In vitro gas production and digestibility of oil palm frond silage mixed with different levels of elephant grass

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Abstract. Oil palm frond (OPF) is an agricultural by-product which abundantly available in Indonesia. Study to enhance its nutritive value and to preserve OPF is necessary. Ensiling is widely used for preserving biomass with addition of lactic acid bacteria as inoculant. Further, to improve nutritive value of OPF, it is ensiled mixed with grass. This research was conducted to evaluate the effect of addition of elephant grass (Pennisetum purpureum) in OPF mixed silages on in vitro gas production and digestibility. Treatments were proportion of elephant grass started from 0, 20, 40, 60, and 100%. Higher grass portion in OPF mix silage decreased pH, increased total acid and lactic acid bacteria (LAB). Highest total gas and maximum gas production were 90.38 mL and 99.50 mL, both resulted from S4 (100% grass). Higher portion of grass increased methane production. Highest methane production was 6.38 %, resulted from S4 (100% grass), significantly higher (p<0.05) than other treatments. The lowest methane production, 1.50% was produced from 100% OPF silage (S0). Highest dry matter and organic matter digestibility were 62.55% and 62.53%, resulted from 100% grass silage (S4). It was concluded that optimum composition of mixed silages for rumen fermentation was OPF 80%+elephant grass 20%.

1. Introduction

Oil palm frond (OPF) is a cheap and abundant by product from oil palm plantation. Oil palm frond has complex structure containing leaves, rachis, and petiole. The petiole contains a large amount of sugars [1] and relatively palatable. The OPF can be used as forage or feed supplement for ruminants [2, 3]. The production of OPF is approximately 550 kg ha⁻¹year⁻¹ [4]. Although available in huge quantities, nutritionally they have limitation in supporting the nutrient and energy requirement of ruminants. The use OPF as ruminant feed is often restricted by high lignin content (199 g kg⁻¹ DM) [5], and low metabolize energy value [6]. Provision of low quality feed with high lignin content, will affect the digestibility of nutrient, and limiting the availability of structural carbohydrates for microbial fermentation in rumen.

Application of technologies to improve its nutrient and digestibility is important. Fermentation is one of the technologies to improve the quality of feed, due to the involvement of microorganisms in degrading crude fiber, reducing lignin levels and anti-nutrient compounds, so that the digestibility value of feed from waste can increase [7]. In practice, the OPF is harvested periodically usually after harvesting of the fruits. Consequently, the freshly harvested OPF has to be preserved to ensure the continuity of roughage supply to the animals. Ensiling is a crop conservation method under anaerobic conditions in which lactic acid bacteria (LAB) convert the water soluble carbohydrate (WSC) to organic acids to decrease the pH value and inhibit the undesirable microorganisms [8, 9].

For high quality of silages, some homofermentative species as well as heterofermentative species, including Lactobacillus plantarum and Lactobacillus brevis, were often used as inoculants to rapidly initiate the ensiling fermentation [10, 11]. High fibrous content of OPF may inhibit fermentation by lactic acid. Furthermore, the concentration of water-soluble carbohydrates (WSC) in OPF...
(approximately 1% DM) may be too low for successful ensiling [12]. Addition of rice bran as source of readily fermentable sugars in ensilage was to ensure nutrient supply for growth of *Lactobacillus plantarum*.

Addition of high quality grass in OPF silage was done in order to increase its nutritive value. Elephant grass (*Pennisetum purpureum*) is common grass for ruminant feed with high yield and high nutrition content, with reasonable amount of soluble carbohydrates. This grass is the most cultivated grass and in Indonesia it is mainly used in cut and carry system for dairy and beef cattle feeding [13]. Mixed silages of OPF and elephant grass is an alternative for making good silage for high fibrous materials. Hence, the objectives of this study were to evaluate the effect of adding elephant grass in OPF mixed silages on in vitro gas production and digestibility.

2. Materials and methods

2.1. Fermentation of OPF silages

Fresh OPF was harvested from the fields of Oil Palm Research Institute (PPKS), Medan, North Sumatra, Indonesia. The OPF was chopped by chopper machine and then used for silage material. Chopped OPF was air dried before use for other purposes. Elephant grass (EG) was collected from field laboratory of Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Cibinong, West Java, Indonesia. The grass was chopped to 3-5 cm long and wilted to lower the dry matter content [14]. Silages were made in Laboratory of Applied Microbiology, Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Cibinong, West Java, Indonesia.

Treatments applied were control, OPF 100% + EG 0% (S0), OPF 80% + EG 20% (S1), OPF 60% + EG 40% (S2), OPF 40% + EG 0% (S3), OPF 100% + EG 0% (S4). Silage inoculum contain *Lactobacillus plantarum* was sprayed after dissolved with in sterile water. Oil palm frond treated with LAB contained at least $1 \times 10^6$ colonies forming units (CFU) per gram fresh weight. Rice bran was added 5% from fresh weight of silage material. The control treatment was 100% OPF without any additive. Thereafter, the experimental OPF were tightly packed in small plastic jars for each treatment until the jars were completely filled. The jars were stored at room temperatures ranging. The silages were opened after 30 days ensiling for further analysis.

After ensiling, a subsample of silage was taken and mixed with sterile water (1:9 w/v) using laboratory blender [15]. The extract was filtered through cheese cloth, and used for pH, total acid, LAB population and yeast population. Samples of silages were dried and ground to pass a 1 mm screen and stored until analysed for in vitro rumen fermentation analysis.

2.2. In vitro rumen fermentation

In vitro rumen fermentation analysis was conducted according to Theodorou et al. [16]. Rumen fluids were obtained from rumin fistulated Ongole breed (PO) cattle, filtered through a double layer of cheese cloth, pooled in pre-warmed bottles, sealed and immediately transported to the laboratory. The substrate used for in vitro rumen fermentation was the OPF-grass mix silage with the same treatments as mention above. Approximately 0.75 g of substrates was put inside the serum bottle glass and filled with 75 mL mixture rumen fluid and Mc'Dougall buffer. The bottle was closed with a rubber cap and an aluminium crimp after it was flushed with CO$_2$ gas for 30 sec to obtain anaerobic conditions. Then, the bottle was incubated in a water bath incubator at a temperature of 39°C. Gas production was measure using plastic syringe at 2, 4, 6, 8, 10, 12, 24, 48 h incubation. Methane production was measured using portable methane analyzer at 48 h incubation. In vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) were analysed according to Tilley and Terry [17]. Briefly, at the end of 48 h incubation, rumen fluid samples were centrifuged at 378 g for 10 min. The pellets were mixed with 50 mL pepsin-HCl solution (containing 2 g/L pepsin and 17.8 mL L$^{-1}$ HCl) in 100 mL serum bottles and incubated at 39°C for 48 h. After incubation, the samples were centrifuged and the precipitated feed was dried at 100°C for 48 h.
2.3. Statistical analysis

Experimental design used for this experiment was Completely Randomized Design to compare parameters using General Linear Model of SPSS (SPSS version 10.0) package programmes. Significance between individual means was identified using the Duncan’s multiple comparison test.

3. Results and discussions

3.1. Quality of OPF silages

Silages were open after 30 days of ensiling. The most common measurement used for evaluating silage fermentation is pH. In this experiment, pH range from 4.26 – 5.18 (Table 1). Lower pH indicating better quality silage, because most contaminant cannot grow at low pH. Addition of elephant grass in OPF mix silage resulted lower pH. Lowest pH was observed from S2 (4.26), which significantly higher (p<0.05) from other treatments which used lower portion of grass (control, S0, S1). Decrease pH was result from accumulation of lactic acid produced by lactic acid bacteria. Total acid also affected by increase of grass portion in mix silage. Highest total acid was 2.343% resulted from 100% grass silage (S4). It can be seen that mixed silage started from S1 already resulted significantly higher total acid (p<0.05%) compare with control or S1 which contain lower grass portion.

*Lactobacillus plantarum* was added as inoculum on this experiment, except for control treatment. Addition of inoculum raised lactic acid bacteria (LAB) population when silages were opened. Control treatment has significantly (p<0.05) lower population of LAB (7.56 log cfu mL\(^{-1}\)), compare to other treatments. The function of LAB inoculants is to ensure a rapid and efficient fermentation of water soluble carbohydrate (WSC) into lactic acid by homolactic fermentation and improve silage preservation with minimal losses. LAB improves the nutritive quality by stimulating lactic acid production, decreasing the pH rapidly, and extending the preservation time. *L. plantarum* is known as homolactic LAB that is able to result in rapid decrease of the pH of the silage, rapid increase in LAB, and inhibition of spoilage microorganisms [18].

| Parameters | Control | S0     | S1     | S2     | S3     | S4     |
|------------|---------|--------|--------|--------|--------|--------|
| pH         | 5.18\(^b\) | 5.08\(^a\) | 4.50\(^b\) | 4.26\(^a\) | 4.33\(^a\) | 4.30\(^a\) |
| Total acid (%)| 0.61\(^a\) | 0.82\(^a\) | 1.86\(^b\) | 1.98\(^b\) | 1.94\(^b\) | 2.34\(^b\) |
| LAB (log cfu) | 7.56\(^a\) | 8.00\(^b\) | 8.22\(^b\) | 7.93\(^b\) | 7.42\(^a\) | 7.94\(^b\) |
| Yeast (log cfu) | 8.13\(^b\) | 7.83\(^b\) | 7.20\(^a\) | 7.07\(^a\) | 7.29\(^a\) | 7.14\(^a\) |

Different superscripts showed significant differences (P < 0.05)

3.2. In vitro gas production

Total gas production from 48 h in vitro rumen fermentation was affected by level of grass mixed with OPF in silage (Table 2). Highest gas production was produced from 100% grass silage (S4). Gas production from in vitro rumen fermentation was correlated with digestibility. Maximum gas production (a+b) showed the same pattern with total gas production, whereas higher grass level in silage increased maximum gas production and gas production rate. Each level of grass portion in OPF mix silage resulted in significantly (p<0.05) higher total gas production and maximum gas production compare to treatments with lower grass portion. Highest gas production rate (c) value was 0.085 mL h\(^{-1}\) resulted from S1 treatment. It was significantly (p<0.05) higher than other treatments except from S2 treatments. It is showed that composition of OPF:elephant grass in S1 and S2 treatments was the easiest to digest by rumen microbes.

Gas production from rumen fermentation resulted from direct substrate fermentation (CO\(_2\) and CH\(_3\)) and indirect gas production (CO\(_2\)) reactions from organic acids with bicarbonate buffers [19, 20,
Therefore, gas production will indicate digestibility of substrate. Increase gas production when proportion of grass was increased in mixed silage showed that grass was easier to digest compare than OPF. High fibrous material in OPF, including lignin which difficult to digest by rumen bacteria, was limitation for OPF to be digest in rumen fermentation resulted in low gas production. From this experiment, OPF-elephant grass mixed silages were proved to increased digestibility of OPF silage as ruminant feed. Higher gas production along with higher elephant grass in mix-silage also reported in elephant grass-dried tamarind residue mix-silage [22].

| Parameters               | Treatments | Control | S0 | S1 | S2 | S3 | S4 |
|--------------------------|------------|---------|----|----|----|----|----|
| Total gas (mL)           |            | 41.50<sup>a</sup> | 39.38<sup>a</sup> | 52.25<sup>b</sup> | 62.00<sup>c</sup> | 72.88<sup>d</sup> | 90.38<sup>e</sup> |
| a+b (mL)                 |            | 41.99<sup>a</sup> | 39.32<sup>a</sup> | 51.79<sup>b</sup> | 62.03<sup>c</sup> | 75.24<sup>d</sup> | 99.50<sup>e</sup> |
| c (mL h<sup>-1</sup>)    |            | 0.071<sup>bc</sup> | 0.074<sup>c</sup> | 0.085<sup>d</sup> | 0.081<sup>d</sup> | 0.068<sup>b</sup> | 0.051<sup>a</sup> |
| Methane 48h (%)          |            | 1.25<sup>a</sup> | 1.50<sup>a</sup> | 2.75<sup>ab</sup> | 3.38<sup>b</sup> | 3.88<sup>b</sup> | 6.38<sup>c</sup> |

<sup>a</sup> = potential gas production;  
<sup>b</sup> = gas production rate;  
<sup>c</sup> = different superscripts showed significant differences (P < 0.05)

Addition of elephant grass in OPF silage increased methane production. The lowest methane production, 1.50% was produced from OPF silage without grass (S0). Grass silage (S4) produced the highest value of methane (6.38%), significantly higher (p<0.05) than other treatments. Gas produced from rumen fermentation consist of 40% carbon dioxide (CO<sub>2</sub>), 30-40% methane (CH<sub>4</sub>), 5% hydrogen, and a small portion of oxygen and nitrogen [20]. An increase of methane emission was observed when higher portion of grass was used in silage. The lowest methane production, 1.50% was produced from OPF silage without grass (S0). Both OPF silage without grass, control and S0, produced significantly lower methane compare with other treatment (p<0.05). Grass silage (S4) produced the highest value of methane (6.38%), significantly higher (p<0.05) than other treatments.

Enteric CH<sub>4</sub> formed by fermentation of feed in the gastrointestinal tract of ruminants constitutes a loss of dietary energy to the animal [23]. Methane emission from enteric fermentation, apart from its association with environmental problem, is also representing a certain amount of energy loss from the animals [24]. Low methane production was estimated caused by tannin content in OPF. Tannin has the ability to inhibit methane production in rumen fermentation by lowering protozoa population which produces methane. Among a number of nutritional attempts for mitigating enteric methane emission, plant secondary compounds such as tannins, saponins and essential oils are considered as the promising substances due to their abundant in nature [25, 26, 27]. With regard to tannins, previous studies have reported that tannin-containing plants or tannin extracts decreased methane emissions both in in vitro and in vivo experiments [28, 29].

### 3.3. In vitro digestibility

The nutritive value of a ruminant feed is determined by the concentrations of its chemical components, as well as their rate and extent of digestion [30]. Measurement of in vitro digestibility has been widely used to assess the nutritional quality of feeds, due to its high correlation with in vivo digestibility [31]. The digestibility coefficient of dry matter and organic matter of OPF-grass mixed silage on in vitro rumen fermentation presented in Table 3. In this experiment observed that increase grass portion in mix silage resulted higher dry matter and organic matter digestibility. Highest IVDMD was 62.55%, resulted from S4 (100% grass silage), significantly higher (p<0.05) than other treatments.

Organic matter digestibility is defined as the proportion of feed organic matter apparently digested in the total digestive tract. Organic matter digestibility is a measure of energy available to ruminants [32]. Organic materials produce energy for the growth and development of livestock. The higher the digestibility value of a feed ingredient, indicated that more nutrients for the animal body absorbs.
value IVOMD can be seen in Table 3. The result showed that OPF mixed silage with different grass level gave significant effect (P <0.05) on the digestibility level of organic matter. The highest IVOMD was found in 100% grass silage treatment (62.53%), while the lowest percentage of IVOMD was found in control treatment (26.95%). The higher the digestibility of the dry matter and the organic material of the high-fibre nutrient feed that can be used to meet the nutrient requirements of livestock. Addition of grass, which easier to digest than OPF, increased digestibility of OPF-elephant grass mixed silages.

Table 3. In vitro dry matter and organic matter digestibility

| Parameters | Treatments | C | S0 | S1 | S2 | S3 | S4 |
|------------|------------|---|----|----|----|----|----|
| IVDMD (%)  |            | 29.67<sup>a</sup> | 31.74<sup>a</sup> | 37.48<sup>b</sup> | 41.34<sup>c</sup> | 49.07<sup>d</sup> | 62.55<sup>e</sup> |
| IVOMD (%)  |            | 26.95<sup>a</sup> | 29.85<sup>a</sup> | 35.60<sup>b</sup> | 38.74<sup>c</sup> | 47.69<sup>d</sup> | 62.53<sup>e</sup> |

IVDM = In Vitro Dry Matter Digestibility; IVOMD = In Vitro Organic Matter Digestibility; Different superscripts showed significant differences (P < 0.05)

4. Conclusions
Addition of elephant grass in OPF mixed silages increased quality, gas production and digestibility. Optimum composition of mixed silages for rumen fermentation was OPF 80%+elephant grass 20% according to quality, gas production, digestibility and methane production.

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6. References
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