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Research Article
In vitro Evaluation of Oil Palm Fronds Fermented with Produren: A Durian Probiotic

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Abstract
Background and Objective: Increasing ruminant productivity requires a protein source not only from feed but also from rumen microbial activity. An increase in the activity of rumen microbes can be accomplished with arumen modifier, which is the role of produren (a durian probiotic). The current study aimed to determine the effect of produren on the *in vitro* fermentation of oil palm fronds in beef cattle rumen fluid. **Methodology:** The study used a completely randomized design with 4 treatments and4 replications. The treatments consisted of oil palm fronds without fermentation (NPF/control), oil palm fronds fermented with 2.5% produren (PF1), oil palm fronds fermented with 5% produren (PF2) and oil palm fronds fermented with 7.5% produren (PF3). The parameters measured were dry matter digestibility, organic matter digestibility, N-NH_3, pH, the total volatile fatty acid (VFA) concentration and the acetic acid, propionic acid and butyric acid concentrations. The data were analyzed by Duncan's multiple range test. **Results:** The oil palm fronds fermented with produren had a higher ($p<0.05$) dry matter digestibility (8.9%) and concentration of total VFAs, (21.6%) than the control but fermentation with produren did not affect ($p>0.05$) organic matter digestibility, pH or NH_3 in the rumen fluid from beef cattle. **Conclusion:** Fermentation of oil palm fronds with produren at 7.5 g% DM improved dry matter digestibility and the total VFA concentration in the rumen fluid from beef cattle.

Key words: Beef cattle, oil palm fronds, produren, rumen fluid, VFA concentration, rumen pH, ruminal ammonia

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There are several byproducts of oil palm, including oil palm trunk (OPT), oil palm fronds (OPF), empty fruit bunches (EFB), palm kernel cake (PKC), palm oil mill effluent (POME) and palm press fiber (PPF). The first three byproducts are from growing oil palm in plantations and the remaining three are from palm oil processing. OPF in particular have been of interest lately, as they have great potential to be utilized as a roughage source or as a component ofa total mixed ration for ruminants. The supply of OPF is substantial and can support a reasonably large population of ruminants locally. This is important for the development of the domestic ruminant industry, especially as the country is only approximately 20% self-sufficient in beef, 6% in mutton and 5% in milk[1]. Dry matter intake declines by up to 30% when fresh oil palm fronds replaced 75% of the grass in Bali cattle rations and oil palm fronds can be used for ruminants at only approximately 30% of the diet[2,3].

Approximately20% of the dry biomass of oil palm fronds is lignin, so the lignin content is a major obstacle when using oil palm fronds as animal feed[4]. A low-quality feed with a high lignin content will cause adverse conditions and will cause the rumen to function less well, so it is necessary to use technology to fix the problem. Fermentation is one of the technologies used to improve by product feed quality because microorganisms degrade fiber, reduce the lignin and anti-nutritive compound contents and can increase feed digestibility[5].

Recent trends have favored the use of natural feed additives to replace additives such as antibiotics and chemicals that can increase the function of ionophores to manipulate fermentation in therumen[6,7]. Feed additives are considered ideal if they meet certain requirements, such as not being harmful to animals, humans, or the environment and not leaving residues in the body of production animals[8]. The main microorganisms contained in probiotics are fungi, such as *Aspergillus oryzae*, *Saccharomyces cerevisiae* and lactic acid bacteria (LAB), including *L. plantarum* and *L. acidophilus*[9].

Efforts to improve the nutritional value of animal feed include using microbes such as lactic acid bacteria (LAB). The LAB isolates can be obtained from plants, fruits and animal products. The LAB isolates derived from animal products are different from those which are derived from plants because plants have secondary metabolite compounds, such as sources of bioflavonoids or antioxidants[10]. Produren (a durian probiotic) is the fermented pulp of durian fruit and it is found in the species *Lactobacillus plantarum*, which is a lactic acid bacteria isolate[11].

The lactic acid bacterial population in fermented durians consists of *Lactobacillus* sp, *Lactobacillus plantarum*, *Weissella paramesenteroides* and *Pediococcus acidilactici*[12]. The majority of the acid-forming bacteria belong to the genera *Lactobacillus*, 40% of which are *Lactobacillus plantarum*, while the remaining strains are still unidentified. For the unidentified strains, there is a need for further examination with regard to phylogenetic determination because biochemical tests are unable to differentiate among the strains *Lactobacillus*. The presence of live LABs suggests that produren has a potential to be used as a probiotic since its consumption could benefit the host by improving the properties of the indigenous intestinal microflora. It is expected that the use of produren for OPF fermentation could increase the digestibility of OPF and improve the microbial population and rumen fluid characteristics. The aim of this study was to determine the *in vitro* digestibility and fermentability of oil palm fronds with produren (a durian probiotic)

MATERIALS AND METHODS

For this research, the oil palm fronds were fermented and analyzed immediately at the Laboratory of Nutrition, Faculty of Animal Husbandry, Jambi University. The analysis of *in vitro* digestibility was conducted in the Laboratory of Nutrition at the Bogor Agriculture Institute. Produren was produced in the Integrated Laboratory, Jambi University and the samples of the oil palm fronds were collected from around the Muaro Jambi District. The parameters measured in this study were dry and organic matter digestibility, the total volatile fatty acid concentration, acetic acid, propionic acid and butyric acid concentrations, pH and NH_3 concentration.

Procedure for making produren: One kilogram of durian skin from Jambi was mixed with 1 L of distilled water and then filtered and heated at 100°C. Ten percent of the *L. lactobacillus* bacteria starter from the durian fruit fermented in MRS broth was added to durian skin extract. Solutions of the extract containing the bacteria were stored inside an incubator for 24-48 h until produren was formed[13].

Fermentation of the oil palm fronds with produren (a durian probiotic): The main materials used were oil palm frond substrate, produren, molasses, bran and urea. The oil palm fronds were cut, dried and then finely milled. The fermentation of the oil palm fronds with produren was carried out for one week. The nutritional compositions of the treatment rations is presented in Table 1.

Table 1: Nutritive value of the feed treatments

Nutrients*	Diets NPF	PF1	PF2	PF3
Dry matter (%)	90.127	90.444	90.711	92.342
Crude protein (%)	14.466	14.927	15.733	15.609
Ether extract (%)	1.688	2.079	2.164	2.219
Crude fiber (%)	35.705	34.732	34.634	33.310
Ash (%)	5.742	5.885	5.826	5.916
ADF (%)	52.889	52.686	52.477	52.737
NDF (%)	71.503	72.371	71.802	72.000

Rumen fluid characteristics: *In vitro* experiments were conducted based on the Tilley and Terry[14] method. Rumen fluid was taken from a cow rumen via a fistula at the Experimental Farm of Faculty of Animal Husbandry, Bogor Agriculture Institute. The measured characteristics of the rumen fluid were the dry matter digestibility coefficient, organic matter digestibility coefficient, N-NH_3 concentration, pH, total volatile fatty acid (VFA) concentration and acetic acid, propionic acid and butyric acid concentrations. Fermenter tubes were each filled with 0.5 g of sample and then 40 mL of buffer solution and 10 mL of fresh rumen fluid were added. After the tube was injected with CO_2, the tubes were closed with a rubber stopper. Then, the tubes were inserted into a water bath shaker and incubated at 39°C for 4 h, followed by an analysis of the dry matter digestibility coefficient, organic matter digestibility coefficient, N-NH_3 concentration, pH, total volatile fatty acid (VFA) concentration and acetic acid, propionic acid and butyric acid concentrations. The N-NH_3 concentration was measured using the Conway microdiffusion technique; the rumen fluid pH was measured with a digital pH meter and the total and individual volatile fatty acid (VFA) concentrations consisting of acetic acid but yrate and propionate were measured using gas chromatography (GC).

Experimental design and statistical analysis: The experiment was conducted via a completely randomized design with 4 treatments and 4 replications. The treatments consisted of NPF: the oil palm fronds without fermentation (control), PF1: the oil palm fronds that were fermented with 2.5% produren, PF2: the oil palm fronds that were fermented with 5% produren and PF3: the oil palm fronds were fermented with 7.5% produren. The collected data were analyzed using statistical analysis system (SAS) and the differences between the treatment means were analyzed using Duncan's multiple range test[15] and statistical significance was set at $p<0.05$ and $p<0.01$.

RESULTS AND DISCUSSION

Dry and organic matter digestibility: Upon ingestion by ruminants, feedstuffs enter the rumen and are degraded to various extents by the rumen microbial population. The ruminal ecosystem consists ofa diverse, symbiotic population of obligatory anaerobic bacteria, fungi and protozoa[16].

The results showed that when the oil palm fronds were fermented with produren, dry matter digestibility was significantly ($p<0.05$) affected. The dry matter digestibility of PF1, PF2 and PF3 was higher ($p<0.05$) than that of NPF (control). Produren at a dose of 7.5% g DM resulted in the highest digestibility and increased dry matter digestibility by as much as 8.9% compared to the control. The dry matter digestibility of the oil palm fronds was 38.26-39.33%. This result was lower than the result of a previous study conducted by Ebrahimi *et al.*[17] who used oil palm fronds fermented with *Lactobacillus* bacteria and cellulose additives; fermentation with the combination of *Lactobacillus* bacteria and cellulose additives resulted in a dry matter digestibility (*in vitro*) as high as 52-55%. Feeding many probiotic microbes could remodel the lignin and crude fiber (cellulose and hemicellulose) in the rumen[18]. Lignin can reduce digestibility by forming hydrogen bonds with cellulose and hemicellulose that limit the ability of the cellulase enzyme to digest fiber.

The organic matter digestibility coefficient of the oil palm fronds fermented with produren is shown in Table 2. The results showed that fermentation of the oil palm fronds with produren had no effect ($p>0.05$) on organic matter digestibility. The results showed that the value of organic matter digestibility was relatively low compared with the value of dry matter digestibility. The average organic matter digestibility value of the oil palm fronds fermented with produren ranged from 34.10-35.90%. These results were higher than those of the study conducted by Wajizah *et al.*[19] who found that the organic matter digestibility value of oil palm fronds fermented with *Aspergillus niger* fungus and with various sources of carbohydrates was between 17.21-21.88%. It can be concluded that the use of *L. plantarum* bacteria can allow for higher digestibility than the use of *Aspergillus niger*. Probiotics stimulate rumen bacterial activity, stabilize the rumen pH and increase the use of ammonia, which is used for microbial protein synthesis[20].

Table 2: Characteristics of the beef cattle rumen fluid

	Diets				
Items	NPF	PF1	PF2	PF3	MSE
pH	7.09	7.11	7.11	7.12	0.074
NH3 (mM)	12.62	13.94	13.94	15.00	3.53
Dry matter digestibility (%)	35.81[a]	38.26[b]	38.61[b]	39.33[b]	1.524
Organic matter digestibility (%)	33.06	34.10	34.61	35.90	2.137
Total VFA (mM)	78.24[a]	94.74[b]	97.22[b]	99.87[b]	2.167
Acetic acid (mM)	12.88	13.10	14.37	14.99	2.597
Propionic acid (mM)	3.40	3.59	3.83	4.05	0.700
Butyric acid (mM)	3.10	3.18	3.45	4.20	0.673

MSE: Mean of the standard error, NPF: Oil palm fronds without fermentation (control), PF1: Oil palm fronds that were fermented with 2.5% produren, PF2: oil palm fronds that were fermented with 5% produren and PF3: Oil palm fronds that were fermented with 7.5% produren. Different letters within a row show significant differences ($p<0.05$)

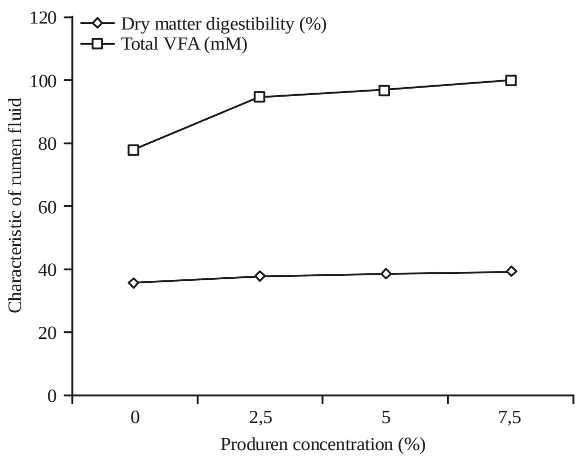

Fig. 1: Graph of oil palm fronds dry matter digestibility (%) and total VFA concentration (mM)

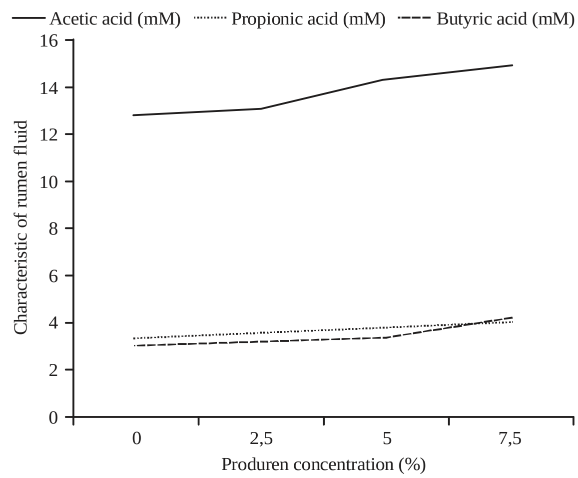

Fig. 2: Graph of the oil palm frond individual VFA concentrations (mM)

The digestibility of ruminant livestock feed depends on the populations and types of rumen microbes, especially the bacteria, because the feed is broken down by enzymes produced by the rumen microbes. Therefore, increasing the population of rumen bacteria will increase the concentration of the enzymes and thereby improve feed digestibility[21]. The P3 treatment had a high dry matter digestibility as shown in Fig. 1 compared to that of the other treatments. A low crude fiber content (Table 1) would increase the digestibility of other nutrients and a high crude fiber content would inhibit the rumen microbial degradation of feed[22].

Total and individual VFA concentrations: Volatile fatty acids (VFAs)are the main products of rumen microbial fermentation. VFA production reflects feed fermentability and is the main energy source for livestock. VFAs are the end products of nutrient fermentation, especially protein and carbohydrates[23]. The total VFA concentration of the oil palm fronds fermented with produren ranged from 78.24-99.87 mM (Table 2). The total VFA production of oil palm fronds fermented with *Aspergillus niger* ranged from 62.15-96.58 mM[18]. The VFA values of all the treatments in this study were within the optimum range for the growth of rumen microbes and for the systems within the animal, which is 60-120 mM[24].

The total VFA concentration of the oil palm fronds fermented with produren was significantly different ($p<0.05$)among the treatments. The total VFA concentrations of PF1, PF2 and PF3 were higher ($p<0.05$) than that of the control (NPF). The PF3 treatment (7.5% produren dose) produced the highest VFA values (Fig. 2), which was approximately 21.6% higher than that of the control. VFAs play an important role in livestock production and are directly related to feed composition[25]. A large amount of VFA production that is followed by a low concentration of ammonia reflects the efficiency of using ammonia for microbial protein synthesis and growth.

The VFA concentration in the rumen indicates whether the feed is easily fermented by rumen microbes (carbohydrates and soluble proteins) or not. If the protein in animal feed has a high level of solubility, then the protein will be fermented in the rumen and produce VFA and ammonia. If the protein in the feed has a low level of solubility, then the protein will be relatively unchanged in the rumen[26].

The individual VFA concentrations such as the acetic acid concentration ranged from 12.88-14.99 mM, propionic acid, 3.4-4.05 Mm and butyric acid, 3.10-4.20 mM (Table 2). The individual VFA results from this research were lower than those of the study conducted by Widiyanto *et al.*[27] who used rations made from water hyacinth with a starter of *Lactobacillus plantarum* and showed that the concentrations of acetic acid, propionic acid and butyric acid were between 50.15-70.35, 20.10-40.02 and 8.75-14.58 Mm, respectively. The fermentability of the feed in the rumen is used as an estimate of rumen microbial gradation; acetic acid has gluconeogenic properties, while butyric acid has ketogenic properties[17]. Propionic acid is converted into blood glucose in the liver. Blood glucose will enter a cell and be used to support fat and protein synthesis in the body and produce VFAs, such as acetic acid (C2), propionic acid (C3) and butyric acid (C4). Propionic acid is a source of ATP[28].

The high total VFA concentration in P3 (Table 2) was caused by increased fermentation due to an increased number of rumen microbes. The VFA results also agreed with the increasing availability of NH_3 in the rumen fluid, which would improve microbial growth through the production of VFAs[20]. Febrina *et al.*[29] reported that the VFA concentration in rumen fluid can indicate the level of feed fermentability, where the higher the feed fermentability level is, the greater is the VFA concentration.

pHandNH$_3$-N: Rumen pH is one of indicator of the activities of bioprocess in the rumen. Treatment of oil palm fronds fermented with produren had no effect ($p>0.05$) on the rumen pH. The rumen pH values in this study were 7.09-7.12 (Table 2). The rumen pH values were higher than those reported by Nagaraja and Titgemeyert[30] who found a rumen pH of approximately5.8-6.5 in beef cattle. A low rumen pH can occur because volatile fatty acids have been formed from the fermentation of high concentraterations[31]. The rumen pH when oil palm fronds were fermented with *Aspergillus niger*, which caused the fermentation process to work properly, ranged from 6.80-6.90[18].

Van Soest[32] stated that a pH greater than 7.1 can drastically reduce the microbial population, which would result from low energy. The conditions for optimal rumen microbial activity are a rumen pH of 6-6.9[33]. In this study, the pH of the rumen fluid was greater than 7.1; therefore, the pH of the rumen fluid was not balanced between the buffer capacity and the basic or acidic properties of the fermentation products. The type of feed given to livestock will affect the pH of the rumen[20].

The fermentation of oil palm fronds with produren had no effect ($p>0.05$) on the rumen N-NH_3 concentration in all treatments, which ranged from 9.84-18.35 mM and were in the optimum range (6-30 mg dL^{-1} or 4-21 mM)[34].Ammonia is a N source for bacterial growth and almost 80% of bacteria can grow with ammonia as the only source of N. The availability of VFAs and sufficient ammonia increase microbial protein synthesis. A decrease in the ammonia concentration in rumen fluid reflects a good level of fermentation and shows a decrease in protein degradation[35].

Ruminal ammonia concentrations tended to decrease from 4.92-3.89 mM when 3.2% sucrose was included in the diet of dairy cows[36]. Rumen ammonia concentrations are inversely related to the rate of energy fermentation and different studies have indicated that the efficiency of dietary N utilization improves with the synchronization of carbohydrate and protein fermentation in the rumen[37].

The NH_3 concentration reflects the amount of ration protein that is available in the rumen and its value is strongly influenced by the ability of the rumen microbes to degrade the ration protein. NH3 is an important source of N for the microorganisms that live in the rumen, where it is used for the synthesis of microbial proteins[20]. In this study, the average NH_3concentration of the rumen fluid was 12.62-15 mM, which was lower than 16.90-19.39 mM[20]. The production of NH_3 depends on the solubility of dietary protein, the protein concentration in the ration, the duration that food is in the rumen and the pH of the rumen[38].

CONCLUSION

In conclusion, the fermentation of oil palm fronds using 7.5% produren improved dry matter digestibility and the concentration of total VFAs in the rumen of beef cattle.

SIGNIFICANCE STATEMENT

This study showed that oil palm fronds from plantation waste fermented by produren containing *L. plantarum* bacteria have the potential to replace grass when there is a scarcity of forage in the dry season. Produren, as an activator of the fermentation process, can increase the digestibility of dry matter and VFA concentration in the rumen.

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