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THE EFFECT OF FERMENTATION TIME AND STARTER TYPE ON ASH AND CRUDE FIBER CONTENT IN COCOA BEAN SHELLS (THEOBROMA CACAO L)

Radiyatunnisah[1*], Ajeng Kartika Pratiwi[2], Kharismafullah[3]
[1]Program Studi Agribisnis Peternakan, Politeknik AMA, Kota Bima, Indonesia

**Corresponding author email: radiyatunnisah@poltekama.ac.id*

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ABSTRACT

Cocoa bean shells are agro-industrial waste that has the potential to be used as alternative animal feed, but their use is still limited due to their relatively high crude fiber content. One effort to improve the nutritional quality of this feed material is through fermentation. This study aims to examine the effect of fermentation time and starter type on the ash and crude fiber content of cocoa bean shells. The study used a 3 × 3 factorial completely randomized design (CRD) with two factors, namely starter type (Aspergillus niger, Penicillium chrysosporium, and Trichoderma sp.) and fermentation time (3, 6, and 9 days), each with three replicates. Data were analyzed using analysis of variance (ANAVA). The results showed that the type of starter had a significant effect ($P<0.05$) on the crude fiber content of cocoa bean shells, while the fermentation time and the interaction between the starter and fermentation time had no significant effect. The crude fiber content tended to decrease with increasing fermentation time, with the lowest value obtained in the Trichoderma sp. starter treatment. Meanwhile, the ash content of cocoa bean shells was relatively stable and was not significantly affected by fermentation time or starter type. Based on the results of this study, fermentation of cocoa bean shells using certain mold starters has the potential to improve their quality as alternative feed ingredients, particularly through a reduction in crude fiber content.

Keywords: Cocoa Bean Husk, Fermentation, Starter, Ash Content, Crude Fiber

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INTRODUCTION

The availability of quality feed is one of the key factors in the success of livestock farming. Many local feed ingredients have high crude fiber content and low digestibility, resulting in suboptimal utilization by livestock. One way to improve the quality of these feed ingredients is through fermentation technology.

Fermentation with the help of microorganisms can improve the nutritional value of feed materials, particularly by reducing crude fiber content and increasing nutrient availability.

Several types of fungi commonly used as fermentation starters include Aspergillus niger, Trichoderma sp., and Phanerochaete chrysosporium. Each microorganism has different enzymatic capabilities in degrading cell wall components such as cellulose, hemicellulose, and lignin.

In addition to the type of starter, the duration of fermentation also plays an important role in determining the final quality of the fermented product. Fermentation that is too short can result in an incomplete degradation process, while fermentation that is too long has the potential to reduce the quality of certain nutrients. Therefore, this study aims to evaluate the effect of fermentation time and starter type on the ash and crude fiber content of fermented feed used as alternative feed.

METHODS

This study was conducted over one month in the animal nutrition and feed laboratory. The fermentation process and proximate analysis were carried out during the research period. The materials used were cocoa bean shells (Theobroma cacao L), three types of fermentation starters (Aspergillus niger, Trichoderma sp., and Phanerochaete chrysosporium), and chemicals for proximate analysis. The equipment used included an analytical balance, oven, furnace, and crude fiber analysis equipment.

Research Procedure

The feed materials were fermented with each starter according to the treatment and fermentation time that had been determined. After fermentation was complete, the materials were dried and analyzed for ash and crude fiber content using the proximate analysis method.

Parameters Observed

The parameters observed in this study included: Ash content (% dry matter) Crude fiber content (% dry matter).

Research Design

The study used a completely randomized design (CRD) with a factorial pattern with two factors, namely: Starter type (S) A.niger, P.crysosporium and Trichoderma Sp, and fermentation time (w) 3 days, 6 days and 9 days. Each treatment was repeated three times. With the following formula model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Data Analysis

The data obtained were analyzed using analysis of variance (ANAVA) to determine the significance of the effect of each factor and the interaction between factors.

RESULT AND DISCUSSION

Ash Content

Based on the analysis results, there was a significant difference in ash content before fermentation of 8.10 percent (Minife, 1984) and ash content after fermentation of 9.63 percent, indicating that ash content increased during the fermentation process. This is thought to be due to an increase in phosphorus content during the fermentation process and the breakdown of inorganic substances in the cocoa bean shell. The fermentation process increases the nutritional content of the cocoa bean shell.

Fermentation of feed ingredients causes changes in chemical composition, characterized by an increase in nutrient content, including ash content. This is due to the activity of microorganisms that break down organic matter and increase the availability of minerals during the fermentation process (Winarno, 2007; Van Soest, 2006). Fermentation treatment using Trichoderma sp. starter produced the lowest ash content in the observed substrate. This phenomenon is likely due to the dissolution and loss of soluble minerals during the

fermentation/processing of samples and/or the transformation of minerals into forms that are less measurable by conventional incineration methods (Olugosi et al., 2019; other SSF studies).

The best ash content treatment with the lowest average was found on day 9 (9.63%) with Trichoderma sp. starter. This is because microorganisms have broken down the nutrients contained in the cocoa bean shells into inorganic materials. In this study, fermentation treatment using Trichoderma sp. inoculum showed the lowest ash content compared to the control and other treatments. This can be explained by several complementary mechanisms: (1) the enzymatic activity of Trichoderma, which breaks down the lignocellulose matrix, causes the release of minerals, some of which become soluble and potentially lost during the washing or sample processing stages; (2) some of the released minerals can rebind to microbial biomass or form organic complexes that behave differently in ash analysis, so they are not recorded as ash fractions; and (3) variations in drying or solid separation procedures during sample processing can cause the loss of soluble mineral fractions, thereby reducing the measured ash value. Because the literature on the effects of fermentation on ash content is varied, some studies report an increase in ash due to a decrease in the organic fraction, while other studies report a decrease related to the loss of soluble minerals. Therefore, the interpretation of these results needs to be supported by further mineral (atomic) analysis to determine the dominant mechanism.

Crude Fiber Content

Based on the analysis results, the crude fiber content of fermented cocoa bean shells on days 3 (14.96%), 6 (14.21%), and 9 (13.91%) was lower than that of unfermented cocoa bean shells (18.60%) (Minife 1984). The crude fiber content decreased as the fermentation time increased. The greatest decrease occurred in the treatment with P. chrysosporium starter. This was due to the ability of the mold to produce ligninase and cellulase enzymes that are effective in degrading lignocellulose. This decrease in crude fiber is very beneficial because it can increase digestibility. Fermentation of cocoa bean shells causes a decrease in crude fiber due to the activity of microorganisms that produce cellulase and ligninase enzymes. Alemawor et al. (2009) reported that fermentation of cocoa shells using lignocellulolytic fungi can reduce the lignocellulose fraction and increase feed digestibility. This process is in line with the opinion of Schwan and Wheals (2004), who stated that cocoa fermentation involves complex microbial activity that changes the chemical structure of the cell wall of the material.

Fermentation of feed using lignocellulolytic fungal starters has been proven to increase the degradation of lignin and structural fiber fractions such as Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF). White rot fungi such as Phanerochaete chrysosporium and Pleurotus ostreatus produce ligninase, manganese peroxidase, and laccase enzymes that play a role in breaking down the lignin matrix, making cellulose and hemicellulose more accessible to cellulase and hemicellulase enzymes (Schwan & Wheals, 2004; Van Soest, 2006).Alemawor et al. (2009) reported that fermentation of cocoa fruit husks using Pleurotus ostreatus significantly reduced NDF and ADF content and increased feed digestibility. Increased cellulase enzyme activity during fermentation causes depolymerization of structural polysaccharides, which results in increased in vitro digestibility (Oduguwa et al., 2008).

In addition to improving fiber content, the fermentation process also contributes to reducing the content of antinutritional compounds such as tannins and theobromine. Adamafio (2013) states that microbial activity during fermentation can reduce theobromine levels in cocoa husks through biodegradation, thereby increasing its safety and potential for use as ruminant feed. Thus, fermentation using lignocellulolytic fungal inoculants not only improves nutritional quality but also expands the use of cocoa waste as an alternative feed source.

Overall, the results showed that the combination of starter type and fermentation time affected the ash and crude fiber content of cocoa bean shells. The Phanerochaete chrysosporium starter with a longer fermentation time produced the best results in reducing crude fiber content.

This was due to the activity of ligninase and cellulase enzymes, which were able to effectively break down the lignocellulose structure. This reduction in crude fiber is very beneficial in the utilization of cocoa bean shells as alternative feed because it can increase digestibility and nutrient utilization efficiency by ruminant livestock.

Table 1. Results of analysis of fermentation time and starter type on ash and crude fiber content of cocoa bean shells

Parameter	S/W	Starter			$\bar{X}$
		A.Niger	P. Crysosporium	Trichodrma S.p	
Kadar Abu	3 hari/w1	9.74	9.79	9.56	**9.69**
	6 hari/w2	9.75	9.63	9.51	**9.63**
	9 hari/w3	9.78	9.58	9.55	**9.64**
Kadar Serat Kasar	3 hari/w1	15.14	14.39	13.86	**14.46**
	6 hari/w2	14.9	14.26	14.05	**14.40**
	9 hari/w3	14.84	13.99	13.84	**14.22**

The results of analysis of variance (ANAVA) on the ash content of cocoa bean shells influenced by fermentation time and starter type. The analysis results show that the starter type (S) factor has a significant effect on ash content, as indicated by a calculated F-value of 5.4399, which is greater than the F-table value of 0.05 (3.55), so that the difference in ash content is caused by the difference in the starter used. Conversely, the fermentation time factor (W) had no significant effect on ash content because the F-count value of 0.6005 was smaller than the F-table value. Similarly, the interaction between starter and fermentation time (S×W) showed an F-count value of 0.7038, indicating that there was no significant interaction on ash content. Overall, ash content variation was more influenced by starter type than fermentation duration, indicating that microbial activity in the starter played a more dominant role in determining changes in mineral composition during the fermentation process, while minerals were relatively undegraded during fermentation (Steel & Torrie, 1993; Tillman et al., 1998; AOAC, 2005).

Table 3. Analysis of variance of ash content based on fermentation time and type of cocoa bean shell starter.

Source of Variation	DB	JK	KT	F Hitung	F0.005	F0.01
Treatment	8	0.2892	0.0362	1.8620*	2.32	3.28
Starter (S)	2	0.2112	0.1056	5.4399*	3.55	6.01
Time (W)	2	0.0233	0.0117	0.6005ns	3.55	6.01
S X W	4	0.0547	0.0137	0.7038ns	2.93	4.48
Error	18	0.3495	0.0194			
Total	26	0.6387	Kk (%)=		1.6405824	

Based on the results of the analysis of variance, which showed a significant effect on crude fiber content, the type of starter had a significant effect ($P<0.05$) on crude fiber content, as indicated by the Fcount value (4.4924) being greater than the Ftable at the 5% level, while the fermentation time factor had no significant effect ($P>0.05$). The interaction between starter

type and fermentation time also did not show a significant effect on crude fiber content. This indicates that differences in the enzymatic ability of microorganisms in each starter play a greater role in influencing the degradation of fiber components than the length of fermentation time, while the absence of interaction shows that the effect of the starter on crude fiber is independent of fermentation time. The coefficient of variation value of 5.68% indicates that the data obtained has a good level of accuracy and the research results are statistically reliable. Van Soest (1994) stated that structural fiber fractions such as lignin-bound cellulose have high resistance to microbial degradation, so that changes in crude fiber content are more determined by the type of microbe than by the duration of fermentation.

Interaction between fermentation time and inoculum type on ash and crude fiber content.

The interaction between fermentation duration and inoculum type is often reported in the literature because these two factors together determine the intensity and type of substrate degradation (Pandey et al., 2000; Singhania et al., 2010). Starter cultures rich in ligninolytic enzymes, such as Phanerochaete chrysosporium, require sufficient incubation time to express oxidative enzymes (lignin peroxidase and manganese peroxidase) that break down lignin, making cellulose and hemicellulose more available to cellulase enzymes. as a result, the reduction in NDF and ADF is generally more pronounced in medium to long treatment times (Kirk & Farrell, 1987; Hatakka, 2001). Conversely, starters such as Aspergillus niger tend to rapidly produce hydrolytic enzymes for non-structural components and hemicellulose, so that changes in fiber fractions can be seen in short to medium fermentation times, depending on the substrate and fermentation environment conditions (de Vries & Visser, 2001).

For ash content, the variations observed among the time × starter combinations may arise from several mechanisms working together, namely the degradation of organic components that increases the relative percentage of ash (Van Soest, 2006; Tillman et al., 1998), the release of soluble minerals that may be lost during fermentation and sample processing, thereby reducing the measured ash content (Oduguwa et al., 2008; Olugosi et al., 2019), and the re-binding of minerals to microbial biomass or the formation of organic complexes that affect the ash analysis response (Pandey et al., 2000; Nielsen et al., 2010). Therefore, the interpretation of ash content changes should be supplemented with liquid phase analysis and elemental analysis using ICP or AAS methods to distinguish between relative increases due to organic matter reduction and actual mineral loss (AOAC, 2019; Skoog et al., 2014).

Table 3. Analysis of variance of crude fiber content, fermentation time, and starter type of cocoa bean shells.

Source of Variation	DB	JK	KT	F Calculate	F0.005	F0.01
Treatment	8	5.6741	0.7093	1.2285	2.32	3.28
Starter (S)	2	5.1873	2.5936	4.4924	3.55	6.01
Time (W)	2	0.2856	0.1428	0.2474ns	3.55	6.01
S X W	4	0.2012	0.0503	0.0871ns	2.93	4.48
Error	18	10.3922	0.5773			
Total	26	16.0663	Kk (%)=		5.679822	

CONCLUSION

The type of starter and fermentation time have a significant effect on the ash and crude fiber content of fermented feed, with an interaction between the two factors. The use of P. chrysosporium for a longer fermentation time resulted in the greatest reduction in crude fiber, reflecting its ability to effectively degrade lignocellulose. These changes in nutrient composition indicate an improvement in feed quality and digestibility, making it a potential feed source for goats. Therefore, selecting the appropriate starter type and fermentation duration is crucial for optimizing the nutritional quality of fermented feed, particularly in reducing crude fiber and managing mineral fractions.

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