In vitro rumen fermentation of oil palm frond with addition of Lactobacillus plantarum as probiotic

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Abstract. The research evaluated the effects of substitution of elephant grass (EG) with oil palm frond (OPF) and addition of probiotic Lactobacillus plantarum on in vitro rumen fermentation. Treatments were T1: Concentrate 60% + elephant grass 40% + OPF 0%, T2: Concentrate 60% + elephant grass 20% + OPF 20%, T3: Concentrate 60% + elephant grass 0% + OPF 40%, T4: Concentrate 60% + elephant grass 40% + OPF 0% + probiotic, T5: Concentrate 60% + elephant grass 20% + OPF 20% + probiotic, T6: Concentrate 60% + elephant grass 0% + OPF 40% + probiotic. Probiotic increased gas production in each OPF level. Highest gas production from non-probiotic treatments was 99 ml, while highest gas production from probiotic treatments was 124.5 ml. Highest methane proportion was resulted from T4 (7.31%). Probiotic did not affect digestibility and OPF decreased dry matter and organic matter digestibility. Thus, substitution of EG with OPF decreased gas production and digestibility. Probiotic increased gas production but cannot maintain rumen digestibility as equal as control.

1. Introduction
Oil palm plantation produces palm oil wastes that have potential prospective as feed providers of ruminant livestock. One of them is oil palm frond (OPF) with production quantity approximately 550 kg ha⁻¹ year⁻¹ [1]. The OPF is a potential feedstuff that can replace the grass. The main problem in using OPF is the low nutrition content, namely 609 g NDF, 581 g ADF, 28 g hemicellulose, 481 g cellulose, and 100 g acid detergent lignin per 1000 g dry matter [2]. The other nutrition content of OPF in dry matter basis are crude protein 4.7%, crude fiber 38.5%, ether extract 2.1%, and ash 3.2% [3]. Rumen fermentation can be optimized by adding feed additives, such as probiotic.

Probiotics are living microbial feed supplements that beneficially affect the host animal upon ingestion by improving its intestinal microbial balance [4]. In ruminants, probiotics are administered to target the rumen (main site of feed digestion) where they have an effect on rumen fermentation especially on feed digestibility and degradability and rumen microbiota [5]. Lactic acid bacteria (LAB) that potential to serve as a probiotic is Lactobacillus plantarum, which produces lactic acid from their metabolism. It is commonly used as probiotic for ruminant to alter rumen fermentation [6]. As probiotic, LAB could interact with rumen microorganisms and enhanced rumen microbial activity, improved rumen degradability [7] and has shown potential to reduce methane emissions [8, 9].
High content of fibrous material in OPF might give negative effect to rumen fermentation. Addition of LAB as probiotic is expected to stimulate rumen microbes to have better adaptation with the substitution of EG with OPF. The aim of this research was to study the effects of OPF in substituting elephant grass (EG) (*Pennisetum purpureum*) in different proportion and the effects of *L. plantarum* addition as probiotic on *in vitro* rumen fermentation.

2. Material and methods

2.1. Material
Oil palm frond was harvested from the fields of Indonesian Oil Palm Research Institute (PPKS), Medan, North Sumatera. It was chopped by chopper machine and then air dried for further treatments. Probiotic used in this experiment was *L. plantarum* TSD-10, obtained from Laboratory of Applied Microbiology, Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI). It was cultured in deMan Rogosa Sharpe (MRS) broth medium (Merk, Darmstadt, Germany) at 39°C under anaerobic conditions.

2.2. Methods

2.2.1. *In vitro* rumen fermentation. Rumen fluid for *in vitro* fermentation was obtained from two rumen fistulated Ongole crossbred cattle before morning feeding, mixed in equal portion. Rumen fluid was filtered through a double layer of cheesecloth, pooled in prewarmed bottles, sealed and immediately transported to the laboratory. One ml of *L. plantarum* TSD-10 strain (10⁹ cfu mL⁻¹) served as probiotic was inoculated to each experimental bottle with probiotic treatments using syringe. *In vitro* rumen fermentation was conducted using serum bottle glass, filled with 75 ml mixture rumen fluid and Mc'Dougall buffer (1:2 ratio). The bottle was closed with rubber cap and aluminum crimp after flushed with CO₂ gas for 30 s to get anaerobic conditions and incubated in water bath incubator at temperature of 39°C [10]. Gas production was measured at 2, 4, 8, 10, 12, 24 and 48 h incubation. Methane production was measured at 24 and 48 h incubation. After 48 h incubation, pH of rumen fluid was measured. Dry matter and organic matter digestibility were measured following Tilley and Terry [11] with some modification. Briefly, at the end of 48 h incubation, rumen fluid samples were centrifuged at 378 g for 10 min and the precipitated sample was washed by distilled water. In the next step, washed samples were mixed with 50 mL pepsin-HCl solution (containing 2 g L⁻¹ pepsin and 17.8 mL L⁻¹ HCl) in 100 mL serum bottles and incubated at 39°C for 48 h.

2.2.2. Statistic. The experiment design was arranged in a completely randomized design with 6 treatments and 4 replications. Treatments used for *in vitro* rumen fermentation was T1: Concentrate 60% + elephant grass 40% + OPF 0%, T2: Concentrate 60% + elephant grass 20% + OPF 20%, T3: Concentrate 60% + elephant grass 0% + OPF 40%, T4: Concentrate 60% + elephant grass 40% + OPF 0% + probiotic, T5: Concentrate 60% + elephant grass 20% + OPF 20% + probiotic, T6: Concentrate 60% + elephant grass 0% + OPF 40% + probiotic.

Data were analyzed by one-way analysis of variance using SPSS 23 (SPSS, Inc., IBM, Chicago). Significant effects of treatments were determined by Duncan’s multiple range test method. Significant differences were accepted if P<0.05.

3. Results and discussion
The gas production was fitted to the Ørskov’s equation [12] to find the potential gas production (a+b) and rate of gas production (c) as shown in Table 1. Substitution of EG with OPF decreased gas production from *in vitro* rumen fermentation, with or without probiotic treatments. Highest gas production was measured from T4, while lowest gas production was measured from T3. Without probiotic treatment, 20% OPF (T2) still produced equal gas production with control (T1) but 40% OPF (T3) significantly decreased (p>0.05) total gas production and potential gas production. Probiotic
treatments significantly increased (p>0.05) total gas production, potential gas production (a+b) and rate of gas production (c) compared with treatments without probiotics. Increased gas production from \textit{in vitro} rumen fermentation by addition of \textit{L. plantarum} as probiotic also reported by other observation [13]. However, probiotic treatments could not avoid decreased of gas production along with higher level of OPF.

Table 1. Gas production from \textit{in vitro} rumen fermentation with different level of OPF and probiotic addition

| Parameters       | T1   | T2   | T3   | T4   | T5   | T6   |
|------------------|------|------|------|------|------|------|
| Total gas (ml)   | 99.000\textsuperscript{b} | 97.875\textsuperscript{b} | 90.625\textsuperscript{a} | 124.500\textsuperscript{e} | 116.750\textsuperscript{d} | 107.875\textsuperscript{c} |
| a+b (ml)         | 102.780\textsuperscript{b} | 100.258\textsuperscript{b} | 90.5358\textsuperscript{a} | 126.334\textsuperscript{d} | 117.517\textsuperscript{c} | 106.988\textsuperscript{b} |
| c (ml h\textsuperscript{-1}) | 0.068\textsuperscript{a} | 0.071\textsuperscript{ab} | 0.087\textsuperscript{d} | 0.076\textsuperscript{ac} | 0.080\textsuperscript{bc} | 0.096\textsuperscript{e} |
| Methane (%)      | 6.187\textsuperscript{bc} | 6.250\textsuperscript{bc} | 5.062\textsuperscript{ab} | 7.312\textsuperscript{c} | 7.062\textsuperscript{b} | 4.312\textsuperscript{a} |

Different superscripts showed significant differences (P < 0.05)

Substitution of EG with OPF decreased methane production (%) from \textit{in vitro} fermentation. Higher OPF produced lowered methane indicated more efficient rumen fermentation. Lowest methane production was measured from T6 which consist of higher level of OPF and probiotics. Methane production from T6 was 4.31 %, significantly lower (P<0.05) than other treatments. Decreased of methane production in this experiment was related with tannin content of OPF. The OPF contains high amounts of tannins and phenolic compounds [14]. Tannin is one of the most abundantly available plant secondary metabolites, which has adverse effects on the rumen microbial population, feed digestibility and animal performance [15]. Tannin-containing forages was reported to decreased methane production from \textit{in vitro} rumen fermentation [16]. Probiotic \textit{L. plantarum} also had beneficial effect in lowering methane production from \textit{in vitro} rumen fermentation [9,17].

Digestibility of \textit{in vitro} dry matter (DM) and organic matter (OM) was significantly decreased (P<0.05) by substitution of EG with OPF (Table 2.), both in treatments with and without probiotic. Highest DM digestibility was 62.70% resulted from T4 (0% OPF + probiotic), while lowest DM digestibility was 62.70% resulted from T6 (40% OPF + probiotic). The low digestibility of DM and OM are probably due to the high fiber content of palm fronds [18]. As animal feed, OPF is fibrous material with high content of lignocellulose. This becomes limitation of OPF as ruminant feed. Other reason for decreased rumen digestibility was tannin content of OPF. High amounts of tannin in the diets usually reduce the digestibility and performance of ruminant animals [19].

Table 2. Dry matter and organic matter digestibility from in vitro rumen fermentation with different level of OPF and probiotic addition

| Parameters       | T1   | T2   | T3   | T4   | T5   | T6   |
|------------------|------|------|------|------|------|------|
| IVDM (%)         | 61.781\textsuperscript{a} | 58.540\textsuperscript{b} | 53.710\textsuperscript{a} | 62.704\textsuperscript{c} | 59.043\textsuperscript{b} | 53.203\textsuperscript{a} |
| IVOMD (%)        | 63.705\textsuperscript{a} | 58.053\textsuperscript{b} | 52.137\textsuperscript{a} | 62.605\textsuperscript{c} | 58.679\textsuperscript{b} | 51.766\textsuperscript{a} |

Different superscripts showed significant differences (P < 0.05)

IVDM: \textit{in vitro} dry matter digestibility; IVOMD: \textit{in vitro} organic matter digestibility

T1: Concentrate 60 % + elephant grass 40% + OPF 0%, T2: Concentrate 60 % + elephant grass 20% + OPF 0%, T3: Concentrate 60 % + elephant grass 0% + OPF 40%, T4: Concentrate 60 % + elephant grass 40% + OPF 0% + probiotic, T5: Concentrate 60 % + elephant grass 20% + OPF 20% + probiotic, T6: Concentrate 60 % + elephant grass 0% + OPF 40% + probiotic.
4. Conclusion
Substitution of elephant grass with OPF decreased gas production and digestibility in all level. Probiotic treatments increased gas production but cannot maintain rumen digestibility as equal as control treatment (without OPF). As ruminant feed, pre-treatment was needed to digest fibrous material and increase rumen fermentation digestibility.

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