Effect of oil palm (*Elaeis Guineensis* JACQ.) leaves methanolic extract on *in vitro* methanogenesis and gas production in the diets of goats

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Abstract

The effects that are implemented by OPLE on the *in vitro* production of methane, total gas and gas production rate were determined. During this phase, the determination of the methane production and gas (*in vitro*) was conducted. The diets (experimental) where Ctrl, T1 and T2 (0, 300 and 600 mg OPLE \ kg DM diet). In this study, a noticeable lessening in the production of methane and gas (*in vitro*) in OPLE groups for treatment was observed and analogized to a group whose feed was not complemented with OPLE, known as the control group. Total gas production was significantly ($P<0.05$) reduced by the experimental OPLE treatments which were for Ctrl, T1 and T2, 41.58, 38.67 and 38.27 (mL/250mg DM), respectively. And the gas production rate for Ctrl, T1 and T2 was 1.73, 1.61 and 1.59 (mL/hour of incubation), respectively, which are showing a significant ($P<0.05$) reduction in OPLE treatment groups. Furthermore, OPLE supplement suppressed methane production after 24 hours of incubation and the effect was significant ($P<0.05$).

Keywords: oil palm; methane emission, greenhouse, biohydrogenation, plant secondary metabolites.

تأثير مستخلص اوراق نخيل الزيت (*Elaeis Guineensis* JACQ.) في انتاج الميثان والغازات مختبرياً في عليقة الماعز

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الخلاصة

تم تقدير تأثير مستخلص اوراق نخيل الزيت OPLE في انتاج الميثان ، انتاج الغاز الكلي ومعدل انتاج الغاز مختبرياً . علائق الاختبار التي تم استعمالها كانت عليقة سيطرة ، عليقة T1 و عليقة T2 (0 ، 300 و 600 ملغم OPLE / كغم مادة جافة من العليقة) على التوالي . وقد لوحظ انخفاظ واضح في انتاج الغاز الكلي ومعدل انتاج الغاز اضافة الى انتاج الميثان عند اجراء الاختبار للهضم المختبري بالنسبة لعلائق المعاملة بالمقارنة مع عليقة السيطرة . حيث انخفظ انتاج الغاز الكلي معنويا ($P<0.05$) في علائق المعاملة بالمقارنة مع عليقة السيطرة فكانت القيم بالنسبة لعليقة السيطرة ، T1 و T2 هي 41.58 ، 38.67 و 38.27 (mL/250mg DM) على التوالي . كما حصل انخفاض معنوي ($P<0.05$) في معدل انتاج الغاز في علائق المعاملة بالمقارنة مع عليقة السيطرة وقد سجلت عليقة السيطرة ، T1 و T2 1.73 ، 1.61 و 1.59 (mL / ساعة) على التوالي . علاوة على ذلك ، فقد كان لاستعمال مستخلص اوراق نخيل الزيت في الهضم المختبري تأثير معنوي ($P<0.05$) في انتاج غاز الميثان بالنسبة لعلائق المعاملة بالمقارنة مع عليقة السيطرة .

كلمات مفتاحية : نخيل الزيت ، انتاج الميثان ، الاحتباس الحراري ، الهدرجة الحيوية ، منتجات النباتات الثانوية .

Introduction

Along with global warming, changes of climate is very important matter with a negative outcomes and effects on livestock generation and the environment. The production of livestock with its by-products responsible of (51%) of global-warming gasses with methane comprising 15-20 % of the overall percentage (Wanapat *et al.*, 2013). The contribution of methane in heat-trapping is 23 times more than the CO2, causes 2 – 12 % dietary gross energy losing in ruminants (Bhatta et al., 2013). So, the mitigation of the produced methane from ruminants, was considered to be a pivotal goal, because it decreases greenhouse gasses (GHG) emanation and progresses the efficiency of feed. In anaerobic fermentation, enteric methane is by-product of the rumen. It is developed by the archaea of methanogenic for discharging of metabolic-hydrogen that produced by the metabolic activity (Toral *et al.*, 2011). A requirement for recognizing feed additive to alter the fermentation of rumen to enhance the utilization of efficiency of feed energy with the decrease of rumen methanogenesis is mandatory (Bhatta et al., 2013).

The bioactive compounds that existed in plants secondary metabolites (PSM), are effective in the reduction of the methanogenesis and the biohydrogenation (BH) in the rumen (Jayanegara, *et al.*, 2011). These compounds and the plant extracts like saponins, tannins and essential oils and flavonoids have shown to develop the metabolism of the rumen, reducing the methanogenesis, and protein degradation, inducing microbial protein production, and protein flow towards duodenum, affecting particular rumen groups(Patra and Saxena, 2011). Plant extracts, because of their antimicrobial properties, have also been used for centuries for many purposes as preservatives of food and traditional medicine (Busquet et al., 2006). The supplementation of tannin to animal feed seems to be capable to curb the BH of unsaturated FA (*in vitro*) without causing any negative response in ruminal fermentation (Carreño et al., 2015)

With antimicrobial activities, plants showcase a vital natural source of compounds, among them, tannins, saponins, organo-sulfur compounds ,and essential oils, which are existing in the plant,

in sectors of plants or in their extracts(da Silva et al., 2016; Durmic and Blache, 2012; Flachowsky and Lebzien, 2012). The usual and practical technique for the purification of secondary metabolites from a plant source is extraction with solvents (Santos-Buelga et al., 2012). Plants phenolic extracts were tested in *in vitro* studies to evaluate its ability to alert rumen fermentation and mitigate gas production (Aiman-Zakaria, et al. 2018; Jafari, 2015).

The tropical plants that usually hold medium or high content of plant secondary compounds with antimicrobial activity, possess capacity in reducing BH of PUFA of ruminants (Miri *et al.,* 2015; Wanapat *et al.*, 2013).

Considering the role of methane emission in GHG, some researches have been carried out on mitigating methane emission (Piluzza et al., 2014), but there is a need to evaluate the activity of oil palm leaves extract (OPLE) on rumen methane emission. Therefore, the present study was accomplished to evaluate the effect of OPLE on *in vitro* gas and methane production.

Materials and Methods

Feed preparation and experimental diets

Fresh OPF, which used in this experiment, were harvested from the fields of MARDI (Malaysian Agricultural Research and Development Institute) which located in (Serdang, Selangor, Malaysia (3°00′18.88″N, 101°42′15.05″E)). The leaves were chopped into (2-3 cm) and transferred to Ladang II farm located in UPM, Serdang, Selangor, Malaysia. According to Lee *et al*., (2009), the chopped leaves were flooded in 10 vol (v/w) of 80% methanol and left for about 72 hour and the mixture was shaken every 3 hours then ,methanolic oil palm leaf extract was collected, then passed through a 10mm sieve, and stored in a 20-liter bottle. The methanolic extract was vacuum evaporated (Heidolph, Germany) and then dried -completely- by using a freeze drier and stored.

Feeding diets – used in *in vitro* trial- comprised 50% of goat commercial concentrate plus 50% of alfalfa hay (w/w) (Table.1). The experimental treatments designed according to (Anyanji *et al*.,

2013; Beauchemin *et al*., 2007) were contained 0, 300 and 600 mg OPLE/ kg DM of diet in control (Ctrl), treatment 1 (T1) and treatment 2 (T2) group consequently.

Animal Management

Six Kacang crossbred male goats -weighing 40.39 ± 0.74 Kg- were rumen fistulated (Bar-Diamond, Parma, ID, USA) that fed -a diet comprising 50% alfalfa hay and 50% goat concentrate (DM basis)- twice daily at 08:00 and 17:00 h , were used for *in vitro* study. The animals were housed individually in cages. Water and mineral blocks were available at all the times.

Animal Welfare

The study undertaken following the Research Policy guidelines of the University of Putra Malaysia, on animal ethics ref: UPM/IACUC/AUP-4040/2015.

Table 1. Ingredients (%) and chemical composition of the diet fed to goats

Ingredients	% (w/w)
Alfalfa hay	50.00
Corn, grain	20.05
Soybean meal	16.64
Palm kernel cake	5.00
Sunflower oil	3.96
Mineral premix	0.50
Vitamin premix	0.50
Ammonium chloride	1.00
Limestone	1.00
Chemical composition (g/kg DM)	
ME (Kcal/Kg)	2.45
DM	850.20
CP	120.00
EE	64.44
NDF	431.00
ADF	287.00
SFA	27.18
MUFA	20.45
n-3 PUFA	13.63
n-6 PUFA	33.92
n-6: n-3	5.27

ME = metabolizable energy ,DM=dry matter ,CP=crude protein ,EE=ether extract ,NDF =neutral detergent fiber ,ADF =acid detergent fiber ,SFA= sum of saturated fatty acids(C14:0 + C16:0 + C18:0); MUFA= sum of monounsaturated fatty acids(C14:1+ C16:1n-7+ C18:1n-9), n-3 PUFA= sum of C18:3n-3 polyunsaturated fatty acids, n-6 PUFA =sum of C18:2n-6 polyunsaturated fatty acids, n-6: n-3 =(C18:2 n-6):(C18:3n-3) ratio.

In vitro Rumen Liquor Collection and Treatment

The liquor of rumen was collected from the rumen fistulated-goats (Bar-Diamond, Parma, ID, USA) before the morning feeding. The contents of the rumen liquor were transferred into warmed thermos flasks which flushed with CO_2 and transferred to the laboratory. The liquor was mixed -for 30 seconds- in a blender (Waring Products Division, New Hartford, USA) and filtered through 4 layers-cheesecloth. The filtered liquor was placed in a 39 °C water bath and the pH was measured and recorded during gassing the headspace with CO_2.

The Preparation Bicarbonate and phosphate Buffer

According to Fievez *et al*. (2005), two kinds of buffers were prepared. The phosphate buffer, includes (28.8 g $Na_2HPO_4 \cdot 12H2O$, 6.1 g $NaH_2PO_4 \cdot H2O$ and 1.4 g NH_4Cl) . The bicarbonate buffer includes (39.2 g of $NaHCO_3$)per liter of distilled water. The pH of the phosphate buffer was adjusted to 6.8 and the buffer flushed with CO_2 for 1.5h.

The *In Vitro* incubation of experimental treatment feeds

A gas-tight 100 ml calibrated plastic syringes containing 250 mg of each experimental treatment feed separately -which explained previously- were prepared and the strained liquor of rumen were mixed (1:4 v/v) with the identical buffer and introduced (30 mL of the mixture) to the syringes. The tips of the syringes were closed after the air was expelled. All syringes were positioned in at the 39^o C-incubator for 24 h and they were gently shaken to ensure complete mixing of the contents. The protocol of gas production, was carried out in accordance to Fievez *et al*. (2005) . At times of 0, 2, 4, 6, 8, 10, 12 and 24 h of incubation, the volumes of the gas produced were determined. Net gas production values were corrected by subtracting blank values from the samples, in which 30 mL of buffered (phosphate buffer and bicarbonate buffer) rumen fluid solution was dispensed into 100 mL calibrated plastic syringes.

Gas production Calculation.

Data were fitted to the equation (GP= $a + b\,(1- exp^{-c.t})$)(Orskov, 1985), where *a*, *b* and *c* are constants and GP is gas production of the substrate at time *t*. Where GP (mL) denotes the cumulative gas production at time *t*, *a* (mL) is the asymptotic gas production, *c* (/h) is the fractional rate of gas production and *c* (h) is the lag time.

Post-incubation Analysis

For the determination of methane production after the 24 h of incubation, 1mL of the gas phase was sampled from the syringe,and analyzed by gas-liquid chromatography (Agilent 5890 Series Gas Chromatograph, Palo Alto, CA, USA) equipped with flame ionization detector. The calibration was completed using the standard methane prepared by Scotty Specialty Gases (Supelco, Bellefonte, PA, USA). The whole procedures were repeated three times.

Statistical analysis

All experimental data were statistically analyzed by using the SAS (version 9.1; SAS Institute Inc., Cary, NC). The MIXED procedure of the SAS, was used to evaluate the effects of the treatment. Multiple comparison of the means among times and treatment, was performed using the method of Tukey's HSD. The differences mean were considered significant at ($P < 0.05$). The polynomial effects were used, to evaluate the effects of the treatments.

Results

Total gas production (Figure 1) and gas production rate (Figure 2),was significantly ($P<0.05$) affected by the experimental treatments in comparison with the control (Ctrl). There were no significant differences between the two treatments in total and rate of gas production. Total gas production for

Ctrl, T1 and T2 was 41.58, 38.67 and 38.27 (mL/250mg DM), respectively. And the gas production rate for Ctrl, T1 and T2 was 1.73, 1.61 and 1.59 (mL/hour of incubation), respectively.

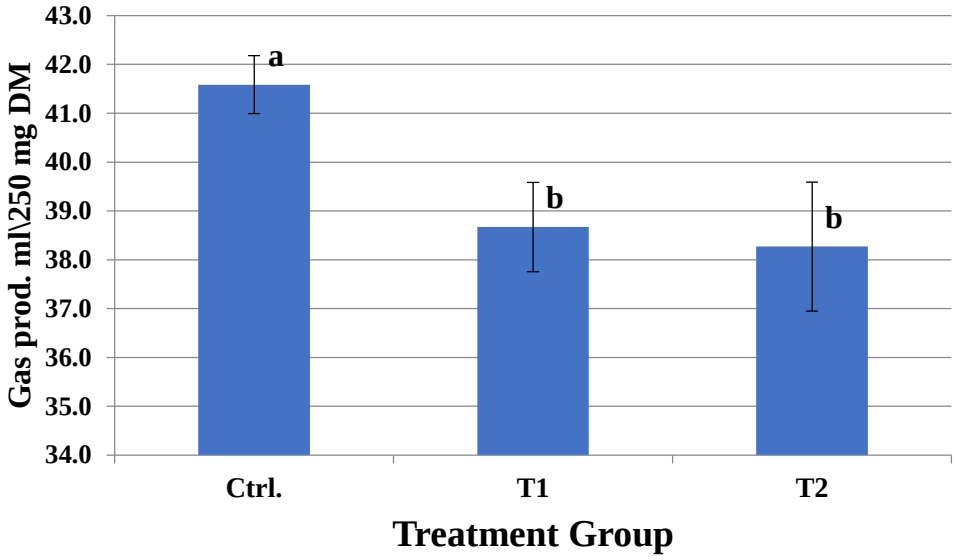

Figure 1: Total *In vitro* gas production of different inclusion levels of OPLE after 24 hours of incubation (Mean±SE; n = 6). Ctrl = Control (0 OPLE), T1 = 300 mg OPLE\ kg DM diet, T2 = 600 mg OPLE\ kg DM diet. Different letters are significantly different (P<0.05). the vertical bars are ±1 standard error.

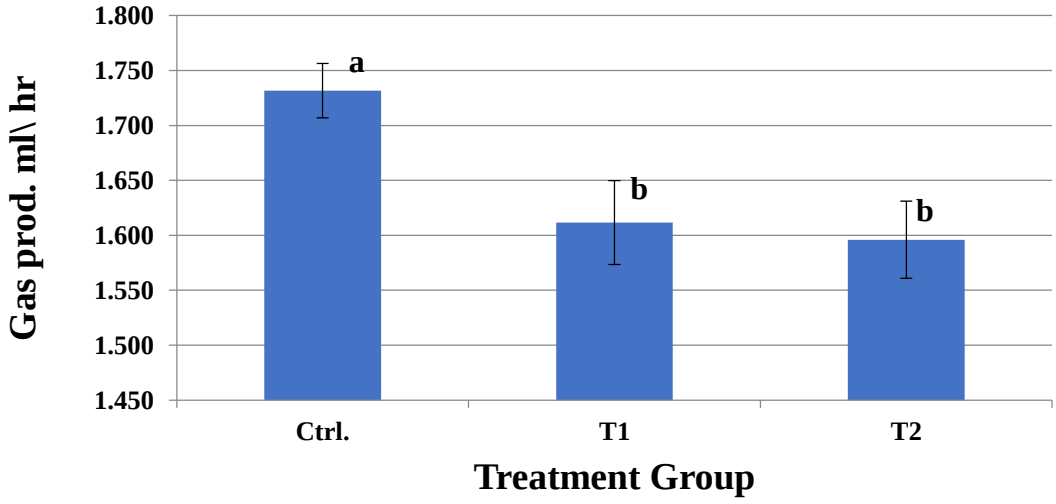

Figure 2: *In vitro* gas production rate of different inclusion levels of OPLE at the 24 hours of incubation (Mean ± SE; n = 6). Ctrl = Control (0 OPLE), T1 = 300 mg OPLE\ kg DM diet, T2 = 600 mg OPLE\ kg DM diet. Different letters are significantly different (P<0.05). the vertical bars are ±1 standard error.

The OPLE supplement suppressed methane production after 24 hours of incubation and the effect was significant (P<0.05). (Figure 3)

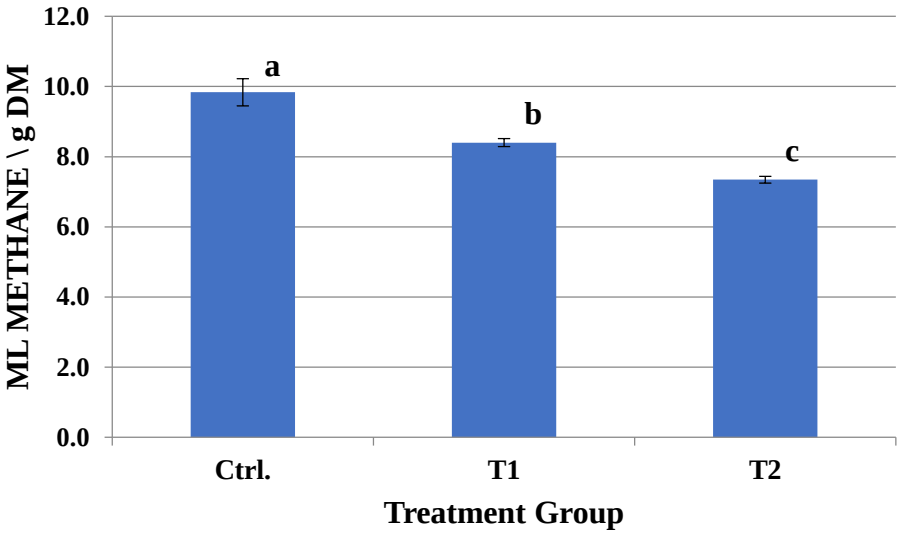

Figure 3: *In vitro* methane production of different inclusion levels of OPLE after 24 hours of incubation (Mean ± SE; n = 6). Ctrl = Control (0 OPLE), T1 = 300 mg OPLE\ kg DM diet, T2 = 600 mg OPLE\ kg DM diet. Different letters are significantly different (P<0.05). the vertical bars are ±1 standard error.

Discussion

In both the total gas production and gas production rate, the outcomes showed a reduction in the treatments of OPLE in comparison to control. This study expressed that the reduction could be due to the polyphenol's presence. Kumar *et al*. (2011) ,showed that the effect of methanolic extract of *E. jambolana* causes a reduction in gas production, which they attributed the results to the high content of condensed tannin (CT) in the extract.

The observed methane reduction for T1 and T2 compared to the Ctrl diet. The significant decreasing of methane production noted in the present study confirmed a previous report by Jayanegara, *et al*. (2011)who showed that, polyphenol containing plants can reduce methane production, therefore, polyphenols could add to the diets for lessening methane emission by ruminants. The polyphenols, which possess antimicrobial events and have suggested to be effective alternatives to antibiotics, would have suppressed the activity of methanogens, thus impressing rumen methanogenesis (Jayanegara *et al.*, 2009; Makkar *et al*., 2007). The polyphenols efficacy to reduce methane emission, is recognizable and an extensive range of plants, have been noticed in many laboratories (Jayanegara *et al.,* 2012). The rumen methane production that reduced in the present study, could be connected to the secondary metabolites of OPLE in reducing the population of methanogenic archaea. A study conducted by Agarwal et al. (2009), in which the methanol extract of the berries depressed *in vitro* methane production compared to the control group. Past research has shown, to control methane production by their activities of anti-microbial and anti-protozoal, plant by-products (extracts) comprising secondary metabolites (PSM) can be added as feed supplements (Halim *et al.,* 2011; Kumar *et al.,* 2011; Wischer *et al.,* 2013). In fact, this also caused the shift of the composition of short-chain fatty acid away from acetate, and hence lower production of hydrogen and low methane emission.

Conclusion

The most obvious finding to emerge from this study is that the OPLE in the experimental treatment feeds reduced *in vitro* methane production.

Using of oil palm leaves extract (OPLE) as a supplement in ruminant diet would benefit addition to ruminant production as it can reduce the problem of greenhouse gas problem by mitigating production of methane and gas in rumen.

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