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Evaluation of the Nutrition and Anti-Nutrition of the Fermentation of Cacao Seed Skins (*Theobromacacao l.*)

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Abstract: ***This study was to evaluate the effect of starter types and different fermentation time on organic matter content, crude protein content, theobromine levels, ADF, NDF, lignin and silica ofcacao seedsskins(Theobroma cacao L). This research wasconductedat the Nutrition Laboratory of the University of Muhammadiyah Malang. This study used a completely randomized design (CRD) factorial (3x3). The first factor was the time of fermentation, consisting of 3 levels (3, 6, and 9 days). The second factor was the type of starter consisting of 3 types namely Aspegillusniger (S1), Phanerochaetechysosporium (S2) and Trichoderma viridae (S3). The results of the analysis showed that there was a significant correlation (P<0.01) among the fermentation time, content of organic matter, and crude protein of fermented cacao seed skin. The best level of organic matter was the interaction of 6 days fermentation time with the type of starter of S2 (85.43%). The best level of crude protein ingredients was the interaction of 6 days fermentation time with the type of starter of S3 (17.80%). This study concludedthatthe 6 daysfermentationtimegavethebest level oforganicmatterandcrude protein ingredient.***

Keywords: cacao seed skin, organic ingredient, crude proteins, anti-nutrient, fermentation

1. Introduction

The feed is one of the most decisive factors in the livestock business. The availability of forage feed fluctuates. It is abundant in the rainy season and briefly occurs during the dry season [1]. This has become a barrier and challenge for farmers to continue to provide food with a high protein content that is cheap and sustainable [2]. The provision of feed has shifted to exploration and utilization of non-conventional feed ingredients with low competition values, such as the utilization of plantation waste or agro-industrial waste that functions as a feed source for ruminants. The effectiveness of the use of waste is the main determinant of the availability of materials, prices, and nutritional content. One type of plantation waste used as animal feed is cacao pods (*Theobroma cacao*) [3]. Puastuti stated that cacao pods are the main waste from processing cacao, which has the potential to be utilized as a source of ruminant feed. Cacao production increases every year and has the potential to produce waste that continues to increase as well. If it is not processed and used properly, it can potentially be a pollutant [4].

The processing that can be done on cacao seed skins is done by fermentation. Fermentation is a method for increasing the nutritional content and value from the original ingredients. It increases the crude protein and palatability because it changes the aroma to be better than the original material and is a way to eliminate anti-nutrients [5].

The seed skins of cacao have not been used optimally. Thus far, the seed skins of cacao beans are only used as animal feed by fermentation. The skin of the cacao bean seed is thin, soft, and slightly slimy. The percentage ranges from 10% to 16% of the total portion of dried cacao seeds [5]. Thus, it is necessary to study the effect of fermentation time and the types of starters regarding the content of organic matter, crude protein, and anti-nutrition of cacao seed skins.

2. Materials and Methods

Research Materials

The material used in this study is the cacao seeds skin (*Theobroma cacaoL*). The research observed the effect of fermentation time and type of starter on the levels of organic matter, crude protein and antinutrient of cacao seeds skin (*Theobroma cacao L*).

Fermented Cacao Seeds

The skin of dried cacao seeds with dry matter content at average 60-70% was used as a raw material sterilized in autoclaves. Dry cacao seeds skin was placed in airtight plastic (10 kg each) and added with 2% bran and molases 1%.The skin of dried cacao seeds was inoculated with 3 types of inoculum (S1: *Aspegillusniger*; S2: *Phanerochaetechysosporium;* and S3: *Trichoderma viridae*). The each composition was 1.0% of dry and mixed ingredients put in an airtight plastic (1.0 kg) [6].Mold starter was obtained from Center for Biotechnology Studies, University of Gajahmada and was grown on rice flour for 7 days. Potato Dextrose Agar (PDA) MERCK was used as a medium for mold growth and bred in rice flour media. Dry cacao seeds skin was mixed with starter and was fermented according to groups treatment W1:3 days; W2:6 days; and W3:9 days. In the end of study, the fermented cacao skin seeds was analyzed for nutritional and anti-nutritional content in the Animal Feed Laboratory of the Faculty of Agriculture-Animal Husbandry and the Biomedical Laboratory of the Faculty of Medicine, University of Muhammadiyah Malang. Analysis of theobromine content was carried out at the Central Laboratory of University of Jember.

The treatment given consisted of two factors, namely the type of starter and thetime of fermentation. The starter type treatment (S) consisted of 3 types of starters, namely:S1:

Aspergillus niger; S2: *Phanerochaetechysosporium;* and S3: *Trichoderma viridae*. The fermentation time treatment (W) consisted of 3 levels, namelyW1:3 days; W2:6 days; and W3:9 Days.This research used experimental methods. The design of this study was a completely randomized factorial (CRD) 3 x 3. Data was analysis using ANOVA and the DuncansTest.

3. Results

Contents of Organic Matter of Fermented Cacao Seed Skin

The organic matter was influenced by the fermentation time by 85.29% for *W*1, 84.71% for *W*2, and 84.62% for *W*3. The organic matter was affected by the type of starter regarding the mean level of organic matter by 84.80% for *S*1, 85.05% for *S*2, and 84.90% for *S*3. The results of the analysis of variance indicated that the treatment of fermentation of the cacao seed skin was not significant (p>0.05) regarding the content of the organic matter. This means that using a fermentation time of 3 days, 6 days or 9 days had the same result for the content of organic matter of cacao seed skins, so using just 3 days of fermentation would be more efficient. Data of organic matter levels are shown in table 1.

Table 1: Combination data among length of time, type of starter, and level of organic matter of fermented organic cacao seed skin (%)

S/W	Starter			Σ	Average (Ȳ)
	S1	S2	S3		
W1	84,74	85,70	85,85	256,29	85,29
W2	84,99	85,02	84,10	254,13	84,71
W3	84,68	84,44	84,75	253,88	84,62
Σ	254,42	255,16	254,72	764,31	
Mean (Ȳ)	84,80±0,16	85,05±0,63	84,90±0,88		

Note :S1=*Aspegillus*niger; S2=*Phanerochaetechysosporium*;S3=Trichoderma; W1=3 days; W2= 6 days; W3=9 days.

Contents of Crude Protein Levels of Fermented Cacao Seed Skins

The mean crude protein level by fermentation time is 14.90% for *W*1, 16.09% for *W*2, and 15.71% for *W*3. The average organic matter effect by starter type is 14.90% for *S*1, 14.14% for *S*2, and 17.13% for *S*3. The crude protein content of fermented cacao seed skins is higher compared to that of fresh cacao seed skins by around 9% to 10%. This is in accordance with the results found by Suparjo et al. (2011). The fermented cacao seed skins have a higher chemical composition than the unfermented cacao seed skins. Fermentation increased the crude protein content of the cacao bean skin to 13.84%, which is an increase of 59.31% of the unfermented protein content. Crude protein levels of fermented cacao seed skins are shown in table 2.

Table 2: Combination data among length of time, type of starter, and level of crude protein of fermented organic cacao seed skin (%)

S/W	Starter			Σ	Average (Ȳ)
	S1	S2	S3		
W1	13,30	14,70	16,72	44,71	14,90
W2	16,25	14,24	17,80	48,29	16,09
W3	15,15	13,48	16,90	45,53	15,17
Σ	44,7	42,42	51,41	138,53	
Mean (Ȳ)	14,9±1,49	14,14±0,62	17,13±0,58		

Note :S1=*Aspegillus*niger; S2=*Phanerochaetechysosporium*;S3=Trichoderma; W1=3 days; W2= 6 days; W3=9 days.

Anti-nutritional Content of Fermented Cacao Seed Skins

The results showed the average antinutrient content of fresh cacao seeds for tannin content (0.36%), ADF (35.77%), NDF (71.61%), lignin (15.38%), silica (0.98%), and theobromine (0.589%). The antinutrient content of fermented cacao bean skins averaged 0.26% tannin content, 45.7% ADF levels, 51.72% NDF, 23.74% lignin, 0.67% silica, and 0.606% theobromine. The complete data is shown in table 3.

Table 3: Anti-nutritional content of fermented and non-fermented cacao seed skin (%)

No	Material	Content Antinutrition (%)						
		NDF	ADF	Hemisellulosa	Sellulosa	Silica	Lignin	Tanin
1	Fermentation	51,72	45,76	5,96	21,35	0.67	23,74	0,26
		50,31	47,35	7,45	20,25	0,69	25,55	0,27
		52,45	43,75	3,55	22,71	0,62	21,34	0,24
	Average	51,49±1,08	45,62±1,81	5,65±1,96	21,44±1,23	0,66±0,03	23,54±2,11	0,26±0,01
2	Non Fermentation	71,61	35,77	35,85	19,42	0,98	15,38	0,38
		73,52	33,57	33,81	22,37	0,95	14,35	0,40
		70,42	37,35	37,75	17,25	0,91	16,51	0,36
	Average	71,85±1,56	35,56±1,89	35,80±1,97	19,68±2,57	0,95±0,04	15,41±1,08	0,38±0,02

4. Discussion

Contents of Organic Matter of Fermented Cacao Seed Skin

Based on table 1, the starter type treatment significantly affected the organic matter content (p<0.05) of the fermented cacao seed skins. The best type of starter for the content of organic matter was *P. chrysosporium*(*S*2) and *T. viride* (*S*3), while the lowest results were obtained from *A. niger*(*S*1). Murni and Okrisandi explained that the fermentation process occurs via bioconversion of organic and inorganic compounds into cell proteins so that the fermented substrate protein content increases. Fiber-breaking by enzymes, such as cellulase and others produced during the fermentation process, plays a role in reducing the fiber content of the substrate [7]. Thus, the treatment using *S*2 is the best option for the content of organic ingredients of cacao seed skins.

According to Mujnisa, the content of fermented cacao pods yields a value that is 87.16% lower when compared to the results of research on cacao seed skin fermentation with the highest content of *S*2 of 85.85%. The increase in organic matter in each treatment ratio is caused by the success at

fermentation. This shows that fermentation can increase the nutritional value of cacao seed skins as an animal feed [3]. Supardjo et al. stated that materials that undergo fermentation have a higher nutritional value than the original ingredients [6].

Contents of Crude Protein Levels of Fermented Cacao Seed Skins

Based on the results of the analysis of variance, it was found that the treatment duration of fermentation of the cacao bean skin had a significant ($p< 0.01$) effect on the crude protein content of the fermented cacao seed skins. The duration of fermentation of 6 days (*W*2) was significantly higher than the fermentation time of 9 days (*W*3) and 3 days (*W*1). The fermentation time of 6 days (*W*2) was the best for the variable of crude protein levels of fermented cacao seed skins. The crude protein content using the *S*3 (*Trichoderma sp*.) treatment was higher than those of *S*2 (*P. chrysosporium*) and *S*1 (*A. niger*) ($p< 0.01$). Thus, the *S*3 treatment (*Trichoderma*) produced the best results for crude protein content. The results of this study are in accordance with the study of Syahrir and Abdeli that the dose and time of fermentation affect the nutritional value of fermented cocoa pods because *T. viride* can utilize organic material in feed ingredients to be overhauled and converted to increase the protein content [8]

The crude protein after fermentation is mostly an amino acid that has been synthesized by *Trichoderma sp*., making it more profitable for livestock. The fungus *T. viride* has been used in the fermentation of several feed ingredients, especially waste. The benefits of fermentation can increase protein content, reduce crude fiber content, and reduce tannin content [9].

The results of this study are higher when compared to the results of Mujnisa's study, where it was reported that the crude protein content of fermented cacao pods was 13.78% [3]. The use of *Trichoderma* mushrooms in fermented rice straw can increase the crude protein content from 4.65% to 6.65% [10]. This is in accordance with the opinion of Harman who stated that the increase in crude protein is due to the fermentation process of the fungi *Trichoderma sp.*, where fermentation is able to improve the nutritional value of the protein content [9]. According to Winarno and Fardiaz, fermented material has a higher nutritional value than the original material because microbes break down complex components into simpler materials [11].

Based from several studies, it was concluded that the use of mold (namely *Aspergillus niger*[12], *Rhizopusoligosporus* and *Trichoderma reseei* [8], *Rhizopusstolonifer* [13], *Phanerochaetehrysosporium*[6]) as a fermentor in the cocoa pods fermentation process may increase nutrient values including protein andfiber digestibility of cocoa pods. Mold produces enzymes (cellulase and hemicellulase) which are able to decompose the fiber fraction so the lignin bonds break. The fraction of cellulose and hemicellulose can be also break down into simple carbohydrate and sugar molecules by the enzymes. The carbohydrate and sugar are converted to volatile fatty acids (VFA) which is useful for ruminants.

Anti-nutritional Content of Fermented Cacao Seed Skins

Based on table 3, the anti-nutritional content in the skin of fresh cacao seeds is higher when compared to the skin of the fermented cacao seed skins. This is in accordance with the opinion of Puastuti and Susana that the skin of cacao seeds should be fermented before being used as animal feed. The content of theobromine compounds in the skins of cacao beans can be reduced by processing (fermentation, grinding, and drying) [4]. Adamafio et al. showed that microbes including *A. niger* could degrade theobromine in cocoa skins seeds fermentation through a fermentation process [14].

Cacao seed skin fermentation provides benefits that improve the texture and taste of the ingredients, enhance digestibility, reduce lignin content, increase protein levels, suppress the adverse effects of theobromine toxins, and increase livestock productivity [15]. Suparjo et al. explained that the decrease in crude fiber content of fermented cacao seed skins was caused by the degradation of cell-wall components, namely lignin, and hemicellulose. The enzymes secreted from *P. chrysosporium* with the help of fermentation can relax the bonds between lignin and polysaccharides and degrade the lignin into simpler materials [6]. Syahrir et al. [16] stated that processing cocoa pods with molds is able to increase crude protein, digestibility of dry matter, and organic matter but the cellulose level decreased.

5. Conclusions

Basedontheresultsoftheresearch, thefollowingfindingscanbeconcluded:

1) Fermentation increases the levels of organic matter and crude protein and decreases the anti-nutrition of cacao seed skin.
2) The type of starter significantly affects the yield of cacao bean skin fermentation ($p < 0.05$). The starter *T. viride* produced the highest crude protein content compared to *A. niger* and *P. chrysosporium*.
3) The fermentation time significantly affects ($p< 0.05$) the yield of fermented cacao seed skins. The fermentation time of 6 days produced the highest protein content compared to 3 days and 9 days.

6. Conflict of Interest

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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