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Abstract. This research aimed to evaluate the use of biochar and liquid smoke as feed additives on gas production and organic matter digestibility of a complete feed in the in vitro rumen fermentation system. Biochar and liquid smoke came from the pyrolysis process of cacao pod husks. Treatments consisted of: control (substrate), biochar BC1 (0.1 mg / ml), BC2 (0.2 mg / ml), BC3 (0.3 mg / ml), BC4 (0.4 mg / ml), liquid smoke LS1 (0.25 ul / ml), LS2 (0.5 ul / ml), LS3 (0.75 ul / ml) and LS4 (1.0 ul / ml). The samples were incubated in vitro with buffered-rumen fluid in four replicates, represented by two incubation units per replicate, and conducted for 48 h at 39oC. Parameters measured in the in vitro evaluation were total gas production and digestibility of organic matter. Data were tested using analysis of variance (ANOVA) and if there was a significant difference at $P<0.05$ then continued with Duncan's multiple range test. The results showed that supplementation of biochar and liquid smoke did not affect gas production. However, the addition of biochar and liquid smoke decreased the digestibility of dry matter and organic matter as compared to a control substrate ($P <0.05$). Furthermore, the addition of biochar and smoke caused a decrease in total protozoa ($p<0.05$). It was concluded that biochar supplementation and liquid smoke as feed additive reduce protozoa without affecting total gas production and gas production rate.

1. Introduction

Utilization of cocoa pod husk as a source of animal feed ingredients in fresh form, however, it also contains antinutrient compounds (lignin, tannins, theobromine) so that their utilization is less than optimal. The high fibre content of 33.19-39.45% causes cocoa pod husk challenging to digest by livestock. Various studies have been carried out to increase the added value of cocoa pod husk in the presence of abundant, inexpensive and renewable bioactive compounds such as dietary fibre, pectin, antioxidant compounds, minerals and theobromine [1].

Utilization of cocoa pod husk will be useful if it is processed into bio-industrial products. There are 2 (two) bio-industrial products that can be developed from cocoa pod husk processing, namely biochar and liquid smoke. Biochar has been known as a good adsorption material because it is easily connected to various types of molecules. In addition, biochar is very effective in removing bacterial toxins in in

vitro studies [2]. Biochar can eliminate toxins from *E. coli.* The use of biochar in livestock as feed supplements to improve animal health, feed efficiency and livestock productivity. Biochar has been shown to have a positive effect on the parameters of toxin adsorption, digestion, blood, feed efficiency, meat quality and/or greenhouse gas emissions [3]. Biochar from nut cashew shells tested for ruminants in vitro reported by Saenab et al was able to mitigate methane by 24.21% and increase propionate, but did not affect feed degradation [4].

Wood vinegar/pyroligneous acid/liquid smoke/bio-oil is a liquid product from the wood pyrolysis process and consists of a complex mixture of water, phenol, guaiacol, vanillin, catechol, syringol, carboxaldehyde furans, isoeugenol, pyrone, acetic acid, formic acid and other carboxylic acids [5]. Liquid smoke with a pH of 2.5-2.8 can function as an insecticide, fungicide, bactericide, and deodorant to treat foul odors from pets [6]. Several studies have shown the usefulness of phenolic compounds contained in liquid smoke derived from lignin useful as an antimicrobial agent (suppressing bacterial growth) [7]. Research on the use of liquid smoke in ruminants is still limited. But, the use of bamboo vinegar as an animal feed additive is a new concept in poultry [8,9]. In addition, a mixture of biochar and liquid smoke can improve intestinal villi function, so that nutrient absorption is better [10]. This research aimed to evaluate the use of biochar and liquid smoke as feed additives on gas production and organic matter digestibility in an *in vitro* rumen fermentation system.

2. Methods

The biochar and liquid smoke were obtained from the Laboratory of Forest Products Research and Development Center, Bogor, West Java. The basal feed consists of pollard and forage. Forage was chopped and dried at 60°C for 2–3 days (until moisture content reached 12%), then ground and sieved into 1 mm particle size.

2.1. Treatment

The in vitro incubation consisted of 9 treatments with four replications by following a randomized block design and analyzed separately between biochar and liquid smoke for control. Treatments consisted of: control (substrate), biochar BC1 (0.1 mg / ml), BC2 (0.2 mg / ml), BC3 (0.3 mg / ml), BC4 (0.4 mg / ml), liquid smoke LS1 (0.25 ul / ml), LS2 (0.5 ul / ml), LS3 (0.75 ul / ml) and LS4 (1 ul / ml).

2.2. Incubation in vitro

The samples were further incubated *in vitro* with buffered-rumen fluid in four replicates, represented by two incubation units per replicate, and conducted for 48 h at 39ºC. Gas production was recorded after incubation of 2, 4, 6, 8, 10, 12, 24, dan 48 h. Gas production kinetics was calculated following the equation $p = a + b\ (1 - e^{-ct})$ [11], (a+b)= theoretical maximum of gas production; c= gas production rate. The dry matter and residual samples after 48 h incubation were used to estimate dry matter digestibility (DMD) and organic matter digestibility (OMD) of feed.

2.3. Parameters measured

Parameters measured in the *in vitro* evaluation were total gas production, dry matter digestibility (DMD), and organic matter digestibility (OMD) [12]. Other parameters measured after the incubation were total protozoa. The *in vitro* procedure was performed according to Theodorou and Brooks [13]. Data were tested using analysis of variance (ANOVA) and if there was a significant difference at $P<0.05$ then continued with Duncan's multiple range test.

3. Results and discussion

3.1. Gas production

Data on gas production after 24 hours and 48 hours of incubation can be seen in Table 1. Total gas production during 24 hours and 48 hours of incubation ranged from 74.8 ml to 113.9 ml. Increasing the level of biochar addition causes a decrease in total gas production to the level of 0.4 mg/ml (BC4). The

highest gas production during 48 hours of incubation time was produced by BC1 (0.1 mg/ml) which was 111.7 ml, while the lowest gas production was produced by control treatment which was 107.1 ml. Increasing the level of addition of liquid smoke causes a decrease in total gas production to the level of 1.00 ul/ml (LS4). Gas production during 48 hours of incubation time was produced by LS2 (0.5 ul/ml) which was 113.9 ml, while the lowest gas production was produced by control treatment which was 74.9 ml. The addition of biochar and liquid smoke decreased gas production compared to controls but it was not significantly different. Not only to total gas production, maximum gas production and gas production rate were also not significantly different amoung treatments. This result was in agreement with the results reported by Saenab et al., using biochar and biosmoke (liquid smoke) from cashew nut shell [4]. Although the pH of biochar and liquid smoke was 10 and 3.75, respectively, they did not cause any negative effect on total gas production as the buffer solution added to the rumen liquor could maintain the pH of rumen fermentation solution.

Table 1. Total gas production at 24 h and 48 h, a+b and c Constanta of the biochar and liquid smoke treatment.

Treatments	Level	24 h (ml)	48 h (ml)	a + b (ml)	c (mlh^{-1})
Biochar (mg/ml)					
Control	0	74.9±13.3	107.1±11.4	124.6±13.1	0.043±0.01
BC1	0.1	78.6±10.9	111.7±8.3	127.8±14.0	0.044±0.01
BC2	0.2	77.2±11.9	109.5±9.5	125.3±12.4	0.045±0.01
BC3	0.3	76.4±7.8	108.5±5.7	122.6±8.7	0.045±0.01
BC4	0.4	75.9±12.8	108.5±10.5	124.3±9.8	0.044±0.01
SE		5.683	8.317	10.236	4.96E-006
P-Value		0.286	0.287	0.277	0.625
Liquid smoke (ul/ml)					
Control	0	74.9±13.3	107.1±11.4	124.6±13.1	0.043±0.01
LS1	0.25	80.9±14.2	111.2±13.2	122.4±14.9	0.051±0.02
LS2	0.5	83.4±12.0	113.9±11.7	124.1±16.7	0.053±0.02
LS3	0.75	78.5±6.0	111.9±7.1	129.1±22.5	0.044±0.01
LS4	1	78.0±11.5	110.7±8.5	122.5±20.4	0.046±0.01
SE		44.369	34.669	42.773	4.00E-005
P-Value		0.484	0.608	0.614	0.154

Note: (a+b) = theoretical maximum of gas production; c= gas production rate.
Statistical analysis of each product is tested separately for control

3.2. Dry matter digestibility, organic matter digestibility and total protozoa

Dry matter digestibility, organic matter digestibility and total protozoa presented in Table 2. The analysis showed that the addition of biochar showed a significant reduction of DMD values compared to controls ($p < 0.005$) starting from BC2, BC3 and BC4, however, BC1 level did not significantly reduce DMD compared to controls (BC0). Biochar levels of 1-4% of dry feed ingredients also did not have a negative effect on rumen fermentation both in total gas production and gas production rate. Liquid smoke addition on the other hand did not affect DMD or OMD. This is supported by Saenab et al who described that the addition of liquid smoke from cashew nut shell did not affect any digestibility and gas production [4].

The content of total phenol in liquid smoke in this experiment was 3.12%, not only did not have a negative effect on total gas production and gas production rate, it also did not affect DMD and OMD. Jayanegara reported that phenols at concentrations of more than 5% depressed livestock performance [14], so their use in rations needed to be low level. Furthermore, phenolic compounds had ability to interact with proteins that present in the cell wall or membrane and various enzymes of pathogenic microbes, hence, destroy microbes [15].

Table 2. Dry matter digestibility (DMD), organic matter digestibility (OMD) and total protozoa of the biochar and liquid smoke treatment.

Treatments	Level	DMD (%)	OMD (%)	Total protozoa, cellml^{-1} (log)
Biochar (mg/ml)				
Control	0	46.4^{a}±2.1	45.5^{a}±2.05	6.8^{a}±0.09
BC1	0.1	45.6^{a}±0.9	43.9^{b}±2.00	6.7^{a}±0.13
BC2	0.2	44.0^{b}±1.3	42.3^{c}±1.63	6.6^{a}±0.11
BC3	0.3	42.6^{c}±1.1	40.2^{d}±1.20	6.5^{b}±0.13
BC4	0.4	41.1^{d}±0.7	37.9^{e}±0.78	6.3^{c}±0.07
SE		0.866	0.825	0.005
P-Value		0.000	0.000	0.000
Liquid smoke (ul/ml)				
Control	0	46.4±2.1	45.5±2.05	6.8^{a}±0.09
LS1	0.25	46.2±1.4	45.5±1.53	6.7^{b}±0.11
LS2	0.5	45.8±0.9	44.9±1.03	6.6^{c}±0.09
LS3	0.75	45.7±2.4	44.7±2.47	6.5^{d}±0.11
LS4	1	46.5±1.5	45.6±1.68	6.4^{e}±0.11
SE		0.797	0.697	0.001
P-Value		0.675	0.485	0.000

Note : DMD = dry matter digestibility, OMD = organic matter digestibility.
Different superscripts in the same column show a significant difference at P<0.05. Statistical analysis of each product is tested separately for control.

Table 2 illustrated the effect of biochar addition and liquid smoke on protozoa count. Increasing the level of biochar and liquid smoke addition both caused a decrease of protozoa count (about 6-7%). This decrease in the number of protozoa causes a decrease in DMD and OMD [16]. Protozoa have a role in the rumen fermentation process because they have the ability to degrade the main components of the feed. One of the ciliated protozoa that have an important role in the rumen is the Diploplastron affine that are common in livestock and have the ability to digest cellulose and carbohydrates from grain [17]. Furthermore, holotrich protozoa, although in small amounts, also have enzymes that are responsible for cellulose and hemicellulose degradation. Mosoni et al the decline in protozoa populations also negatively impacts the digestion of fiber which is the main function of the rumen [18].

4. Conclusions

It is concluded that the bio industrial product of cocoa pod husk (biochar and liquid smoke) had the potency as feed additives in rumen. Biochar supplementation and liquid smoke as feed additive reduce protozoa without affecting total gas production and gas production rate.

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References

[1] Iflah T, Tarigan E B, and Pranowo D 2014 *Bunga Rampai: Inovasi Teknologi Bioindustri Kakao, ed* Rubiyo, Harni R, Martono B, Wardiana E, Izzah N K, Hasibuan A M (Jakarta : IAARD Press) pp 155-166
[2] Du X N, Niu Z, Zhou G Z, and Li Z M 1987 *Biomater. Artif. Cells. Artif Organs*. **15** 229-235
[3] Schmidt H P, Hagemann N, Draper K, and Kammann C 2019 The use of biochar in animal feeding

PeerJ. 7 e7373
[4] Saenab A, Wiryawan K, Retnani Y, and Wina E 2018 *JITV*. **23** 61-70
[5] Mohan D, Pittman C U, and Steele P H 2006 *Energy Fuels* **20** 848-889
[6] Akakabe Y, Tamura Y, Iwamoto S, Takabayashi M, and Nyuugaku T 2006 *Biosci Biotechnol Biochem.* **70** 2797-2799
[7] Beker S A, Machado M E, Maciel G P S, Silva R, Cataluña R, Caramão E B, and Bento F M 2016 *J. Braz. Chem. Soc.* **27** 91-98.
[8] Ruttanavut J, Yamauchi K, Goto H, and Erikawa T 2009 *Int. J. Poult. Sci.* **8** 229-236
[9] Yamauchi K, Ruttanavut J, and Takenoyama S 2010 *J. Anim. Feed Sci.***19** 257-268
[10] Qomariyah N, Retnani Y, Jayanegara A, Wina E, and Permana I G 2019 *Wartazoa* **29** 171-182
[11] Orskov E R and McDonald 1979 *J. Agric. Sci. Cambridge.* **92** 499-503
[12] Association of Official Analytical Chemists 2005 *Official Methods of Analysis* (Washington DC: AOAC International)
[13] Theodorou M and Brooks A 1990 *Evaluation of A New Laboratory Procedure for Estimating the Fermentation Kinetics of Tropical Feeds. Annual Report* (Meidenhead: AFRC Inst)
[14] Jayanegara A 2009 *Trop. Anim. Sci. J.* **32** 53-62
[15] Kondo M, Hirano Y, Ikai N, Kita K, Jayanegara A, and Yokota H-O 2014 *Asian-Australasian J. Anim. Sci.* **27** 1571-1576
[16] Newbold C J, Fuente G D L, Belanche A, Morales E R, and McEwan N R 2015 *Front. Microbiol.* **6** 1-14
[17] Wereszka K and Michałowski T 2012 *Folia Microbiol (Praha)* **57** 375-377
[18] Mosoni P, Martin C, Forano E, and Morgavi D P 2011 *J. Anim. Sci.* **89** 783791