Fermentation Characteristics (*In Vitro*) of Palm Oil Trunk Waste as Feed for Lactating Dairy Cow

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ABSTRACT

This study aimed to evaluate the applicability of palm oil trunk waste utilization as a feed source for lactating dairy cows using an *in vitro* approach. This study used a randomized complete block design with 4 treatments and 3 groups. Treatment consisted of P0 (control diet), P1 (control diet supplemented with 12.5% palm oil trunk), P2 (control diet supplemented with 25% palm oil trunk), P3 (control diet with commercial concentrate). The parameters observed were rumen pH, NH_3 concentration, total VFA concentration, and *in vitro* dry matter and organic matter digestibility (IVDMD and IVOMD) coefficients. The findings of this study showed a significant 41.73% in NH_3 concentration, 24.96% in total VFA concentration, 10.47% in IVDMD, and 10.91% in IVOMD, upon introducing 25% palm oil trunk waste into the ration (p<0.05), except for rumen pH. It can be concluded that palm oil trunk waste can be used at a level of up to 25% in the diet of lactating dairy cows.

Keywords: digestibility, fermentation, *in vitro*, palm oil trunk, unconventional feed

ABSTRACT

Penelitian ini bertujuan untuk mengevaluasi penerapan pemanfaatan limbah batang kelapa sawit sebagai sumber pakan sapi perah laktasi dengan pendekatan in vitro. Penelitian ini menggunakan rancangan acak kelompok lengkap dengan 4 perlakuan dan 3 kelompok. Perlakuan terdiri atas P0 (pakan kontrol), P1 (pakan kontrol ditambah 12,5% batang kelapa sawit), P2 (pakan kontrol ditambah 25% batang kelapa sawit), P3 (pakan kontrol dengan konsentrat komersial). Parameter yang diamati adalah pH rumen, konsentrasi NH3, konsentrasi VFA total, dan koefisien kecernaan bahan kering dan bahan organik (IVDMD dan IVOMD) in vitro. Hasil penelitian ini menunjukkan adanya perbedaan yang signifikan sebesar 41,73% pada konsentrasi NH3, 24,96% pada konsentrasi total VFA, 10,47% pada IVDMD, dan 10,91% pada IVOMD, setelah memasukkan 25% limbah batang kelapa sawit ke dalam ransum (p<0,05), kecuali untuk pH rumen. Dapat disimpulkan bahwa limbah batang kelapa sawit dapat dimanfaatkan hingga 25% dalam pakan sapi perah laktasi.

Kata Kunci: batang kelapa sawit, fermentasi, *in vitro*, kecernaan, pakan inkonvensional

INTRODUCTION

The society's demand for fulfilling the main source of animal protein, particularly milk, has been continuously increasing. However, the national milk production can only meet 30% of the domestic milk demand. According to data from the Badan Pusat Statistik (2022), national milk production was only 968,980 tons, while milk demand reached 4.4 million tons. Efforts to increase milk production often encounter obstacles due to the decreasing environmental carrying capacity to provide quality feed supply. The fundamental issue that occurs in almost all regions of Indonesia is the poor feed management, resulting in low nutrient intake by livestock, especially to optimize milk production. The fluctuating availability of feed, both in quality and quantity, leads to suboptimal livestock productivity (Nuraina *et al.* 2021). The presence of low-quality forage and the inability of farmers to meet the needs of quality cattle concentrates have become classic problems in this country. Therefore, alternative feed that can substitute limited-seasonal livestock feed is highly needed.

Agricultural waste can be utilized as an alternative feed solution, possessing nutritional value equivalent to commercial feed, such as palm oil. According to data from the Indonesian Palm Oil Statistics (BPS 2021), the palm oil plantation area in Indonesia was approximately 14.26 million hectares in 2021, with 12.05 million hectares being productive. Palm oil trees have a productive lifespan of about 25 years and a 4% annual rejuvenation rate, resulting in an annual waste of 81.5 million tons of palm oil trunks, making it the largest waste biomass in palm oil plantations. In one hectare, an average of 128 palm oil trees is planted, generating a total waste of 783.17 kg of trunks (82.97%), 50.06 kg of fronds (9.01%), and 22.88 kg of leaves (6.03%) per tree (Muhdi *et al.* 2015). The palm oil trunk contains 55.5% starch, 1.6% protein, 36% crude fiber, and 0.6% fat at a height of 0-1 meter from the top of the trunk (Sinurat *et al.* 2012). Typically, the waste from rejuvenated palm oil trunks is burned since farmers no longer utilize it. Starch is valuable for energy production due to its carbohydrate content. Therefore, based on the nutritional composition found in palm oil trunks, it is expected that they can be used as an unconventional feedstuff to formulate a concentrate for dairy cows containing high energy.

The suitability of a feed to be tested for dairy cattle can be determined by assessing its fermentation outcomes *in vitro*. Several parameters can be evaluated, such as dry matter and organic matter digestibility, VFA concentration, NH_3 concentration, total gas, and methane gas. *In vitro* studies also serve to evaluate feed, and examine its response to anti-nutritional factors, feed supplements, and additives (Despal *et al.* 2022; Permana *et al.* 2022). This research aimed to assess the utilization of palm oil trunk waste as a source of concentrate feed for lactating dairy cows through *in vitro* analysis.

METHODS

Sample Preparation

An over 25-year-old unproductive oil palm tree was cut down, and the top 50 cm of the trunk was extracted. The harvested trunk was split, separating the outer peel and middle layer (parenchyma). Subsequently, the parenchyma part was thinly sliced and sun-dried for three days, followed by oven-drying at 60°C for one day until it reached a stable weight. The material was then ground into powder and used for proximate analysis (AOAC 2005). The data obtained from the proximate analysis were used to formulate the ration that would be further tested *in vitro*.

Formulation of Experimental Ration

The experimental ration consisted of forage and concentrate in a ratio of 40:60. The forage used was elephant grass, while the commercial concentrate (GT-03) was provided by PT. Indonesia Formula Feed, comprising yellow corn, wheat bran, rice bran, soybean meal and coconut meal, as well as vitamins and minerals. The nutrient content of palm oil trunk and commercial concentrate is presented in Table 1.

Palm oil trunk and commercial concentrate were then formulated using the Solver application in MS Excel, referring to the nutritional requirements standard for lactating dairy cows according to NRC (2001). The NRC (2001) nutritional standards used were for a 450 kg body weight (BW) cow with nutrient requirements of 64% total digestible nutrients (TDN) and 13% crude protein. Other feed ingredients used in formulating the treatment ration included coconut meal, soybean meal, corn, pollard, cassava waste, cassava meal, $CaCO_3$, and dicalcium phosphate (DCP). The composition of feed ingredients in the ration used is presented in Table 2, and the nutrient composition of the experimental diet used is presented in Table 3.

Table 1 Nutrient content of palm oil trunk and commercial concentrate (%)

Nutrient	Palm oil trunk[1]	Commercial Consentrate[2]
Dry matter	6.70	-
Ash	13.20	-
Crude protein	2.65	16
Ether extract	0.85	4
Crude fiber	34.0	7
Nitrogen-free extract	50.01	-
Ca	1.007	-
P	0.619	-
TDN[3]	62.2	68

[1]The analysis results were conducted at the Biotech Laboratory, IPB; [2]Produced by PT. Indonesia Formula Feed; [3]TDN was Ca = calcium; P = phosphor; TDN = total digestible nutrient.

Feed ingredient	Treatments			
	P0	P1	P2	P3
Elephant grass	40.00	40.00	40.00	40.00
Palm oil trunk	0.00	12.50	25.00	0.00
Commercial concentrate	0.00	0.00	0.00	60.00
Coconut meal	15.00	12.50	5.00	0.00
Soybean meal	6.60	7.01	15.00	0.00
Corn	10.0	7.00	6.40	0.00
Pollard	10.0	12.00	1.00	0.00
Cassava waste	16.08	7.50	5.87	0.00
Cassava meal	0.00	0.00	1.00	0.00
$CaCO_3$	1.19	0.96	0.00	0.00
DCP	1.12	0.52	0.73	0.00

P0 = control diet; P1 = control diet supplemented with 12.5% palm oil trunk; P2 = control diet supplemented with 25% palm oil trunk; P3 = control diet with commercial concentrate; DCP = dicalcium phosphate.

In Vitro Procedures

Collection of rumen fluid and preparation of McDougall's solution

The rumen fluid was collected using a thermos filled with hot water. The rumen fluid was obtained from three Peranakan Ongole cows with fistula at the Livestock Center (BRIN) in Cibinong, Bogor, West Java. The collected rumen fluid was then filtered using a cloth filter and transferred into the thermos, where the hot water was removed to condition it at a temperature of 39 °C. Subsequently, the rumen fluid was transported to the Dairy Cattle Nutrition Laboratory, Faculty of Animal Sciences, IPB University, Bogor.

For the preparation of the McDougall solution, 2 liters of aquadest were poured into a flask, and the following ingredients were added: $NaHCO_3$ (19.6 g), $Na_2HPO_4.7H_2O$ (7.42 g), KCl (1.14 g), NaCl (0.94 g), $MgSO_4.7H_2O$ (0.24 g), and $CaCl_2.2H_2O$ (0.08 g). $CaCl_2.2H_2O$ was added last after ensuring that the other ingredients had completely dissolved. Then, the solution was slowly flushed with CO2 gas until the pH reached 6.8.

Fermentative Digestion Procedure

Fermentative digestion was carried out *in vitro* using the method proposed by Tilley & Terry (1963). Fermenters, in the form of 50 mL polyethylene tubes, were filled with 0.5 grams of the sample, 40 mL of McDougall buffer solution, and 10 mL of fresh rumen fluid. The tubes were then flushed with CO_2 for 30 seconds and sealed with vented rubber stoppers. Subsequently, the tubes were placed in a shaker water bath at a temperature of 39 °C to create an environment similar to that inside the rumen and incubated for 4 hours. After 4 hours, the fermentation process was halted by adding 2 drops of saturated $HgCl_2$ solution. The samples were centrifuged at a speed of 3,000 rpm for 15 minutes. The supernatant was collected and stored in a freezer for further analysis of ammonia (NH_3) concentration and total volatile fatty acids (VFA) concentration.

Rumen pH Measurement

Rumen pH measurements were conducted at the end of the 4-hour incubation period using a pH meter. The pH meter was calibrated beforehand using standard pH solutions (buffers), and then the cathode was inserted into the rumen fluid, and its value was observed on the monitor screen.

Analysis of NH_3 concentration

The NH_3 concentration analysis was conducted using the Conway micro-diffusion technique (Conway, 1957). Before use, the Conway cell lips were coated with vaseline. A 1 mL sample of the supernatant resulting from fermentation was taken and placed at one end of the Conway cell groove, while at the other end, 1 mL of saturated Na_2CO_3 was introduced. Care was taken to ensure that the supernatant and Na_2CO_3 did not mix. A 1 mL solution of borate acid with methyl red indicator was placed in a small cup located at the center of the Conway cell. Subsequently, the Conway cell was immediately sealed airtight to avoid air leakage. The Conway cell was gently shaken to ensure thorough mixing of the supernatant and Na_2CO_3, and then left at room temperature for 24 hours. After 24 hours, the borate acid with the methyl red indicator was titrated with 0.0059 N H_2SO_4 until the blue color changed to red. The NH_3 concentration was calculated based on the following formula:

$$NH_3 \text{ concentration (mM)} = \frac{\text{mL } H_2SO_4 \times \text{N } H_2SO_4 \times 1000}{\text{sample weight (g)} \times \% \text{ DM of sample}}$$

Analysis of Total VFA Concentration

The total VFA concentration was determined using steam distillation. The VFA measurement procedure involved preparing the distillation apparatus by boiling water and directing the steam to the condenser or cooler. Subsequently, 5 mL of the sample and 1 mL of 15% H_2SO_4 were added to the distillation apparatus. The generated VFAs were captured with 5 mL of 0.5 N NaOH, which was placed in an Erlenmeyer flask. The liquid was collected until it reached 250—300 mL, and then 2—3 drops of an indicator were added. It was titrated with 0.5 N HCl solution until the titrant color changed from pink to colorless. The following formula was used to calculate the total VFA concentration:

$$\text{Total VFA concentration (mM)} = \frac{(a - b) \times \text{N HCl} \times 1000 \ (5 \text{ mL}^{-1})}{\text{Sample weight (g)} \times \% \text{ DM of sample}}$$

where: a = volume of HCl used in the blank titration (mL); b = of HCl used in the sample titration (mL).

Digestibility Measurement

The *in vitro* digestibility coefficients of dry matter (IVDMD) and organic matter (IVOMD) were measured using the method described by Tilley & Terry (1963). The method comprised two stages, namely the fermentation stage and the digestibility stage. The fermentation process as the initial stage was carried out in the same manner as the fermentative digestion procedure, except that the incubation time was extended to 48 hours. The fermenter tubes were then centrifuged at a speed of 3,000 rpm for 15 minutes, and the supernatant was

discarded, while the residue was used for the digestibility process in the second stage.

In the second stage, the residue was mixed with 50 mL of 0.2% pepsin-HCl solution. This mixture was then incubated aerobically for another 48 hours. After the digestion, the resulting mixture was filtered using pre-weighed Whatman No. 41 filter paper and a vacuum pump. The residue was then placed in porcelain crucibles and dried in an oven at 105 °C for 24 hours. After obtaining the dry weight, the sample was ashes in a muffle furnace at 650 °C for 4 hours. After ashing, the ash weight and the organic matter weight were determined. To calculate the IVDMD and IVOMD, the following formulas were used:

$$\text{IVDMD (\%)} = \frac{\text{initial DM sample (g)} \times \left(\text{DM residue (g)} - \text{DM blank (g)}\right)}{\text{initial DM sample (g)}}$$

$$\text{IVOMD (\%)} = \frac{\text{OM sample (g)} \times \left(\text{OM residue (g)} - \text{OM blank (g)}\right)}{\text{initial OM sample (g)}}$$

Experimental Design and Statistical Analysis

The research was conducted using a Randomized Complete Block Design (RCBD) with 4 treatments and 3 blocks. The treatments were as follows: P0 = control diet; P1 = control diet supplemented with 12.5% palm oil trunk; P2 = control diet supplemented with 25% palm oil trunk; P3 = control diet with commercial concentrate. The rumen fluid from three Peranakan Ongole cows with fistula was used as the blocks. The data obtained were analyzed using analysis of variance (ANOVA), and when significant differences were observed ($p<0.05$), further Duncan's test was performed (Steel & Torrie 1993).

RESULTS AND DISCUSSION

Rumen Fermentation Characteristics

The characteristics of rumen fermentation of the treatment diets were observed through the values of rumen pH, ammonia (NH_3) concentration, and total volatile fatty acids (VFA). The appropriate rumen pH value is crucial for sustaining the life of anaerobic microorganisms involved in the fermentation process. Rumen fermentation of feed generates volatile fatty acids (VFA) as the main products, providing energy and carbon for the growth and maintenance of the microbial community. Understanding VFA production is vital in comprehending carbohydrate fermentation processes and their implications for livestock productivity, as most VFAs in the rumen originate from the fermentation of dietary carbohydrates (Rosmalia *et al.* 2023). Ammonia (NH_3) is a critical component for the synthesis of amino acids and microbial cell protein. It serves as the primary center for nitrogen metabolism in the rumen, representing the final product of protein fermentation (Dewhurst & Newbold 2022). The characteristics of

Table 3 The rumen fermentation characteristics of the experimental rations

Treatments	Rumen pH	NH_3 concentration (mM)	Total VFA concentration (mM)
P0	6.73±0.05	5.80±1.53[b]	110.39±17.33[b]
P1	6.77±0.05	7.33±1.06[a]	127.82±17.41[a]
P2	6.70±0.00	8.22±0.95[a]	137.94±16.66[a]
P3	6.73±0.05	5.50±0.65[b]	110.54±17.95[b]

P0 = control diet; P1 = control diet supplemented with 12.5% palm oil trunk; P2 = control diet supplemented with 25% palm oil trunk; P3 = control diet with commercial concentrate; Different superscript letter in the same column indicate statistically significant on $p<0.05$

rumen fermentation in the treatment ration are presented in Table 3.

The research findings indicate that the rumen pH values among the treatment groups did not exhibit significant differences and remained within the ideal range of 6.0—7.0 to support the growth and activity of rumen microorganisms (McDonald *et al.* 2010; Rosmalia *et al.* 2022c). This aligns with a study by Astuti & Yelni (2015), which examined the fermentation characteristics of palm oil waste using local microbial species, resulting in a rumen pH of 6.85 — 6.88. Maintaining a normal rumen pH ensures undisturbed microbial growth and metabolism, thus facilitating optimal microbial activities and efficient feed digestion. Conversely, a rumen pH below 6.0 can negatively impact fiber digestion in the rumen (Sung *et al.* 2007).

Regarding ammonia (NH_3) concentrations, the analysis of variance revealed significant variations ($p<0.05$) due to the addition of palm oil trunk in the rations. There was an increase in NH_3 by 26.38% and 41.73% in treatments P1 and P2, respectively, compare to the control diet. Noershidiq *et al.* (2020) reported that *in vitro* ammoniation of palm oil trunk, yielding an NH_3 concentration ranged from 6.11 mM to 10.00 mM. The inclusion of palm oil trunk, up to 25%, increased NH_3 concentration due to the high availability of non-protein nitrogen (NPN) as part of rumen degradable protein (RDP) (Ginting *et al.* 2018; Noersidiq *et al.* 2020). High RDP supply lead to increased ammonia levels within the rumen, potentially enhancing microbial protein synthesis (Rebelo *et al.* 2019; Rosmalia *et al.* 2022b). Nevertheless, including palm oil trunk in dairy ration should be couple with other protein-rich feedstuffs such as soybean meal. Soybean meal possesses a high biological value, approximately 63% - 76%, significantly surpassing the value for palm kernel meal, which stands at 42.8% (Stein *et al.* 2015). The NH_3 values in all treatments remained within the normal range. According to McDonald *et al.* (2010), the optimal concentration of NH_3 in the rumen falls within the ranges from 5 - 18 mM.

The total VFA concentration showed significant differences ($p<0.05$) due to the addition of palm oil trunk flour, with values ranging from 127.82- 137.94 mM. The

total VFA concentration in all treatments remained within the optimum range of 70 - 150 mM, supporting rumen fermentation processes and microbial growth (McDonald *et al.* 2010). This result was significantly higher compared to the study of Astuti & Yelni (2015), reporting a VFA concentration of 54.46 - 72.26 mM in palm waste silage. The high VFA content in treatments P1 (15.79%) and P2 (24.96%) can be attributed to the abundant starch content and RDP in palm oil trunk, which easily undergoes rumen degradation, resulting in increased energy production. According to Yusra *et al.* (2020), palm oil trunk contained 95.56% starch. Besides its high fermentability for microbial growth and ease of rumen digestion, it also offers high digestibility for the host. The rumen's VFA concentration is crucial for dairy cattle production, providing 50 - 70% of the digestible energy. Brooks *et al.* (2012) also reported that high RDP content in the diet increased the total VFA production. Other factor affected total VFA concentration were the availability of soluble substrate and mineral content in the diet especially sulfur (Judd & Kohn 2018; Rosmalia *et al.* 2022a).

In Vitro Digestibility of Dry Matter and Organic Matter

Digestibility values are among the essential indicators in determining the quality of feed for livestock. The higher the digestibility values, the greater the potential for nutrients to be absorbed and metabolized by the animal's body. Table 4 presents the impact of treatments on the dry matter digestibility coefficient (IVDMD) and organic matter digestibility coefficient (IVOMD).

Results revealed a significant impact ($p < 0.05$) of palm oil trunk utilization in the diet of dairy cows on the values of IVDMD and IVOMD. The digestibility values in treatment P1 increased by 4.9% and 5.45% for IVDMD and IVOMD, respectively, compared to the control diet (P0). Furthermore, as the addition of palm oil trunk flour increased to 25% in treatment P2, the IVDMD and IVOMD values rose to 82.49% and 81.62%, respectively, showing an increment of 10.47% for IVDMD and 10.91% for IVOMD.

Table 4 The IVDMD and IVOMD of experimental rations (%)

Treatments	IVDMD	IVOMD
P0	74.67±4.26[bc]	73.59±4.06[bc]
P1	78.33±4.50[ab]	77.60±4.37[ab]
P2	82.49±1.81[a]	81.62±1.97[a]
P3	73.31±2.40[c]	72.50±2.38[c]

P0 = control diet; P1 = control diet supplemented with 12.5% palm oil trunk; P2 = control diet supplemented with 25% palm oil trunk; P3 = control diet with commercial concentrate; IVDMD = *in vitro* dry matter digestibility; IVOMD = *in vitro* organic matter digestibility; Different superscript letter in the same column indicate statistically significant on $p < 0.05$

The IVDMD and IVOMD values obtained in this study were higher than those reported Tafsin *et al.* (2018), who investigated palm oil trunk treated by local microorganism and obtained IVDMD and IVOMD values of 33.45%–59.71% and 50.31%–74.91%, respectively. Harahap *et al.* (2021) reported that the inclusion 60% palm oil trunk treated with fiber cracking technology resulted 50.97% IVDMD and 54.94% IVOMD. The high digestibility in the diets based on palm oil trunk can be attributed to the high content of cellulose and hemicellulose in the palm oil trunk, which can serve as an energy source for livestock. According to Lai & Idris (2013), the cellulose and hemicellulose content in the palm oil trunk is 50.78% and 30.36%, respectively. The easily soluble carbohydrates (starch) and crude protein present in the palm oil trunk can also enhance nutrient digestibility (Yusra *et al.* 2020). The addition of alkali to the palm oil trunk can make it suitable for up to 30% of the total feed in the diet, resulting in a live weight gain of 0.66 - 0.72 kg per head in beef cattle (Prabowo and Susanti 2016).

CONCLUSION

The utilization of 25% palm oil trunk waste in the dairy ration based on the forage: concentrate ratio of 40:60 was found to effectively increase the NH_3 concentration by 41.73%, the total VFA concentration by 24.96%, the dry matter digestibility coefficient by 10.47%, and the organic matter digestibility coefficient by 10.91%. Moreover, it did not have any significant impact on the rumen pH value.

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