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The Effect of Different Time to Fiber Fraction on Cocoa Pod Fermentation

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Abstract

This study aims to determine the duration of the effect of cocoa pod fermentation on changes in fiber fractions (NDF, ADF, Cellulose, and Hemicellulose) in cocoa pod fermentation. This study lasted for 2 months, in October-November 2024 in the laboratory of the Faculty of Agriculture, Universitas Veteran Bangun Nusantara. The study used a Completely Randomized Design (CRD) with a one-way pattern with 4 treatments and 4 replications. The materials used were cocoa pods without fermentation and fermented cocoa pods. The treatments were P1 = cocoa pods fermentation 0 days, P2 = cocoa pod fermentation 3 days, P3 = cocoa pod fermentation 6 days, and P4 = cocoa pod fermentation 9 days. The parameters observed were changes in fiber fractions including Neutral Detergent Fiber (NDF), Acid Detergent Fiber (NDF), Cellulose and Hemicellulose. The results showed that the highest NDF content was found in P3 = 65.75 ± 0.3%; the highest ADF content was found in P3 = 57.00 ± 0.4%; the highest cellulose content was found in P0 = 27.75 ± 0.3%, and hemicellulose showed that the treatment had no significant effect (P> 0.05). The conclusion of this study is that pod cocoa fermentation for 9 days showed the best results on changes in the cocoa pod fermentation fiber fraction.

Keywords: fermentation, cocoa pod, day, fiber fraction

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1. Introduction

Cocoa, which has the Latin name Theobroma cacao or what we know as chocolate, is a plant that grows abundantly in tropical areas. Indonesia is one of the largest cocoa producing countries in the world, ranking 3rd after 2 other countries, namely Ivory Coast and Ghana. The recorded area of cocoa plants in Indonesia is 1.4 million hectares with a production of approximately 500 thousand tons per year. Ivory Coast, which ranks first as the largest cocoa producing country, has an area of 1.6 million hectares with a production of 1.3 million tons per year and is followed by Ghana with a production of 900,000 tons per year (Saputra, 2019).

Cocoa is widely used as a raw material in the food industry, especially for making chocolate. However, in the production process, cocoa fruit consists of 75.52% cocoa pod, 2-3% placenta 2-3% and seeds 22-24%. Of the waste, cocoa pod contributes the largest part, which is around 60-70%. This waste is often not utilized properly and is simply thrown away, which can cause environmental problems such as emitting an unpleasant odor and can become a nest for mosquitoes..So it is necessary to carry out more optimal management of pod cocoa waste (Semiawan et al., 2015).

Cocoa pods have great potential as an alternative feed for livestock, especially because of their abundant and cheap availability. However, in the utilization as animal feed, cocoa pods have major obstacles, namely high lignin content and low protein. Cocoa pods are lignocellulosic waste containing the main components of cellulose 36.23%, hemicellulose 1.14% and lignin 20-27.95%. Lignin that binds to cellulose causes cellulose to not be utilized by livestock. So it requires special treatment before being used as animal feed (Pallawagau et al., 2019).

One method to improve the nutritional quality of cocoa pods is through the fermentation process. In the context of cocoa pods, fermentation can reduce the content of crude fiber and antinutrient compounds, as well as increase the availability of nutrients that are beneficial to livestock. In addition, fermentation can also increase palatability (the level of livestock's preference for feed) and digestibility of feed ingredients (Kamelia and Fathurohman, 2017).

The study aims to determine changes in fiber fractions including NDF (Neutral Detergent Fiber), ADF (Acid Detergent Fiber), cellulose and hemicellulose in cocoa pod fermentation with different fermentation days.

2. Materials and Methods

2.1. Time and Place of Research

This research was carried out for 2 months, in October-November 2024 and was carried out in the laboratory of the Faculty of Agriculture, Veteran Bangun Nusantara University.

2.2. Tools and materials

The equipment used for the study are as follows: Scales to weigh sample materials, stationery to record all research series, plastic to wrap samples, and a set of fiber fraction analysis equipment. The materials used for this study are: unfermented cocoa pods and fermented cocoa pods.

2.3. Method

The research method used is an experimental method using a Completely Randomized Design (CRD). Which consists of 4 treatments and 4 replications, namely:

P0 = Cocoa Pod Fermentation 0 Days
P1 = Cocoa Pod Fermentation 3 Days
P2 = Cocoa Pod Fermentation 6 Days
P3 = Cocoa Pod Fermentation 9 Days

2.4. Research Design

Making Cocoa Pod Fermentation:

Cocoa pod fermentation can be an alternative feed for ruminants, especially to increase its nutritional value and palatability. Here is the process of cocoa pod fermentation for livestock feed. Wilt the cocoa pod for 5-6 hours. Then chop it into 1 cm x 5 cm pieces. Mix urea and probion in a bowl until evenly mixed.

Prepare a fermentation place, it can be on the ground under the house or on a stack of boards. Try to make sure that the place is not exposed to splashes of water or rain. The base of the fermentation place should be slightly sloping to facilitate the flow of waste water from fermentation. Roll out plastic tarpaulin measuring 1 x 1.5 m. Spread the fruit peel on the tarpaulin so that it forms a layer 10-15 cm thick. Sprinkle the layer with a mixture of urea and probion to taste. Then sprinkle water until it is even and looks wet. Then make a second layer using the same method and thickness as the first layer. For 100 kg of raw materials, it can be made into 4 layers. Then cover the pile of fruit peels with plastic tarpaulin. Place rocks or pieces of wood to keep the tarp from opening in the wind.

The fermentation period is adjusted according to the treatment carried out in the research. After fermentation, samples were taken to be examined for fiber fraction analysis in the laboratory.

2.5. Variables / Parameters

2.5.1. Neutral Detergent Fiber (NDF)

To determine the NDF content, the first thing to do is to make a Neutral Detergent Soluble (NDS) solution. How to make an NDS solution:

1. Aquadest is prepared approximately 800 mL in a 1,000 mL beaker.
2. Each chemical is weighed to make the NDS solution and then put into a beaker filled with distilled water.
3. The material was heated until dissolved and distilled water was added to 1,000 mL.

NDF Analysis Working Procedure:

1. A sample weighing 0.5 grams (a) was placed in a crucible that had been weighed (b).
2. The crucible was placed on the Fibertec Hot Extraction, 50 mL of NDS solution was added and heated until boiling, after boiling, octanol was dripped onto the foaming sample, then the heat was optimized and extraction was carried out for 1 hour.
3. The samples were filtered by immersion in Fibertec Hot Extraction and then rinsed with hot water.
4. The crucible cup is transferred to Fibertec Cold Extraction then rinsed with acetone or 96% alcohol.
5. The crucible and sample were oven-dried at 135^{o}C for 2 hours, then cooled in a desiccator and weighed (c).

Formula: % NDF $=\frac{c-b}{a}$x 100%

Information :

a) = sample weight
b) = weight of filter paper/crucible cup

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c) = weight of sample after oven and desiccator.

2.5.2. Acid Detergent Fiber (ADF)

To determine the ADF content, the first thing to do is to make an ADS solution. How to make an ADS solution:

Make by dissolving 20 grams of Cetyl Trimethyl Ammonium Bromide (CTAB) in 1 N sulfuric acid.

How to make 1N sulfuric acid :

N. Greek = x mL . BJ . %

1 . (98.08/2)= x mL . 1.84 . 0.96

49.04 = 1.7664 x

x = 49.04/1.7664 = 27.76 mL

ADF Analysis Working Procedure:

a. The sample was weighed at 0.5 gram (a) and put into a crucible which had been weighed (b).
b. The crucible was placed on the Fibertec Hot Extraction, 50 mL of ADS solution was added and heated until boiling, after boiling, octanol was dripped onto the foaming sample, then the heat was optimized and extraction was carried out for 1 hour.
c. The material is filtered by vacuuming on Fibertec Hot Extraction then rinsed with hot water.
d. The crucible cup was transferred to Fibertec Cold Extraction then rinsed with 96% acetone/alcohol.
e. The crucible and sample were oven-dried at 135^0 C for 2 hours, then cooled in a desiccator and weighed (c).

Formula: % ADF $=\frac{c-b}{a}$ x 100%

Information :

a) = sample weight
b) = weight of crucible
c) = weight of sample after oven and desiccator

2.5.3. Cellulose

Formula: Hemicellulose content = % NDF - % ADF

2.5.4. Hemicellulose

Determination of cellulose content is a continuation of ADF analysis, where the ADF residue (cg) is soaked with 72% H_2SO_4 for 3 hours, then rinsed with hot water and finally with acetone. The residue is dried in an oven at 135°C for 2 hours, then cooled in a desiccator and weighed (dg).

Formula: % Cellulose $=\frac{c-d}{a}$ x 100%

Information :

a) = Sample weight
b) = ADF residue
c) = Sample weight after being in the oven and desiccator

2.6. Data analysis

All data obtained in this study were analyzed using analysis of variance based on a Completely Randomized Design (CRD) with a one-way pattern. If there is a difference between treatments, it is continued with the Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

3.1. NDF (Neutral Detergent Fiber)

The results of determining the NDF content during the fermentation period of cocoa pods are listed in Table 1.

Table1. Average of NDF with different time on cocoa pod fermentation during the study (% DM)

Replication	Treatment			
	P0	P1	P2	P3
1	63.44	61.49	63.76	65.44
2	63.30	61.34	63.87	65.76
3	63.59	60.63	64.79	66.27
4	62.70	60.71	63.14	65.53
Average	63.25^{b}±0.3	61.04^{a}±0.4	63.89^{b}±0.6	65.75^{c}±0.3

^{abc} *Superscript* different in the same row indicates a difference (P<0.01)

The results of the advanced DMRT test showed that the treatments had a very significant effect (P<0.01) on NDF. The average NDF during the study was P0=63.25% P1=61.04%, P2=63.89% and P3=65.75%. The highest NDF content was obtained in treatment P3, which was 65.75% and the lowest was obtained in treatment P1, which was 61.04%.

Based on the results of experiments using Completely Randomized Design (CRD), it shows that cocoa pods fermented with long-day treatment showed a very significant effect (P<0.01) on the NDF content of cocoa pods. The results of Duncan's further test showed that the NDF content in the P3 treatment was very significantly different (P<0.01) from the P0, P1 and P2 treatments.

The results of fermentation for 9 days showed the highest NDF value because the fermentation time for 9 days was the peak of mold growth (P. chrysosporium) so that the enzyme production produced was also high, thus affecting the degradation of NDF in the cocoa pod fermentation process. In the fermentation process, the lignocellulose and hemicellulose bonds of the cocoa pod were broken. Microbes produced during fermentation help break down the lignocellulose bonds so that cellulose and lignin can be released from these bonds by the lignase enzyme.

This is in accordance with what was conveyed (Lumbantoruan and Sitorus (2023), The decrease in NDF is caused by the stretching of lignocellulose bonds and hemicellulose bonds during fermentation, which causes the bound cell contents to dissolve in neutral detergent solution. This causes the cell contents (NDS) to increase, while the feed components that are not soluble in detergent solution (NDF) decrease.

3.2 ADF (Acid Detergent Fiber)

The results of determining ADF levels with the fermentation time of cocoa pods using P. chrysosporium are listed in Table 2.

Table2. Average of ADF with different time on cocoa pod fermentation during the study (% DM)

Replication	Treatment			
	P0	P1	P2	P3
1	55.44	54.49	55.76	56.44
2	55.30	53.34	55.87	56.76
3	54.59	53.63	56.79	57.27

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4	55.70	53.71	55.14	57.53
Average	55.25^{b}±0.4	53.79^{a}±0.4	55.89^{b}±0.6	57.00^{c}±0.4

$^{a\ bc}$*Superscript* different in the same row indicates a difference (P<0.01)

The results of the advanced DMRT test showed that the treatments had a very significant effect (P<0.01) on ADF. The average ADF during the study was P0=55.25% P1=53.79%, P2=55.89% and P3=57.00%. The highest ADF content was obtained in treatment P3, which was 57.00% and the lowest was obtained in treatment P1, which was 53.79%.

Based on the results of experiments using Completely Randomized Design (CRD), it shows that cocoa pods fermented with long-day treatment showed a very significant effect (P<0.01) on the ADF content of cocoa pods. Duncan's further test results showed that the ADF content in the P3 treatment was very significantly different (P<0.01) from the P0, P1 and P2 treatments.

The results of fermentation for 9 days showed the highest ADF value because the fermentation time of 9 days was the peak of mold growth (P. chrysosporium) so that the enzyme production produced was also high, thus affecting ADF degradation in the cocoa pod fermentation process.

The decrease in ADF levels occurs due to the decomposition of ADF content into simpler and more soluble compounds. Where there is a loosening of the bonds so that cellulose increases and causes the ADF content to decrease. This indicates that the fermentation time usesmold (P. chrysosporium) can reduce NDF content. This statement is supported by Lumbantoruan and Sitorus (2023)which states that due to the stretching of lignocellulose bonds and lignohemicellulose bonds, the cell content (NDS) will increase.

3.3 Cellulose

The results of determining cellulose content during the fermentation period of cocoa pods using P. chrysosporium are listed in Table 3.

Table3. Average of cellulose with different time on cocoa fermentation during the study (% DM)

Replication		Treatment		
	P0	P1	P2	P3
1	27.44	26.69	27.76	25.44
2	28.30	27.34	26.87	25.76
3	27.59	26.63	26.79	25.27
4	27.70	26.71	27.14	26.53
Average	27.75c±0.3	26.84b±0.3	27.14bc±0.4	25.75a±0.5

$^{a\ bc}$ *Superscript* different in the same row indicates a difference (P<0.01)

The results of the DMRT test further showed that the treatment had a very significant effect (P<0.01) on Cellulose. The average Cellulose during the study was P0 = 27.75% P1 = 26.84%, P2 = 27.14% and P3 = 25.75%. The highest Cellulose content was obtained in the P0 treatment, which was 27.75% and the lowest was obtained in the P3 treatment, which was 25.75%.

Based on the results of experiments using Completely Randomized Design (CRD), it shows that fermented cocoa pods with long-day treatment showed a very significant effect (P<0.01) on the cellulose content of cocoa pods. Duncan's further test results showed that the cellulose content in the P0 treatment was very significantly different (P<0.01) from the P1, P2 and P3 treatments.

Cellulose is a polysaccharide consisting of a straight chain of glucose units that have a high molecular weight so that cellulose is more resistant to chemical reactions compared to other glucans. The structure of cellulose is difficult to destroy in the digestive system but because of the presence of microorganisms in the rumen of ruminant livestock, cellulose can be digested and utilized properly (Minggo et al., 2024).

3.4 Hemicellulose

The results of determining the hemicellulose content during the fermentation period of cocoa pods using P. chrysosporium are listed in Table 4.

Table4. Average of hemicellulose with different time on cocoa pod fermentation during the study (% DM)

Replication		Treatment		
	P0	P1	P2	P3
1	27.44	26.49	26.76	25.44
2	26.30	25.34	26.87	26.76
3	27.59	26.63	26.79	25.27
4	27.70	28.71	28.14	29.53
Average^{ns}	27.25±0.8	26.79±1.4	27.14±0.6	26.75±26.2

^{ns}the same line shows non significant (P>0.05)

The results of the DMRT test further showed that the treatment had no significant effect (P>0.05) on Hemicellulose. The average Hemicellulose during the study was P0 = 27.25%, P1 = 26.79%, P2 = 27.14%, and P3 = 26.75%. The highest Hemicellulose content was obtained in the P0 treatment, which was 27.25% and the lowest was obtained in the P3 treatment, which was 26.75%. Based on the results of the experiment using a Completely Randomized Design (CRD), it showed that cocoa pods fermented with the long-day treatment showed no significant effect (P>0.05) on the Hemicellulose content of the cocoa pods. The results of the Duncan's further test showed that the Hemicellulose content in the P0 treatment was not significantly different (P>0.05) from the P1, P2 and P3 treatments.

Hemicellulose is easier to hydrolyze with acid into monomers containing galactose, glucose, xylose, mannose and arabinose. Hemicellulose binds cellulose fiber sheets that form microfibrils thereby increasing the stability of the cell wall (Ismail Pasue and Salah, 2019).

4. Conclusion

Based on the discussion and analysis that has been carried out, it can be concluded that fermentation carried out for 9 days showed the best results.

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