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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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# Research Article
# *In-vitro* Digestibility of Palm Leaf Waste Treated with Different Processing Methods

Suyitman, Lili Warly and Evitayani

Faculty of Animal Husbandry, Andalas University Padang, West Sumatra, Indonesia

## Abstract

**Background and Objective:** Leaf waste from palm oil plants has not been widely utilized by farmers due to the low biological quality of the palm leaf midrib. Efforts to optimize the utilization of waste-derived feed include physical, chemical or biological processing or a combination of those techniques. This research was conducted to determine the best palm leaf processing method to increase the nutrient content and digestion of the palm leaf midrib. **Materials and Methods:** This study used completely randomized design with 5 treatments and 4 replications. Treatments were = A: Control (without treatment), B: Physical processing (steam), C: Chemical treatment (ammonia), D: Biological treatment (ensilage) and E: Chemical-physical combination (steam-ammonia). The parameters that were measured included the nutrient content, dry matter digestibility, organic matter, crude protein, *in vitro* fibre fraction and rumen fluid characteristics. **Results:** The processing of oil palm leaf pole with ammonia (treatment C) resulted in better nutrient contents and *in vitro* digestion for cut palm leaves than other treatment methods ($p<0.05$). Leaves in treatment C had the following nutrient contents: Dry matter (40.51%), organic matter (84.25%), crude protein (13.75%), neutral detergent fibre (NDF) (54.76%), acid detergent fibre (ADF) (42.54%), cellulose (20.77%), hemicellulose (12.22%) and lignin (10.74%). *In vitro* digestion resulted in the following nutrient contents: Dry matter (34.53%), organic matter (41.65%), crude protein (45.32%), NDF (30.71%), ADF (24.28%), cellulose (31.39%) and hemicellulose (51.78%). The rumen fluid characteristics were as follows: pH (7.02), VFAs (135.93 mM) and $NH_3$-N (58.90 mg/100 mL). **Conclusion:** Treating cut palm leaf poles with ammonia results in better nutrient contents and *in vitro* digestion than physical processing (steam), biological treatment (ensilage) or a combination of physical and chemical processing (steam-ammonization).

Key words: Palm leaf stem, steam, ammonization, ensilage, steam-ammonization, nutrient digestibility

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Corresponding Author: Suyitman, Faculty of Animal Husbandry, Andalas University Padang, West Sumatra, Indonesia



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

Leaves from palm oil plants are a waste product of palm oil plantations that could potentially become a source of forage for ruminant livestock. The maintenance of palm plants typically involves pruning leaves or leaf midribs. This activity first occurs in the eighth month after the first harvest and pruning is subsequently rotated every six months on plants that are under ten years old and every 8 months for plants that are over ten years old[1]. Furthermore, it involves away bark that is old and no longer efficient in assimilating nutrients and cutting stems attached to fruit bunches. In addition to the pruning programme, palm fronds are also cut regularly every harvest. Palm oil production will continue to increase as the palm oil plantation acreage grows. This indicates that palm has considerable potential as feed forage for ruminants.

Although palm leaf midribs have substantial potential as a feed forage, their utilization as an animal feed is still very limited. This limit is partly due to the low biological quality of palm leaf midribs. The lignin content of oil palm leaves is quite high at 27.6% and this high content leads to a low digestibility of palm leaf stems[2]. The *in vitro* digestibility of small palm oil leaves is approximately 50% and it is recommended that only 15-20% of palm leaves are used in rations. To use more than 40% of palm leaves in rations, it is necessary to process them using physical, chemical or biological treatment or a combination of these methods[3]. This study was conducted to determine the best palm leaf processing method to increase the nutrient content and digestibility of palm leaf midribs.

## MATERIALS AND METHODS

**Materials:** Palm leaves were collected at the Smart Farmers Group located at Block A Sitiung II, Jorong Koto Hilalang II, Nagari Sungai Langkok, Tiumang District, Dharmasraya Regency. Analysis of the processed palm leaf bark was conducted in the Ruminant Nutrition Laboratory of the Faculty of Animal Husbandry at Andalas University of Padang from January-June, 2017. Research materials included chopped palm leaves, urea fertilizer, 5-kg plastic bags, plastic straps, fine bran and chemicals for proximate analyses. The following factors were measured according to the Van Soest method: *In vitro* digestibility and rumen fluid characteristics. The equipment used in this study included a chopper, machetes, autoclave and the equipment for performing proximate analyses, the Van Soest method, *in vitro* digestibility assays and other analysis.

**Methods:** This study used a completely randomized design with 5 ration treatments and 4 groups. The treatments include the following:

A = Control (without treatment)
B = Physical processing (steam)
C = Chemical treatment (ammonization)
D = Biological treatment (ensilage)
E = Physical-chemical combination (steam-ammonization)

The design model used is Steel and Torrie [4]:

$$Yij = \mu + P_i + K_j + \epsilon_{ij}$$

Where:

$Y_{ij}$ = Observed value on the ith treatment and jth repetition
U = Common middle value
$K_j$ = Influence of group j
$P_i$ = Effect of the ith treatment
$E_{ij}$ = Effect of the rest of the ith treatment of the kth group

**Parameters observed:** The observed parameters included the nutrient content of processed palm leaf preparations (proximate analyses: Dry matter, organic matter, crude protein; Van Soest method: Neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose and hemicellulose) and measurements of dry matter digestibility, organic matter, crude protein and *in vitro* fibre fractions (NDF, ADF, cellulose, hemicellulose). Characteristics of the rumen fluid were also measured (i.e., pH, volatile fatty acids (VFAs) and ammonia-nitrogen ($NH_3$-N)).

**Statistical analysis:** All data obtained were analyzed by one-way analysis of variance and the differences between treatments were tested by Duncan's multiple range test (DMRT), with $p<0.05$ indicating a significant difference.

## RESULTS AND DISCUSSION

**Processing of palm oil leaves:** In this study, several methods of processing palm oil leaf midribs were tested, including physical, chemical and biological processing and combinations of those methods. The processed products were analyzed for nutritional content and tested for *in vitro* digestibility. The goal of the analyses was to determine the best processing method that can improve the digestibility and nutritional value of palm leaf midribs.

The nutritional content of palm leaves processed using the different methods can be seen in Table 1, which shows that the contents of processed palm oil leaves ($p<0.05$) are affected by treatment. The dry matter content of processed palm leaves in this study ranged between 40.51 and 61.32%. The DMRT results showed that treatment with steam and steam-ammonization significantly increased the dry matter content compared to the control, whereas ammonization and ensilaging significantly decreased the dry matter content compared to the control. However, the difference in dry matter content between ammonization and ensilaging treatments was not significant and there was a similar result for steam-ammonization.

The increase in dry matter content in the steam and steam-ammonization processing is caused by the loss of some water from the material through evaporation. During steaming, cell walls are stretched by the vapor pressure from the hot steam. This causes the cell walls to become looser, reducing the amount of water in the cavity between the cell walls. This loss of water from the material leads to an increase in the dry matter content. In this study, the dry matter increased by 10.14-12.29% in palm leaf stalks compared to the control group.

The ammonization and ensilaging treatment significantly reduced the dry matter content of palm leaves. This occurs due to the dissolution of some soluble fractions as a result of a chemical reactions in the ammonization process and dissolution in the effluent that is lost in the ensilaging process.

The organic matter content of processed palm leaf bark was significantly decreased compared to control cells. However, there was no significant difference between the treatments. The organic matter content decreased by 2.85-4.29% compared to the control. This decrease occurred due to the loss of some organic material during the processing.

The protein content of roughly processed palm leaf gel significantly ($p<0.05$) increased by 46.07-83.09% compared to the control. The steam treatment increased the crude protein content by 51.40% compared to the control due to the increase in dry matter content, while ensilaging increased the protein content by 46.07% due to the contributions of lactic acid bacteria during processing. The highest increase in protein content occurred in the ammonization and steam-ammonization treatments (78.56-83.09%) due to the addition of urea, which is a source of N and according to the opinion of Suyitman *et al.*[5], ammonia with urea in fibrous feed can loosen the bonds in the lignocellulose that is then more easily digested by rumen bacteria and is also capable of supplying nitrogen for the growth of these bacteria.

Steam treatment, ammonization and ensilaging significantly ($p<0.05$) decreased the fibre fraction content, i.e., NDF, ADF, cellulose and hemicellulose content. This result consistent with Warly[6], who reported that pre-treatment could be used for highly fibrous material to increase the potential digestibility of crude fibre. According to a previous research[7], pre-treatment is useful for increasing the rate of lignocellulose hydrolysis. The greatest decrease in the fibre fraction occurred with the ammonization and ensilaging treatments.

The fibre fraction content in the steam-ammonization (E) treatment was similar to that of the control (A) and there was no decrease in the fibre fraction. This outcome suggests that steam treatment followed by ammonization is ineffective for reducing the fibre fraction since the steam processing removes some soluble substances through evaporation, which leaves only soluble and ammoniacal substances, which are unable to reduce the fibre fraction content.

Treatments significantly ($p<0.05$) decreased the lignin content of palm leaves by 25.29-32.34% compared to the control. The decrease in lignin content was due to the influence of heat generated during processing. Lignin can be broken down by heat. The heat produced during the processing can cause the expansion of cell walls, causing some of the lignin and silica deposits present in the cell wall to fall out and making the material more open to digestion by rumen bacteria. This result is in line with Warly[6], who stated

Table 1: Nutritional content of processed palm oil leaf preparations (% DM)

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| Parameters | A | B | C | D | E | SE |
| Dry material | 54.61$^{b}$ | 60.15$^{a}$ | 40.51$^{c}$ | 41.36$^{c}$ | 61.32$^{a}$ | 0.21 |
| Organic materials | 87.15$^{a}$ | 83.41$^{b}$ | 84.25$^{b}$ | 84.15$^{b}$ | 84.67$^{b}$ | 0.24 |
| Crude protein | 70.51$^{c}$ | 11.37$^{b}$ | 13.75$^{a}$ | 10.97$^{b}$ | 13.41$^{a}$ | 0.13 |
| NDF | 63.76$^{a}$ | 58.93$^{b}$ | 54.76$^{c}$ | 53.82$^{c}$ | 62.15$^{a}$ | 0.15 |
| ADF | 45.64$^{ab}$ | 43.91$^{bc}$ | 42.54$^{c}$ | 41.34$^{c}$ | 47.27$^{a}$ | 0.13 |
| Cellulose | 25.76$^{a}$ | 21.84$^{b}$ | 20.77$^{b}$ | 21.47$^{b}$ | 22.07$^{b}$ | 0.03 |
| Hemicellulose | 18.12$^{a}$ | 15.02$^{b}$ | 12.22$^{c}$ | 12.48$^{c}$ | 14.88$^{b}$ | 0.17 |
| Lignin | 15.74$^{a}$ | 11.36$^{b}$ | 10.74$^{b}$ | 10.65$^{b}$ | 11.76$^{b}$ | 0.19 |

Values with different superscripts in the same row have significant differences ($p<0.05$), A: Control, B: Steaming, C: Ammonization, D: Ensilaging, E: Steam-ammonization
SE: Standard error

Table 2: *In vitro* digestibility of cut palm oil leaves treated with different treatment methods (%)

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| Parameters | A | B | C | D | E | SE |
| Dry material | 30.55$^{c}$ | 35.91$^{a}$ | 34.53$^{a}$ | 30.32$^{c}$ | 33.25$^{b}$ | 0.14 |
| Organic materials | 37.57$^{b}$ | 40.34$^{a}$ | 41.65$^{a}$ | 36.47$^{c}$ | 38.78$^{b}$ | 0.09 |
| Crude protein | 18.71$^{d}$ | 40.72$^{b}$ | 45.32$^{a}$ | 40.57$^{b}$ | 33.78$^{c}$ | 0.11 |
| NDF | 30.01$^{c}$ | 34.69$^{b}$ | 30.71$^{c}$ | 32.75$^{b}$ | 35.91$^{a}$ | 0.10 |
| ADF | 17.68$^{c}$ | 35.29$^{a}$ | 24.28$^{b}$ | 24.74$^{b}$ | 26.72$^{b}$ | 0.29 |
| Cellulose | 28.76$^{b}$ | 40.85$^{a}$ | 31.39$^{b}$ | 27.49$^{c}$ | 39.31$^{a}$ | 0.06 |
| Hemicellulose | 60.72$^{b}$ | 40.87$^{d}$ | 51.78$^{c}$ | 52.97$^{c}$ | 63.98$^{a}$ | 0.15 |

Values with different superscripts in the same row are significantly different ($p<0.05$)

that lignin cannot be digested by rumen microbes and is a limiting factor in the utilization of fibrous feed, especially for agricultural waste. Lignin in the cell wall binds to cellulose and hemicellulose to form lignocellulose and lignohemicellulose complexes. This causes cellulose and hemicellulose, which are a source of energy, to become less useful. Waste feed processing is an effort to reduce the lignin content to increase the feed material's digestibility.

**Digestibility of palm oil leaves:** There was an increase in the nutrient content, as seen in Table 2, which was followed by an increase in dietary digestibility. The data for the *in vitro* digestibility of the processed leaves are shown in Table 2.

The results of the statistical analysis showed that treatment of palm leaf blight significantly ($p<0.05$) influenced the digestibility of the leaves. DMRT results revealed that the steam treatment ($p<0.05$) improved the digestibility of the dry matter, organic matter, crude protein and the fibre fraction compared to unprocessed palm leaves (control). The increased digestibility of the leaves is due to the stretching of the cell wall surface structure due to the influence of steam pressure during heating. This allows easier digestion by rumen microbes. This result agrees with Sa'id[8], who stated that the working principle of applying vapor pressure to a substrate is to develop the fibres or complex bundles of feed material so that it can be easily digested by microorganisms. Breaking glycosidic bonds or lignocellulosic bonds increases the surface area of the substrate, facilitating the penetration of microbial enzymes into the substrate. Treatment with steam pressure is quite effective for improving the palatability and digestibility of foodstuffs.

Treatment C (ammonization) significantly ($p<0.05$) improved the digestibility of dry matter, organic matter, crude protein and the fibre fraction compared to unprocessed palm leaves (control = A). Degradation, especially of protein, was highest for the ammonium treatment compared to the other treatments. This result agrees with the study of Suyitman *et al.*[5], who showed that ammonization with urea of fibrous feed can loosen the lignocellulose bonds and make it more easily digestible by rumen bacteria and can supply nitrogen for bacterial growth.

Dietary digestibility of palm leaf ensilage (D) was lower than the control. This decrease is due to complex molecular structures being simplified by the activity of lactic acid bacteria during ensilaging process. Ensilage is freshly preserved forage kept under anaerobic conditions. In the ensilaging process, fermentation occurs through the activity of anaerobic lactic acid bacteria and lactic streptococcus that live at pH 4. Due to the activity of these bacteria and the decrease in pH, the growth of other bacteria that causes decay in the forage from silos can be prevented[7].

The steam-ammonization (E) treatment in this study improved the digestibility of palm leaves. As indicated by the degradation of dry matter and improvement in organic matter relative to treatment A (control), there was a significant improvement in the digestibility of crude protein and the fibre fraction. This decrease occurred because during the steam treatment some soluble substances were dissolved and lost so that only the insoluble fraction remained in the ammonization process and the content of soluble substances could be increased again. In this study, treatment with steam-ammonization was shown to improve NDF and ADF digestibility significantly compared to ammonization alone and the improvement was significant enough to increase cellulose and hemicellulose digestibility.

**Characteristics of rumen fluid:** Rumen fluid characteristics are important to ensure the survival and activity of rumen microorganisms. Characteristics of rumen fluid in this study are shown in Table 3.

The rumen fluid pH ranged from 6.49-7.02. The highest pH value was obtained with treatment C (Ammonization), which was 7.02, whereas the lowest pH value was found with treatment B (steam), which was 6.49. The results of the statistical analysis showed that the treatment had a highly significant effect ($p<0.01$) on the rumen fluid pH. DMRT results

Table 3: Characteristics of rumen fluid

| Characteristics of rumen fluid | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | SE |
| pH | 60.81[a] | 6.49[b] | 7.02[a] | 6.89[a] | 6.81[a] | 0.03 |
| Prod. VFA (mM) | 86.69[c] | 133.49[a] | 135.93[a] | 119.89[b] | 122.90[b] | 3.76 |
| $NH_3$-N (mg/100 mL) | 80.93[d] | 9.21[d] | 58.90[a] | 14.76[c] | 25.87[b] | 1.21 |

Description: Values with a different superscript in the same line are significantly different ($p<0.05$)

showed that the pH for treatment B was significantly lower ($p<0.01$) than that of other treatments, whereas among the other treatments, there were different ($p>0.05$) but inconsistent effects on rumen fluid pH *in vitro*.

The low pH value for the steam treatment (B) is due to the high concentration of VFAs resulting from the changes in the digestibility of the feed ingredients. This result agrees with Arora[9], who found that the pH of rumen fluid is affected by the production of VFAs and $NH_3$. An increase in free fatty acids (FFAs) will cause a decrease in rumen fluid pH and in contrast, an increase in $NH_3$ will cause an increase in rumen fluid pH. According to previous research[2], pH of the rumen fluid represents a balance of fermentation products (VFAs and $NH_3$). The pH value in this study was still within the optimal range for the growth of rumen microbes. According to a previous study[6], the pH range required for rumen microbes to live and engage in fermentation is 6-6.8, while the optimum pH range for protein synthesis is 6 to 7 and protein synthesis is impaired at a pH that is below the optimum value. According to Arora[9], the optimal rumen pH range for cellulose digestion is 6.4-6.8 and if the pH of the rumen is below 6.2, then the cellulolytic bacteria will be disrupted, decreasing fibre digestion.

The concentration of $NH_3$-N was highest (58.90 mg/100 mL) in rumen fluid obtained with the C (ammonization), followed by the E (steam-ammonization) treatment with 25.87 mg/100 mL of rumen fluid. These values are significantly higher ($p<0.01$) than treatments A and B. This difference occurs because of the addition of urea in the ammonization process for the treatment of C and D, which resulted in increased levels and digestibility of palm leaf protein, so the concentration of N-$NH_3$ is high.

The concentration of $NH_3$ obtained in this study ranged from 8.93-58.90 mg/100 mL of rumen fluid, which was sufficient for the growth and activity of rumen microbes. This is in agreement with a previous study[9] which indicated that the concentration of $NH_3$ in the rumen varies between 0 and 130 mg/100 mL of rumen fluid, while the minimum level for optimum rumen microbial protein synthesis is 5 mg/100 mL of rumen fluid. However, another report[6] indicated that the concentration of $NH_3$ required for maximum consumption, digestibility and microbial protein synthesis is between 10 and 23 mg/100 mL of rumen fluid.

According to Djajanegara *et al.*[1], factors that affect levels of N-$NH_3$ in rumen fluids include nitrogen sources in food, protein solubility and digestibility, feed nitrogen content, rate of discharge of rumen contents, ammonia absorption or urea recycling and nitrogen from bacteria.

The total VFA production in this study ranged from 86.69-135.93 mM. The results of the statistical analysis showed that treatment significantly increased ($p<0.01$) total VFA production. DMRT results showed that the C treatment (ammonization) resulted in the highest (135.93 mM) total VFA concentration. The VFA results of this study are an indicator that the processing was sufficient to fulfil the energy requirements and support the growth and activity of rumen microbes. According to the results of Arora[9], the amount of VFAs required for the activity and growth of rumen microbes is 80-160 mM.

Increased levels of VFAs reflect increased solubility of soluble feed carbohydrates. VFAs, especially acetic acid, propionate and butyrate are the main results of carbohydrate digestion in ruminants and are a major source of energy for ruminants. VFA can provide 55-60% of a ruminant's energy requirements[6].

VFAs in ruminants have a dual role as a source of energy for livestock and a carbon framework for microbial protein synthesis[10]. Rumen microbes utilize VFAs to produce adenosine triphosphate (ATP) as an energy source and they use the ammonia from protein degradation and non-protein nitrogen as nitrogen sources. VFAs are used to form microbial cell proteins. A balance between the production of ammonia and VFAs in the rumen will increase the synthesis of microbial proteins that can be used as a beneficial source of proteins for ruminants[7].

## CONCLUSION

The ammonization method of processing palm oil leaf bark produces the best levels of nutrients and *in vitro*

digestibility, as well as results in beneficial rumen fluid characteristics, compared to the effects of steam, ensilage and steam-ammonization treatments.

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