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

Saenab A1, Wiryawan KG2, Retnani Y2, Wina E3

1Indonesian Center for Animal Research and Development, Jalan Raya Pajajaran Kav. E59, Bogor 16128, Indonesia
2Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University
3Indonesian Research Institute for Animal Production, P.O. Box 221 Bogor 16002, Indonesia

Email: enabak37@yahoo.co.id

(Received 28-02-2020; revised 17-06-2020; accepted 17-06-2020)

ABSTRACT

Saenab A, Wiryawan KG, Retnani Y, Wina E. 2020. Synergistic effect of biofat and biochar of cashew nutshell to mitigate methane in the rumen. JITV 25(3): 139-146. DOI: http://dx.doi.org/10.14334/jitv.v25i3.2475

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 (biindustrial 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 CH4 production in the ruminal fluid. Compared to control, the CH4 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 NH3 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 CO₂ to greenhouse gases in the atmosphere layer, and it has the capability of heat retention 23 times greater than CO₂. 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 CO₂ and H₂ to CH₄ (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 (C₁₅ - 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°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, *Glicidica sepium* leaves, yellow corn, coconut cake, molasses, bran, urea, salt (NaCl), limestone (CaCO₃), and premix used as a substrate in the *in vitro* rumen fermentation. The CF 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°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°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, NH₃, 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 image diagnosis (ID). The carrier gas was Nitrogen 25 mL/min and the burning gas was Hydrogen 30 mL/min. Injector temperature was 250°C, while the column temperature gradient was 70 – 150°C in 11 minutes. The detector used was Fingernail Image Diagnosis (FID) with temperature of 275°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 *in vitro* or in *in vivo* systems have been reported (El-Zaia et al. 2014; Yogianto et al. 2014). El-Zaia 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
Table 1. The effect addition of biofat/biochar combination at different ratios on methane (ml), total gas (ml), ammonia production (nm/g DM) and pH value in the in vitro rumen

| Treatment | Level | Methane (ml) | Total Gas (ml) | NH₃ (nm/g DM) | pH                |
|-----------|-------|--------------|----------------|---------------|-------------------|
| Control   | 0 %   | 45.15±3.42a  | 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) |
|-----------|-------|---------|------------|----------|----------|-------|-----|----------------|
| Control   | Control | 62.06±2.40 | 20.14±2.20 | 10.33±1.75 | 2.18±0.76 | 5.27±0.24 | 3.11±0.43 | 72.42±3.01 |
| 1         | BFBC1  | 59.63±2.46 | 22.16±1.63 | 11.73±1.77 | 1.93±0.41 | 4.88±1.39 | 2.71±0.29 | 84.83±9.78 |
| 2         | BFBC2  | 55.59±4.33 | 22.87±2.16 | 12.36±3.09 | 2.78±0.39 | 5.56±1.33 | 2.45±0.34 | 87.10±10.53 |
| 3         | BFBC3  | 56.43±2.40 | 23.79±1.38 | 10.31±1.34 | 2.89±0.80 | 6.58±0.69 | 2.38±0.18 | 84.47±5.53 |
| 4         | BFBC4  | 53.96±6.49 | 25.90±1.85 | 10.68±2.96 | 2.50±0.81 | 6.96±1.14 | 2.10±0.40 | 91.61±7.09 |
| 5         | BFBC5  | 57.96±4.14 | 23.34±1.67 | 11.86±1.69 | 1.80±0.35 | 5.03±0.86 | 2.50±0.34 | 79.57±8.18 |

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 CO₂ (about 65%), and methane (26%) followed by nitrogen (7%) and small amount of O₂, H₂ and H₂S (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 mcR gene related to methanegens 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

142
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.

Even though 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. Even though 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 (Seelmonas 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). Even though the present experiment didn’t 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 did not 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 very similar 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 compare 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 continuous rumen fermentation done by Watanabe et al. (2010) and Oh et al. (2017) found that addition of CNSL/biofat
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. Even though 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).

**ACKNOWLEDGEMENT**

The author would like to thank Indonesian Agency for Agricultural Research and Development Ministry of Agriculture for financing this research through the research grant No. 242/KPts/KU.010/I.1/07/2015 dated 9 July 2015.

**REFERENCES**

[AOAC] Association of Official Analytical Chemists. 2005. Official Methods of Analysis. 17th ed. Washington DC (USA): AOAC International.

Al Kindi A. 2015. Influence of quebracho tannin extract and activated charcoal on nutrient intake and digestibility, digesta passage, nitrogen balance, and quality of faecal excreta in goats. Kassel (DE): Kassel University Press.

Apajalahti J, Vienola K, Raatikainen K, Holder V, Moran CA. 2019. Conversion of branched-chain amino acids to...
corresponding isoacids - an in vitro tool for estimating ruminal protein degradability. Front Vet Sci. 6:311.

Bhatta R, Enishi O, Yabumoto Y, Nonaka I, Takusari N, Higuchi K, Tajima K, Takenaka A, Kurihara M. 2013. Methane reduction and energy partitioning in goats fed two concentrations of tannin from Mimosa spp. J Agric Sci. 151:119–128.

Cottle DJ, Nolan J V, Wiedemann SG. 2011. Ruminant enteric methane mitigation: a review. Anim Prod Sci. 51:491–514.

El-Zaaiat HM, Araujo RC, Soltan YA, Morsy AS, Louvandini H, Pires A V, Patino HO, Correa PS, Abdalla AL. 2014. Encapsulated nitrate and cashew nut shell liquid on blood and rumen constituents, methane emission, and growth performance of lambs1,2. J Anim Sci. 92:2214–2224.

Gandhi T, Patel M, Dholakiya BK. 2012. Studies on effect of various solvents on extraction of cashew nut shell liquid (CNSL) and isolation of major phenolic constituents from extracted CNSL. J Nat Prod Plant Resour. 2 :135-142.

Gerber PJ, Hristov AN, Henderson B, Makkar H, Oh J, Lee C, Meinen R, Montes F, Ott T, Firkins J, et al. 2013. Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: a review. Animal. 7:220–233.

Hansen HH, Storm IMLD, Sall AM. 2012. Effect of biochar on in vitro rumen methane production. Acta Agric Scand Sect A - Anim Sci. 62:305–309.

Jayanegara A, Marquardt S, Wina E, Kreuzer M, Leiber F. 2013. In vitro indications for favourable non-additive effects on ruminal methane mitigation between high-phenolic and high-quality forages. Br J Nutr. 109:615–622.

Jayanegara A, Wina E, Soliva CR, Marquardt S, Kreuzer M, Leiber F. 2011. Dependence of forage quality and metanogenic potential of tropical plants on their phenolic fractions as determined by principal component analysis. J Anim Feed Sci Tech.163:231-243

Kamra DN, Pawar M, Singh B. 2012. Effect of Plant Secondary Metabolites on Rumen Methanogens and Methane Emissions by Ruminants. In: Patra A, editor. Diet Phytochem Microbes. Dordrecht: Springer Netherlands; p. 351–370.

Lejonklev J, Storm AC, Larsen MK, Mortensen G, Weisbjerg MR. 2013. Differences in rate of ruminal hydrogenation of C18 fatty acids inclover and ryegrass. J Anim Sci. 7:1607–1613

Leng R, Inthapanya S, Preston T. 2012a. Methane production is reduced in an in vitro incubation when the rumen fluid is taken from cattle that previously received biochar in their diet. Livest Res Rural Dev. 24:211.

Leng R, Preston T, Inthapanya S. 2012b. Biochar reduces enteric methane and improves growth and feed conversion in local “Yellow” cattle fed cassava root chips and fresh cassava foliage. Livest Res Rural Dev. 24:199.

Maia MR, Chaudhary LC, Figueres L, Wallace RJ. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Antonie Van Leeuwenhoek. 91:303–314.

Makkar HPS. 2003. Quantification of Tannins in Tree and Shrub Foliage. Dordrecht: Springer Netherlands.

Mitsumori M, Enishi O, Shinkai T, Higuchi K, Kobayashi Yosuke, Takenaka A, Nagashima K, Mochizuki M, Kobayashi Yasuo. 2014. Effect of cashew nut shell liquid on metabolic hydrogen flow on bovine rumen fermentation. Anim Sci J. 85:227–232.

Oh S, Suzuki Yasuyuki, Hayashi S, Suzuki Yutaka, Koike S, Kobayashi Y. 2017. Potency of cashew nut shell liquid in rumen modulation under different dietary conditions and indication of its surfactant action against rumen bacteria. J Anim Sci Technol. 59:27.

Patra AK, Yu Z. 2012. Effects of Essential Oils on Methane Production and Fermentation by, and Abundance and Diversity of, Rumen Microbial Populations. Appl Environ Microbiol. 78:4271–4280.

Saenab A, KG W, Y R, Wina E. 2018. Manipulation of rumen fermentation by bioindustrial products of cashew nut shell (Anacardium occidentale) to reduce methane and enhance other products. JITV. 23:61–70.

Shinkai T, Enishi O, Mitsumori M, Higuchi K, Kobayashi Y, Takenaka A, Nagashima K, Mochizuki M, Kobayashi Y. 2012. Mitigation of methane production from cattle by feeding cashew nut shell liquid. J Dairy Sci. 95:5308–5316.

Rodrigues FHA, França FCF, Souza JRR, N. M. P. S. Ricardo NMP, Feitosa JPA. 2011. Comparison between physico-chemical properties of the technical Cashew NutShell Liquid (CNSL) and those natural extracted fromsolvent and pressing. Polímeros 21: 156–160

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 74:3583–3597.

US EPA. 2005. Greenhouse Gas Mitigation Potential in U.S. Forestry and Agriculture. EPA 430-R-05-006. Washington DC (USA): United States Environmental Protection Agency.

Watanabe Y, Suzuki R, Koike S, Nagashima K, Mochizuki M, Forster RJ, Kobayashi Y. 2010. In vitro evaluation of cashew nut shell liquid as a methane-inhibiting and propionate-enhancing agent for ruminants. J Dairy Sci. 93:5258–5267.

Wina E. 2012. The use of plant bioactive compounds to mitigate enteric methane in ruminants and its application in Indonesia. Wartazoa. 22:24–34.
Yogianto Y, Sudarman A, Wina E, Jayanegara A. 2014. Supplementation effects of tannin and saponin extracts to diets with different forage to concentrate ratio on in vitro rumen fermentation and methanogenesis. J Indones Trop Anim Agric. 39.

Yuliana P, Laconi E, Wina E, Jayanegara A. 2014. Extraction of tannins and saponins from plant sources and their effects on in vitro methanogenesis and rumen fermentation. J Indones Trop Anim Agric. 39:91–97.