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Nutritional Improvement of Palm Oil Fronds for Ruminant Feedstuffs Using a Local Biotechnological Approach

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Abstract: This study aimed to evaluate the nutrient contents of palm fronds fermented with microorganisms derived from the rumen contents, feces and urine of local cows. The study was conducted using a completely randomized, two factorial 2 x 3 design and three replications for each treatment. Factor A was the length of incubation (A1 = 7 days and A2 = 14 days) and factor B was the type of medium (B1 = rumen contents, B2 = cattle feces and B3 = cattle urine). The observed variables were the dry matter, organic matter, crude protein and crude fiber content and the digestibilities of the dry matter and organic matter in the fermented palm fronds. The experimental results revealed that fermentation reduced the dry matter, organic matter and crude fiber content ($p<0.05$). However, fermentation increased the crude protein content (<0.05). Neither the incubation length nor the microorganism source influence ($p>0.05$) the dry matter or organic matter digestibilities. There were interaction effects between the factors on the dry matter and organic matter digestibilities. In conclusion, palm oil fronds fermented with rumen contents for 7 days produced in the highest protein content. The greatest dry matter digestibility was observed in the palm oil fronds that were fermented with rumen contents and the greatest organic matter digestibility was observed in the palm oil fronds that were fermented with cattle urine for 7 days.

Key words: Palm oil fronds, nutrient content, local microorganisms

INTRODUCTION

The palm oil crop is the most widely planted oil tree crop in Indonesia. A palm oil plant has 48 pieces of petiole and 250-300 leaves (Syarif, 2007). Simanihuruk *et al.* (2008) stated that palm fronds have less crude protein than natural grasses. Palm oil fronds contain 609 g NDF, 581 g ADF, 28 g hemicellulose and 481 g cellulose (Bengaly *et al.*, 2010) and are composed of 4.07% crude protein and 94.47% organic matter (Akbarillah and Hidayat, 2009).

Fermentation plays an important role in the nutritional improvement of feeds. Fermentation yields the bioactive peptides that result from protein cleavage therefore this process increases the biological properties of feedstuffs (Steinkraus, 2002). Additionally, fermented products are a good source of peptides and amino acids (Rajapakse *et al.*, 2005). Experimental results have revealed that fermentation can increase crude and soluble protein levels increase the cleavage of protein to peptides and amino acids (Susi, 2012; Lahay and Rinduwati, 2007), increase the crude protein and fat digestibilities, improve the amino acid balance (Ari *et al.*, 2012; Kaleka and Haryadi, 2013; Susi, 2012) and reduced the crude fiber (Lahay and Rinduwati, 2007).

Aspergillus sp and *Lactobacillus* sp might have the potential to improve nutritional values and digestibility. Astuti *et al.* (2014) reported that the usage of local wastes, such as local microorganism sources is beneficial due to, lower costs and easier processes. Therefore, the present study was conduct to evaluate the effects of microorganisms from local sources and different lengths of incubation on the nutritional compositions of palm oil fronds. Astuti and Amir (2013) reported that the organic matter digestibility of banana peels is increased from 45.08 to 57.23% when they are fermented with rumen contents. Paul *et al.* (2004) reported that anaerobic fungi isolated from the feces of wild ruminants exhibit strong ligno-cellulolytic activity and increase nutrient availability when inoculated into other ruminants. Vimal *et al.* (2007) also reported that anaerobic fungi derived from the wild blue bull exhibit cellulolytic activity compared with anaerobic fungi from sheep and cattle. Although some studies have reported that bacterial inoculants affect the fiber concentrations

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and the digestibilities of grass-legume forage and corn silage (Harrison *et al*., 1989; Schaefer *et al*., 1989), local microorganism sources are still expected to be a good source of microorganisms for the fermentation of feedstuffs. The present study was performed to analyze the utility of local microorganism sources, specifically rumen contents, cattle feces and cattle urine for improving the nutritional compositions of palm oil fronds. The usage of these wastes as fermenters would partially solve the problems that result from these wastes.

MATERIALS AND METHODS

Local microorganism sources: Three types of microorganisms, i.e., those sourced from rumen contents, cattle feces and cattle urine, were used in the present study.

The isolation techniques involved the pour plate method and the scratch plate method. The gram staining tests used a set of gram stains, i.e., crystal violet, lugol, 96% alcohol and safranin. Purple results indicated that the bacteria were gram-positive and red results indicated that the bacteria were gram negative. The gram staining tests involved special stains, i.e., malachite green and safranin. Endospora staining used malachite green reagent and safranin. A green color indicated that the bacteria had endospores (Pratita and Rosa Putra, 2012).

Fermentation process: The rumen contents, feces and urine of cattle were collected from the fields. These materials were placed in tubes and sugar and coconut water were added. Next, the tubes were incubated for 10 days in anaerobic conditions. The fronds and leaves of the palm oil fronds were crushed into small pieces using a manual chopper. The crushed palm oil fronds were then incubated with the rumen contents, cattle feces or cattle urine for 7 or 14 days, respectively.

Experimental design: The study was undertaken with a completely randomized design with two factors of 2 x 3 and each treatment was replicated three times. Factor A was the incubation length, i.e., 7 (A1) or 14 days (A2). Factor B was the source of the local microorganism based on the rumen contents (B1), cattle feces (B2) or cattle urine (B3).

The observed variables included the dry matter, organic matter, crude protein and crude fiber contents of the palm oil fronds. The above nutritional components were analyzed using the AOAC method (2012). The digestible nutrients were analyzed according to the methods of Tilley and Terry (1963). The identification of the microbes was performed base on morphological identification.

Statistical analysis: All of the data were subjected analysis of variance (Toutenburg and Shalabh, 2009) and significant differences were further tested with using Duncan's multiple range test.

RESULTS AND DISCUSSION

Local microorganisms in the rumen contents, feces and urine: The experimental results revealed that there were 8 thermophilic bacteria isolates from the rumen, 2 bacterial isolates from the fecal samples and 2 isolates from the cow urine samples. Gram staining results revealed that the bacteria of two of the samples (i.e., the rumen contents and cow urine samples) were gram-positive bacteria, whereas the fecal samples were gram-negative. The outer membranes of the gram positive bacteria were composed of peptidoglycans as indicated by the finding, that the gram staining produced a purplish blue color.

The gram-negative bacterial cell walls were composed of several layers of peptidoglycans and outer membrane. The outsides of the cell walls of the gram-negative bacteria were the outer membranes because the gram staining produced a pink color. Pink was the color of the safranin staining of the outer membrane. The outer membrane structure was similar to that of the cell membrane (Purwoko, 2009).

The endospore staining test results revealed that the rumen contents contained bacteria that produced endospores. Tarigan (1988) stated that endospores are found only in bacteria with thick-walled, highly refractive and very resistant structures. Such structures are produced by all species of *Bacillus*, *Clostridium* and *Sporosarcina*. The most important of bacteria that form endospores are the members of the genera *Bacillus* and *Clostridium*. Endospores are structures that are resistant to extreme environmental conditions such as dry, warm and acidic conditions. Endospores are very dense and refractive due to their very low water content. (Pelczar and Chan, 1986).

The catalase test results revealed that all of the isolates (12 isolates) exhibited positive results, as indicated by the emergence of oxygen bubbles from the surfaces of the isolates when they were dropped into H_2O_2. According to Lay in Sutiamihardja (2008), a catalase test proved that the catalase enzyme from the bacteria was capable of converting H_2O_2 to H_2O and O_2.

Composition of palm oil fronds: Table 1 illustrates the composition of the palm oil frond before fermentation. The palm oil frond contained 4.84% crude protein, 33.47% crude fiber, 92.15% dry matter and 90.27% organic matter.

Effect of fermentation on the dry matter content: The experimental results revealed that the length of incubation significantly ($p<0.05$) affected the dry matter contents (Table 2), but the local microorganism sources exhibited no effect on the dry matter content of the palm

Table 1: Dry matter, organic matter, crude protein and crude fiber contents of the palm oil fronds before fermentation

Nutrients	Nutrient content (%)
Dry matter	92.15
Organic matter	90.27
Crude fiber	33.47
Crude protein	4.84

oil fronds. There was no interaction effect of the incubation length and the local microorganism sources on the dry matter contents.

The palm oil fronds that were fermented over 14 days exhibited lower dry matter contents than those that were fermented for 7 days. The fermentation required O_2 for respiration and produced CO_2 and H_2O. Gervais (2008) stated that the change in dry matter content resulted from the growth of microbes and changes in moisture content. Changes in moisture content occurred due to substrate hydrolysis or metabolic water production. A change in the dry matter content of the feedstuffs occurred due to microbial growth and water content changes. Fardiaz (1989) stated that during the fermentation process, the microorganisms use carbohydrates as an energy source to produce H_2O and CO_2 molecules. Most of the H_2O stay in the product and the moisture content of the product thus increases. The present research demonstrated that longer incubation resulted in a decrease in the dry matter content of the palm oil fronds. Additionally, these results are similar to those of Sasongko (2009) who demonstrated that longer fermentation times increase both the breakdown components and the amount of independent water. Longer fermentation times increase the degradation of materials which results in an increase in the amount of liberated bound water.

Effects fermentation on the organic matter content: The incubation length had no effect ($p>0.05$) on the organic matter content or the microorganism sources. There was no interaction effect ($p>0.05$) between the length of incubation and the local microorganism sources on organic matter content of the palm oil fronds. The average organic matter content following incubation for 7 days was 89.12% and that following incubation for 14 days was 86.44%. The fermentation of palm oil fronds with different sources of local microorganism rumen contents, cattle feces and cattle urine resulted in no differences in the organic matter contents. The greatest organic matter contents of the palm oil fronds resulted from fermentation with local microorganisms sourced from rumen contents (88.34%), followed by cattle feces (88.09%) and cattle urine (86.91%).

Effects of fermentation on the crude fiber content: There was no interaction ($p>0.05$) between the factors on the crude fiber content of palm oil fronds (Table 2).

Table 2: Average dry matter, organic matter, crude protein and crude fiber contents of the palm oil fronds after fermentation

	Local microorganism source			
Incubation length	B1	B2	B3	Average
Dry matter				
A1	89.52	92.23	91.08	90.94^{A}
A2	92.17	89.90	89.56	90.54^{A}
Average	90.84	91.06	90.32	
Organic matter				
A1	89.46	88.74	89.15	89.12^{A}
A2	87.23	87.43	84.67	86.44^{B}
Average	88.34	88.09	86.91	
Crude fiber				
A1	31.89	31.72	30.96	31.52^{A}
A2	30.14	28.71	26.39	28.41^{A}
Average	31.01	30.21	28.68	
Crude protein				
A1	6.15^{aB}	5.61^{aB}	5.38^{bA}	5.71
A2	7.06^{aA}	7.06^{aA}	5.27^{bA}	6.46
Average	6.61	6.34	5.32	

A1: Incubation for 7 days, A2: Incubation for 14 days, B1: Rumen contents, B2: Cattle feces, B3: Cattle urine. The superscripts A and B indicate significant differences between the columns and the superscripts a, b and c indicate significant differences within the rows ($p<0.05$)

The local microorganism source had no significant effect ($p>0.05$) on the crude fiber content of the palm oil fronds, whereas the incubation length significantly affected ($p<0.05$) the crude fiber content. The data revealed that the longer incubation times for fermentation decreased the crude fiber in the palm oil frond. In Indeed, the results of this research are similar to those of Hernaman *et al*. (2007), who stated that the activity of cellulolytic microbes changes crude fiber into soluble compounds. Hernawati *et al*. (2010) stated that microbes can use crude fiber for nutrition for optimal growth. Local microorganism sources, such as rumen contents and cattle feces and urine, are rich in microbes that act to ferment feedstuffs.

Effects of fermentation on the crude protein contents: There was a significant interaction ($p<0.05$) between the factors, i.e., incubation length and the source of the local microorganisms, on the crude protein content of the palm oil fronds. The greatest results were observed with the palm oil fronds that were fermented with the rumen contents and the cattle feces, with incubation times as long as 14 days. The crude protein content of the unfermented palm oil fronds was 4.46, whereas those of the palm oil fronds that were fermented with the rumen contents, cattle feces and cattle urine were 6.61, 6.34 and 5.32%, respectively. The crude protein contents of the palm oil fronds that were fermented for 7 and 14 days were 5.7 and 6.5%, respectively. The lower protein contents of the palm oil fronds that were fermented with cattle urine might have been caused by the lower numbers of microbes in the cattle urine.

Table 3: Effects of incubation length and microorganism source on the dry matter and organic matter digestibilities

	Local microorganism source			
Incubation length	B1	B2	B3	Average
Dry matter				
A1	17.29^{bA}	29.87^{aA}	27.14^{aA}	24.77
A2	17.76^{bA}	21.69^{aB}	24.44^{aA}	21.30
Average	17.53	25.78	25.79	
Organic matter				
A1	15.70^{bA}	16.48^{bB}	27.55^{aA}	19.91
A2	20.60^{bB}	25.63^{aA}	21.71^{bB}	22.65
Average	18.15	21.06	24.63	

A1: Incubation for 7 days, A2: Incubation for 14 days, B1: Rumen contents, B2: Cattle feces, B3: Cattle urine. The superscripts A and B indicate significant differences within the columns and the superscripts a, b and c indicate significant differences within the rows ($p<0.05$)

The results of this study agree with the observations of Astuti and Amir (2013). Krishna and Devi (2005) stated that increases in crude protein might be affected by the additional protein of the microbes, which produce approximately 40-65% protein. Wahab *et al.* (2014) found that decreases in crude fiber content were accompanied by increases in other nutrients, such as organic matter and crude protein.

Effects of fermentation on the digestibility of the dry matter: Incubation length had no effect on dry matter digestibility ($p>0.05$). A similar result was found for the microorganism source. There was a significant interaction effect ($p<0.05$) of the factors (Table 3) on the dry matter digestibility of the palm oil fronds. The greatest dry matter digestibility of the palm oil fronds was observed for the palm oil fronds that were fermented with cattle feces for 7 days and the lowest digestibility was observed for the palm oil fronds that were fermented with the rumen contents for 7 days. These findings indicate that the cattle feces and urine had more microbes than the rumen contents. Zain *et al.* (2011) stated that the supplementation of diets with probiotics (microbes) improves the growth of microbes in the rumen and increases nutrient digestibility. These finding contrast with those of the research of Afdal and Toha (2007), who found that the dry matter digestibility of feedstuffs that are fermented with rumen contents is higher than that of those that are fermented with feces. Lee *et al.* (2014) found that the anaerobic fermentation of rice straw with rumen and abomasum contents increases the dry matter digestibility.

Effects of fermentation on the digestibility of the organic matter: The incubation length had no effect on the organic matter digestibility ($p>0.05$). A similar result was found for the microorganism source. The experimental results revealed that there was a significant interaction effect ($p<0.05$) between the factors on the digestibility of the organic matter of the palm oil fronds. Longer incubation lengths combined with fermentation with local microorganism sourced from the rumen contents and cattle of feces resulted in increases in the digestibility of the organic matter of the palm oil fronds. The greatest digestibility of the organic matter of the palm oil fronds was observed for the palm oil fronds that were fermented with cattle urine over 7 days of incubation.

Conclusion: The results of this study demonstrated that the fermentation of palm oil fronds with microorganism from local sources decreased the dry matter, organic matter and crude fiber contents. However, this fermentation increased the crude protein contents. In conclusion, the palm oil fronds that were fermented with rumen contents for 7 days exhibited the greatest protein contents. The greatest dry matter digestibility was observed in the palm oil fronds that were fermented with the rumen contents for 7 days and the greatest organic matter digestibility was observed for the palm oil fronds that were fermented with cattle urine for 7 days.

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