:biochar combination at different ratios on methane (ml), total gas (ml), ammonia production (mm/g DM) and pH value in the *in vitro* rumen

Treatment	Level	Methane (ml)	Total Gas (ml)	NH_3 (mm/g DM)	pH
Control	0 %	45.15±3.42d	182.25± 13.52	7.6± 1.35b	6.70±0.00
1	BFBC1	39.25± 2.60c	144.17±20.01	8.1±0.68bc	6.68±0.01
2	BFBC2	28.47 ±5.94a	191.85±16.73	8.3±0.72bc	6.70±0.02
3	BFBC3	27.77 ±6.41a	194.87±7.09	8.1±0.87bc	6.74±0.01
4	BFBC4	26.25 ±3.27a	200.87±13.59	8.8±1.01bc	6.67±0.01
5	BFBC5	33.32 ±2.44b	190.87±16.97	5.9±0.52a	6.74±0.01

1: Level BFBC 1 (0%BF:100%BC), 2= BFBC2 (25%BF:75%BC), 3= BFBC3 (50%BF:50%BC), 4= BFBC4 (75%BF:25%BC), 5= BFBC5 (100%BF:0 %BC), DM= dry material
Different letters in the same column show significant (P<0.05) or very significant (P <0.01) difference. Statistical analysis of each product was tested separately against the control

Table 2. The effect of biofat/biochar and biofat/biosmoke combination at different ratios on molar proportion of acetate, propionate, butyrate, valerate, branched chain short chain fatty acids (BCVFA) and total volatile fatty acids (VFA) (mm) concentration, and acetate/propionate of feed incubated 48 hours in the *in vitro* rumen fermentation

Treatment	Level	Acetate	Propionate	Butyrate	Valerate	BCVFA	A/P	Total VFA (mm)
		.....mol/100mol.....						
Control	Control	62.06±2.40	20.14±2.20^{a}	10.33±1.75	2.18±0.76	5.27±0.24^{ab}	3.11±0.43^{c}	72.42±3.01^{a}
1	BFBC1	59.63±2.46	22.16±1.63^{ab}	11.73±1.77	1.93±0.41	4.88±1.39^{a}	2.71±0.29^{bc}	84.83±9.78^{ab}
2	BFBC2	55.59±4.33	22.87±2.16^{ab}	12.36±3.09	2.78±0.39	5.56±1.33^{ab}	2.45±0.34^{ab}	87.10±10.53^{b}
3	BFBC3	56.43±2.40	23.79±1.38^{bc}	10.31±1.34	2.89±0.80	6.58±0.69^{bc}	2.38±0.18^{ab}	84.47±5.53^{ab}
4	BFBC4	53.96±6.49	25.90±1.85^{c}	10.68±2.96	2.50±0.81	6.96±1.14^{c}	2.10±0.40^{a}	91.61±7.09^{b}
5	BFBC5	57.96±4.14	23.34±1.67^{bc}	11.86±1.69	1.80±0.35	5.03±0.86^{a}	2.50±0.34^{ab}	79.57±8.18^{ab}

BCVFA= branched chain short chain fatty acids, A/P= Acetate/Propionate ratio. 1: Level BFBC 1 (0%BF:100%BC), 2= BFBC2 (25%BF:75%BC), 3= BFBC3 (50%BF:50%BC), 4= BFBC4 (75%BF:25%BC), 5= BFBC5 (100%BF:0 %BC)
Different letters in the same column show significant (P<0.05) or very significant (P <0.01) difference. Statistical analysis of each product was tested separately against the control.

methane in the rumen. The synergistic effect of the combination of biofat and biochar on methane reduction may be explained as follows. Biofat reduced methane production through the major effect of anacardic acid as the main bioactive compounds in biofat. Anacardic acid is composed of phenolic and unsaturated fatty acids group **(**Lejonklev et al**.** 2013). Both groups were negatively affected rumen microbes as it was reported that phenolic group is able to suppress certain rumen microbes growth (Jayanegara et al. 2011; Hansen et al. 2012) and unsaturated fatty acids are toxic to ruminal microorganism (Maia et al. 2007). Biochar that added together with biofat may reduce methane production through its pores that absorbed gas from fermentation products including methane. Then, methane may be used by methane utilizing bacteria (methanotroph bacteria) which possibly live in the surrounding pores of biochar (Leng et al. 2012a; Leng et al. 2012b). Both substances, biofat and biochar with different mechanisms may work together and synergistically reducing methane in the rumen.

Table 1 shows the addition of different combination of biofat and biochar did not significantly increase (P>0.05) total gas production in the *in vitro* rumen compared to the control. Total gas was produced as the result of feed degradation by rumen microbes activity and it consisted of several gasses with the major CO_2 (about 65%), and methane (26%) followed by nitrogen (7%) and small amount of O_2, H_2 and H_2S (Yang 2017). The unaffected total gas produced in addition of biofat and biochar combination may be due to the absence of these additives affecting the activity and total population of rumen microbes that degrade feed. Even though this experiment did not measure gas composition and mcrA gene related to methanogens but the same material biofat/ CNSL was used by Mitsumori et al. (2014) showed that the addition of CNSL not only reduced methane, but also increased

hydrogen gas. Another experiment done by Shinkai et al. (2012) showed an inhibition activity toward methanogens by decreasing the copy and expression of mcrA in the rumen, hence, methane production was reduced. Therefore, in this experiment, total gas production was not changed but there may be a shift of gas composition due to combination of biofat and biochar.

Eventhough there was an increased on ammonia production at the BFBC1- BFBC4, but the increase was not significantly different from control (Table 1). At BFBC5 (100% of biofat and 0% biochar), however, a very significant decrease in ammonia production ($P<0.01$) occurred compared to other treatments and control. This explains that biofat at the level of 0.25 µL/mL (100% biofat) depressed feed protein degradation in the rumen. The level of ammonia increased at BFBC4 (75:25) was the highest in numeric compared to other treatments. It is interesting to note that 75% biofat in the combination with 25% biochar in BFBC4 did not negatively affect ammonia production, instead BFBC4 enhanced ammonia production. It shows that there is a synergistic effect on ammonia level caused by combination of biofat and biochar. Eventhough some phenolic was reported to decrease ammonia production in the rumen (Jayanegara et al. 2011; Kamra et al. 2012), biochar might be able to entrap ammonia in its pores so that it contributed higher effect on increasing ammonia level in the rumen.

Table 2 shows the combination of biofat and biochar caused a significant increase ($P<0.05$) on propionate and total VFA production in the rumen over the control. The increased propionate was consistent with the previous result (Saenab et al. 2018) when administration of biofat and biochar separately had caused a significant increase ($P<0.05$) on propionate production and total VFA in the rumen. The combination of biofat and biochar may cause a synergistic effect which resulted in a higher increase of propionate and total VFA. Higher increase of propionate was related to reduce methane production. There may be a competition in utilizing hydrogen by propionate producing bacteria and methanogens to form propionate and methane, respectively. A study by Watanabe et al. (2010) showed that population of several propionate producing rumen bacteria (*Selemonas ruminantium*, *Megasphaera elsdenii*,) increased in the presence of CNSL (biofat) while study of (Shinkai et al. 2012) showed that mcrA gene related to methanogens was depressed by the additions of CNSL (biofat). Eventhough the present experiment didnot measure the population of those bacteria and methanogens in the rumen, there may be possible that combination of biofat and biochar changed the composition of rumen bacteria toward higher propionate producing bacteria and lower methanogens resulted in higher propionate and lower methane produced in the rumen fermentation.

Meanwhile, the production of BCVFA (branched chain volatile fatty acid) showed a significant increase ($P<0.05$) only at the combination level of BFBC4 compared to control. BCVFA (isobutyrate and isovalerate) is a product from feed protein degradation in the rumen. This result indicates that addition of combination of biofat and biochar didnot negatively affect the process of protein degradation by rumen microbes. Protein as popypeptides in the feed will be degraded in the rumen by different microbes to become peptide, amino acids (normal chain amino acid and branched chain amino acids) and finally ammonia and branched chain fatty acids. These branched chain fatty acids (BCVFA) came from branched chain amino acids (valine, leucine and isoleucine) that were oxidatively deaminated, but its production depended on the type of protein source and level of inclusion in the diet (Apajalahti et al. 2019). Ammonia and branched chain fatty acids were the indicator for protein degradation, therefore ammonia and branched chain fatty acids were strongly correlated (Apajalahti et al. 2019). This experiment also showed that both ammonia and BCVFA were low concentration in the rumen with the addition of BFBC5 (100% biofat and 0% biochar). Addition of the lowest level biochar mixed with biofat seemed not only to reduce the negative effect of biofat but also give a beneficial effect on protein feed degradation, hence increase ammonia and BCVFA production.

The effect of ruminal dry material digestibility (DMD), organic material digestibility (OMD) and neutral detergent fibre digestibility (NDFD) from *in vitro* fermentation with the addition of biofat:biochar combination at different ratios are presented in Table 3.

Table 3 shows the result of DMD and OMD in the presence of combination of biofat and biochar at different ratios were the similar as the control ($P>0.05$) except at the BFBC4 (25:75%), in which DMD and OMD were slightly higher in numeric than those at other combinations. As it was shown in Table 1 and 2 that ammonia and BCVFA increased at BFBC4 addition, indicated that protein as part of organic matter could be degraded without any inhibition.

The NDF degradation decreased significantly at BFBC1, BFBC2 and BFBC5 compere to control. It seemed that fiber degrading bacteria in the rumen and protozoa were very sensitive to addition of biofat and biochar. It seemed that fiber degrading bacteria in the rumen and protozoa were very sensitive to addition of biofat and biochar. In the semi continous rumen fermentation done by Watanabe et al. (2010) and Oh et al. (2017) found that addition of CNSL/biofat

Tabel 3. The effect of dry material digestibility (ml/g sampel), organic material digestibility (ml/g sampel), and neutral detergent fibre digestibility (ml/g sampel), values of feed incubated 48 hours in the *in vitro* rumen fermentation with addition of biofat : biochar combination at different ratios

Treatment	Level	DMD (ml/g sampel)	OMD (ml/g sampel)	NDFD (ml/g sampel)
Control	0 %	72.15±1.69ab	75.21±0.81ab	54.41±2.58c
1	BFBC1	72.66±3.25ab	76.02±2.63b	41.57±2.33ab
2	BFBC2	73.79±1.36b	76.68±0.35b	42.97±1.57ab
3	BFBC3	74.57±3.39b	77.29±1.79b	46.20±2.98bc
4	BFBC4	75.91±3.13b	78.03±2.43b	46.58±1.53bc
5	BFBC5	69.25±4.44a	71.68±4.76a	38.44±5.85a

1: Level BFBC 1 (0%BF:100%BC), 2= BFBC2 (25%BF:75%BC), 3= BFBC3 (50%BF:50%BC), 4= BFBC4 (75%BF:25%BC), 5= BFBC5 (100%BF:0 %BC), DMD= dry material digestibility, OMD= organic material digestibility, NDFD= neutral detergent fibre digestibility
Different letters in the same column show significant (P<0.05) or very significant (P <0.01) difference. Statistical analysis of each product was tested separately against the control

reduced the population of *Ruminococcus flavefaciens*, *Ruminococcus albus* that represented fiber degrading rumen bacteria and also reduced the protozoa. Although in the present experiment did not observe or count the population of fiber degrading bacteria, it can be assumed that NDF degradation decreased may be due to the inhibition effect of biofat on the growth of those fibrolytic rumen microbes. But NDF degradation at BFBC3 and BFBC4 was not significantly different to control. Eventhough biofat and biochar negatively affected interestingly the combination biofat and biochar at level 50%:BF:50% BC and biofat and biochar at level 75%BF:25% BC could increase NDF degradation similar to that of control.

The phenolic compounds and unsaturated fatty acids in biofat were reported to negatively affect bacteria responsible for feed degradation in the rumen, even when biofat was combined with biochar, the negative effect of phenolic on feed degradation was reduced. This result was in agreement with Al Kindi (2015) who reported that the addition of biochar or activated charcoal together with tannin or phenolic containing leaves would eliminate the reducing effect of phenolic compounds on feed degradation especially fiber degradation.

The use of biofat from cashew nut shell as feed additive reduced methane and increased propionate but combination of biochar with biofat showed more beneficial effect on higher reduction of methane, higher propionate, BCVFA and ammonia production without disturbing feed degradation. It is suggested that the best combination of biofat and biochar of BFBC4 (75% BF:25%BC) obtained from *in vitro* trial should be evaluated as feed additives in the *in vivo* trial to observe the animal responses with different types of feed. Utilizing of biofat and biochar as feed additive, would give more value on cashew nut shell which previously considered as a waste with less value from cashew nut industry. It is also expected that combination of biofat and biochar as feed additive in livestock feeding would improve the environment to cleaner and greener one as it would reduce greenhouse gases.

CONCLUSION

It was concluded that the best combination of biofat and biochar that produced a synergistic effect on enhancing rumen fermentation products and reducing methane production was at the ratio of 75% Biofat:25% Biochar. It is suggested to conduct further studies on evaluating this combination of biofat and biochar as feed additive with different types of feed and study the animal responses (*in vivo* study).

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