Effect of banana and mango waste product as malic acid source on methane gas production

W S Saputro, C Hanim, L M Yusiati, Z Bachruddin and A Pertiwiningrum

Faculty of Animal Science, University of Gadjah Mada, Jl. Fauna No. 3 Bulaksumur, Yogyakarta 55281, Indonesia

Corresponding author: c.hanim@ugm.ac.id

Abstract. The research aimed to determine the effect of banana (Musa acuminata cavendish subgroup) and mango (Mangifera indica, var. Gedong) waste product on methane gas production. Gas production test was used in this research. The fruits waste products were added based on tannin content that consist of 0, 1, 2, 3% concentration. A completely randomized design (CRD) with factorial pattern (2x4) were used and designed based on the type of fruit waste and used concentrations. The result showed that waste product from banana and Mango could reduce on methane gas production (P<0.01) at the concentration 2%. Number of protozoa and digestibility rate did not show significant effect (P>0.05), but reduced pH value at the level 1%. It can be concluded that waste product from banana and mango that contains secondary metabolites can reduce in vitro methane gas production without affecting on digestibility.

1. Introduction
The process of methane gas formation in the rumen causes loss of energy from food by 2 - 12% [1]. This energy should be used for the synthesis of propionic acid. The negative impact of the synthesis of methane gas is not only detrimental to the livestock but also to the environment. The negative impact of Greenhouse Gas (GHG) needs to be taken seriously to reduce the worst risks that might occur. An alternative that can be done to reduce the amount of methane gas formation is by manipulating fermentation in the rumen using components of secondary metabolites which can be found in plants.

Secondary metabolites are classified based on their biosynthetic pathways: phenolic compounds, terpenes and steroids, and alkaloids [2]. These secondary metabolites have function as anti-microbial, anti-methanogenic and antiprotozoal which can affect the fermentation process in the rumen. The amount of these components is very small and they are not continuously produced. Some types of secondary metabolites are commonly used to inhibit the process of methane gas formation in the rumen, e.g. tannins, saponins and organic acids (i.e., malic acid and fumaric acid). Secondary metabolites can be found in a plant or fruit and its waste are potentially used as the source of secondary metabolites.

Malic acid (MA) (C_4H_6O_5) is one of dicarboxylic organic acids that have acidic taste. It is commonly used to modify rumen condition. Organic acid is a precursor to the formation of propionate in the rumen succinate-propionate cycle in the rumen metabolic process. Furthermore, organic acid also acts by capturing H_2 in the rumen, so that it has the potential to reduce the number of methanogenic bacteria [3]. Tanin is a polyphenol compound derived from plants that has ability to
bind the protein. This ability will reduce the production of methane gas by inhibit the usage of nitrogen for development of microbe, especially methane producer[4]. Saponin is a triterpenic compound or steroid glycoside, a secondary metabolite that is commonly found in most plants. It has anti-methanogen and anti-protozoa effects which can reduce methane gas production [2]. In addition, the steroid in saponins can destroy the membrane cell of protozoa, and eventually will decrease methane gas production as protozoa has symbiotic relation which support methanogens in producing methane gas [4].

This study aimed to determine the effect of the addition of secondary metabolites from agricultural waste especially from banana and mango on in vitro methane gas production.

2. Material and methods

2.1. Materials

The materials were banana powder that contains 91.50% dry matter (DM), 94.92% organic matter (OM), 3.10% crude protein (CP), 1.66% extract ether (EE) and 1.49% crude fibre (CF) and mango powder which contains 92.93% DM, 97.13% OM, 2.13% CP, 2.49% EE and 7.82% CF. Substrates that were used in this study consist of rice bran with 92.07% DM, 83.14% OM, 9.69% CP, 6.87% EE and 17.77% CF and king grass which contains 93.49% DM, 85.75% OM, 12.62% CP, 2.22% EE and 27.34% CF. The secondary metabolite compounds contained in banana powder are 0.30% MA, 6.00% tannin; and 6.22% saponin and in mango powder are 0.08% MA, 7.20% tannin and 7.52% saponin. In vitro gas production conducted based on [5]. As much as 300 mg of DM were added to fruit powder. The addition was carried out in the amount of 0, 1, 2 and 3% of the substrate dry matter. This concentration based on the tannin content on the fruit powder. Rice bran and king grass were used as substrate with concentration 40:60% respectively. The rumen liquid then collected from fistulated Bali cattle aged approximately 3 years old which had been adapted to the feed.

2.2. Methods

Banana and mango waste were obtained from traditional markets and fruit distributors. Fruits wastes were dried out using an oven at 55° C for 72 hours. The dried fruit wastes were then grinded into powder.

2.2.1. Methane gas production. Gas production observation was carried out at 0, 1, 2, 4, 6, 8, 12, 18, 24, 36 and 48 hours after incubation. Sample for measurement of methane gas production was taken at 12th and 48th hours. Samples were collected using 10 ml vennject plain tubes with a total amount of 10 ml of gas. Then the measurement of methane concentration was carried out using Portable Micro GC CP 4900 Varian, Inc.’s. Thermal Conductivity Detector (TCD) was used as the detector and gas carrier using Helium. The production of methane gas was calculated by multiplying the CH₄ concentration with total gas production.

2.2.2. Acidity (pH). Samples from the 48-hour incubation were taken for pH testing using a pH meter (HI 2210, Hanna Instruments).

2.2.3. Protozoa. Preparation and calculation of the number of protozoa were conducted based on [6].

2.2.4. Statistic. Completely randomized design (CRD) with factorial pattern (2x4) was used in this study that consist of groups based on type of waste product from fruits and the concentration of secondary metabolites. The collected data were tested to analyze the variance (ANOVA). The significant results were tested using Duncan Multiple Range Test (DMRT) to compare treatment means. Data were analyzed using IBM SPSS Statistics 21 software.
Table 1. The average production of methane gas (CH$_4$), acidity (pH) level, and number of protozoa (means ± SD).

| Fruit Type | Level (%) | Average |
|------------|-----------|---------|
| CH$_4$ Production / DOM (ml/300 mg DM) | 0 | 1 | 2 | 3 | |
| Banana | 0.040 ± 0.00 | 0.042 ± 0.00 | 0.031 ± 0.00 | 0.033 ± 0.00 | 0.037 ± 0.01$^{ms}$ |
| Mango | 0.040 ± 0.00 | 0.036 ± 0.01 | 0.032 ± 0.00 | 0.030 ± 0.00 | 0.035 ± 0.01$^{ms}$ |
| Average | 0.040 ± 0.00$^b$ | 0.039 ± 0.01$^b$ | 0.032 ± 0.00$^a$ | 0.032 ± 0.00$^a$ | |
| pH | | | | | |
| Banana | 7.05 ± 0.01 | 6.98 ± 0.01 | 6.89 ± 0.03 | 6.83 ± 0.04 | 6.94 ± 0.09$^x$ |
| Mango | 7.05 ± 0.01 | 6.94 ± 0.01 | 6.86 ± 0.02 | 6.79 ± 0.02 | 6.91 ± 0.10$^x$ |
| Average | 7.05 ± 0.01$^d$ | 6.96 ± 0.02$^c$ | 6.88 ± 0.03$^b$ | 6.81 ± 0.04$^a$ | |
| Protozoa (10$^4$ cell/ml) | | | | | |
| Banana | 6.70 ± 1.56 | 6.14 ± 2.03 | 5.97 ± 1.63 | 5.77 ± 0.99 | 6.14 ± 1.53$^{ms}$ |
| Mango | 6.70 ± 1.56 | 5.39 ± 0.81 | 5.31 ± 0.67 | 5.26 ± 0.18 | 5.66 ± 1.07$^{ms}$ |
| Average$^{ms}$ | 6.70 ± 1.49 | 5.77 ± 1.53 | 5.64 ± 1.24 | 5.51 ± 0.73 | |

$^{a,b,c,d}$ Different superscripts on the same row show significant differences (P <0.01)

$^{x,y}$ Different superscripts in the same column show significant differences (P <0.01)

$^{ms}$ Different superscripts in the same row or column do not show significant differences (P >0.05)

DOM : digested organic matter

3. Results and discussion

3.1. Methane gas production

Total methane gas production by digestible organic matter showed significant difference (P <0.01) in between groups. The result showed lower methane gas production when the level of tannin increase. Addition 1% of tannin showed higher production of methane gas compare to 2% and 3% groups. This result might be altered by higher number of methanogen bacteria that survived in 1% concentration. Thus, methanogen bacteria still had ability to growth and produced methane gas in this concentration. The mechanism of reducing methane gas production might be altered by tannins and saponins found in fruits. Tannin and saponin were toxic for protozoa; it was clear that higher levels of tannins caused decreasing protozoa concentration even though it was not significant. Protozoa was attached by methanogen bacteria so that lower concentration of protozoa also reduced methanogen bacteria [2].

Other mechanism is related to malic acid which had functions as the hydrogen electron receptor. On the glycolysis, glucose is degraded to pyruvate which then will be degradedoxaloacetate and hydrogenated to be malate which then becomes fumarate. The fumarate is hydrogenated to be succinate which will become propionate [7]. The addition of malic acid caused hydrogen needs in the process of hydrogenating fumarate to succinate increase, because of this activity, there is competition between methanogen bacteria and malic acid in the process of succinate formation into propionate [7]. The different types of fruit in this study did not show a significant effect on the methane gas production. It possibly due to both fruits has similarity on the chemical composition.

3.2. pH

In this study, pH showed significant difference (P <0.01) between each concentration and also between each type of fruits. The higher concentration of fruits followed by more acid pH. Factors that affected rumen pH consist of physical properties, chemical component types and composition of consumed feed. The difference pH was possibly due to differences in the composition of the treated feed. Higher concentration of fruit waste could alter the composition of feed. Furthermore, decreasing acidity level may be related to the decreasing protozoa concentration, even though the difference of protozoa concentration between groups was not significant. Lower concentration of protozoa can be
alters the acidity level as it plays a role as hydrogen producer that is important in stabilizing rumen pH [2].

3.3. Protozoa
The number of protozoa did not show a significant difference in between treatment groups (P> 0.05). However, descriptively, the higher concentration of fruit waste reduced the number of protozoa. A decrease of protozoa might be caused by the influence of tannins and saponins from the fruit. Saponins can reduce the number of protozoa as it is toxic to protozoa. Cholesterol compounds in saponins have effect on damaging the membrane of protozoa cells, especially eukaryotic membranes [4]. In addition, tannins are influential because they are phenol compounds that can be toxic to protozoa, especially ciliated protozoa [4].

4. Conclusion
Utilization of waste product from banana and mango which containing secondary metabolites can reduce in vitro methane gas production. However, to be applied in vivo it is necessary to conduct the further study of the optimum dosage so it will not cause negative impact on animals.

References
[1] Makoto M and W and Sun 2008 Asian-Australasian J. Anim. Sci. 21 pp 144–54
[2] Calabrò S 2015 Rumen Microbiology: From Evolution to Revolution ed Puniya, A K, R Singh and D N Kamra (New Delhi: Springer India) Rumen Mani pp 153–9
[3] Foley P A, Kenny D A, Callan J J B T M and O F 2009 J. Anim. Sci. 87 pp 1048–57
[4] Yusiati L M 2011 Global warming, methan dan peternakan (Yogyakarta: Universitas Gadjah Mada)
[5] Menke B Y K H, Raab L S A and S H 1979 J. Agric. Sci. 93 pp 217–22
[6] Diaz A A M and E A 1993 Res. Rural Dev. 5 pp 1–6
[7] Carro M D and Ungerfeld E M 2015 Rumen Microbiology: From Evolution to Revolution ed Puniya, A K, R Singh and D N Kamra (New Delhi: Springer India) Rumen Mani pp 177–97