Improving quality and digestibility of cocoa pod with white rot fungi

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Abstract. White rot fungi is a type of fungus that is able to degrade lignin in the feed material from waste, so it can be used to increase the added value of cocoa pod as alternative feed ingredients to meet the nutritional needs of cattle. The purpose of this study is to investigate the use of white rot fungi in improving the quality and digestibility cocoa pod as feed. The study consisted of two phases, namely fermentation using three isolates of white rot fungi (Coprinus comatus, Corilopsis polyzona and Lentinus torulosus) on pod husks and quality testing in vitro digestibility of fermented. Results of analysis of variance show that the treatment was highly significant on the content of lignin, cellulose and hemicellulose pod husks. Fermented cocoa husks with white rot fungi can degrade lignin content of 1.42% - 12.28% and highly significant improved on in vitro digestibility of dry matter and organic matter. The conclusion, isolates of white rot fungi most active in degrading lignin was Lentinus torulosus isolates and less ability to degrade cellulose and hemicellulose.

1. Introduction
Exploration and resource types of feed derived from agro-industrial residual, is important in terms of its use as a substitute for the main feed, especially feed costs are the largest expense for farmers. Various studies have been conducted to utilize waste into valuable feed materials of high biological value and provide added value. Mustabi et al. [1] has conducted research on the use of rice straw fermented white rot fungi as feed substitution grass in goats.

Residue plant cocoa pod utilization as alternative feed ingredients to meet the nutritional needs of cattle. Agro-industrial wastes as feed material have always been associated with low prices and poor quality. The amount of utilization depends on the potential both in quantity and quality that can be utilized. Quantity aspects related to the amount of waste generated from a percentage of the production process and ration formulate. More emphasis on the quality aspects of nutritional value that can be utilized by livestock to increase production and productivity.

Cocoa Pod utilization as feed is generally limited by several factors such as the low nutritional quality due to high fiber content and their networks on a feed from waste has undergone a process lignification (hardening). Lignin and cellulose are often formed lignosellulose compounds in plant cell walls, which is a strong bond [2]. The older the plant, the higher lignin content consequently digestibility decreases with the increasing lignification. In addition to cellulose and hemicellulose lignin binding also bind to the cell wall proteins.

Some groups of microorganisms were reported capable of degrading lignin compounds, being able to use cellulose as a carbon source for their growth substrate, whereas the lignocellulosic components
that can be used by cattle are cellulose and hemicellulose. Problems often arise in the processing of lignocellulosic materials using microorganisms is the loss of organic material substrates used by microorganisms as a source of nutrients in the bioconversion process. Murni et al. [3] suggest that microorganisms ideal in bioconversion of lignocellulose into livestock feed is a microorganism that has the great ability to decompose lignin but low power degradation of the cellulose and hemicellulose. Most fungi lignolitic don’t have the ability to use lignin as a single source for energy and carbon as well as a lot depends on the polysaccharide easily digested in the substrate. Based on the consideration that white rot fungi degrade lignin is the most active, so as to improve the quality and digestibility cocoa pod as feed for sustainable livestock development.

2. Materials and Method

2.1. Location and Description of Study
The experiment consisted of two stage, namely (1) fermentation using 3 isolates of white rot fungi on the substrate cocoa pod was carried out in laboratory animals valorisasi waste Hasanuddin University Faculty of Animal Husbandry; (2) Analysis of digestibility of dry matter and organic matter digestibility of fermented cocoa pod conducted at the Laboratory of Feed Chemistry Hasanuddin University Faculty of Animal Husbandry.

2.2. Sample Preparation and Treatment
The material used in this research is cocoa pod, isolated white rot fungus (Coprinus comatus, Cantharellus freissii and Lentinus Torulosus), cocoa pod, water, Lime (CaCO₃), plastic polypropylene (PP), aluminium foil and the materials used in the analysis digestibility. While the tools used are glass bottles (height 9 cm and 5 cm in diameter), pipe, measuring cup, oven, autoclave, analytical balance and laboratory tools in the analysis digestibility.

The first phase of the research was the fermented cocoa pod. Prior to fermentation, first performed seed media making a mushroom growth of substrate cocoa pod 92%, rice bran 6% and lime 2% [4]. The water is added 70% of the mixture. And then inserted into a glass bottle (9 cm high, 5 cm diameter) 50 g or ± 3/4 of the height of the container, sealed and sterilized into the autoclave at a temperature of 121°C with a pressure of 1.1 atmospheres for 20-30 minutes 2 times. This process is done so that all spores and pathogen microbes are really dead. After that, the fungal isolate was inoculated into the planting medium at the level of 10% of the weight of the substrate (W/W), and incubated at 15 and 30 days. Each treatment was repeated 3 times, so there are 54 treatment units. At this stage the data is processed using analysis of variance completely randomized design [5], and then test the contrast [6] to determine the isolates, and the incubation period of the best influence on variables. After incubation, the samples were dried fermented with 55°C oven for 3-4 days, then ground with a hole diameter of 1 mm filter, and then used for the determination of [7] to determine the levels of lignin, cellulose and hemicellulose, the analysis of dry matter and in vitro organic matter [8].

2.3. Statistical Analysis
The data is processed using analysis of variance completely randomized design according to [5]. Contrast tested [6] to determine the isolates, the level and period of incubation of the best effects on variables.

3. Results and Discussion
Effect of fermented cacao pod with 3 white rot fungal isolates with an incubation period of 15 and 30 days on the content of lignin, cellulose and hemicellulose cocoa pod can be shown figure 1.
Figure 1. Effect of the fermented cocoa pod with some white rot fungi with an incubation period of 15 and 30 days on the content of lignin, cellulose, and hemicellulose.

Based on the analysis of variance results, treatment showed a highly significant effect on the lignin content of the cocoa pod. The cocoa pod that was not inoculated white rot fungi containing higher lignin compared with the pods that treated with white rot fungi inoculation. Further trials showed isolate C (Lentinus torulosus) had lower levels than isolates A (Coprinus comatus) and B (Cantharellus friesii), while at the 15-day incubation period there was no significant different with an incubation period of 30 days in isolates C (Lentinus torulosus).
Figure 1 shows that at the incubation period of 15 days, the lowest lignin content was shown by inoculated cocoa pod with isolates C (Lentinus torulosus), up to 30-day incubation period. The longer the fermentation, the more lignin degraded. Changes in lignin content of the substrate occur due to the utilization of lignin structure for the growth of white rot fungi. This is in accordance with the opinion of Risdiyanto [9] that stated that white rot fungi degrade lignin has a high ability and a microorganism capable of degrading lignin in the wood weathering processes into CO$_2$ and H$_2$O.

In this study, the skin fermented cocoa with white rot fungi can degrade lignin content of 1.42% - 12.28%. This value is higher than the research of Nelson and Suparjo [10] that found a decrease of 9.52% of the lignin content in fermented cocoa pod with Phanerochaete chrysosporium, but lower compared to Laconi [11] and Alemawor et al. [12] that found a decrease of 18.36% and 17.06%, respectively, in lignin content of cocoa pod treated with Phanerochaete ostreatus.

The results of calculation of variance showed that the treatment was highly significant to the cellulose content cocoa pod. Best isolates were less power degradation of the cellulose content. In contrast test showed isolate C (Lentinus torulosus) lower power degradation of the cellulose content than isolates B (Cantharellus friesii) and isolates of A (Coprinus comatus). The results of the analysis were not significant between 15 and 30 days of incubation of the cellulose cocoa pod.

The results of the analysis of the cocoa pod substrate incubation period of 15 days on the cellulose content of the lowest decline in isolates C (Lentinus torulosus), while fermented cocoa pod on a 30-day incubation isolate A (Coprinus comatus) increased cellulose content. The longer the incubation period, the content of cellulose substrate decreases except in isolates C (Lentinus torulosus) increased cellulose content in the incubation period of 30 days. This is caused by fungi outlines cellulose into simpler substances to be used as a nutrient in growth, so the production of enzymes by the fungus is increasing including cellulose digesting enzyme. According to Zeng et al. [13] results overhaul lignocellulosic components will be used by the fungus to meaningful growth will suppress the degradation of lignin and degradation activity will occur again if the availability of nutrients in the media is reduced. While according to Nelson and Supardjo [10], that the fungus will utilize the existing nutrients, including cellulose for growth and reduced lignin degradation activity.

In this study of white rot fungi inoculation can improve cellulose content of 0.8% of controls. This suggests that the increase is lower when compared to the research conducted by Nelson and Suparjo [10] using Phanerochaete chrysosporium seen that the rind contains cellulose fermented cocoa increased 2.91%. The results of calculation of variance seen that the treatment was highly significant to the hemicellulose content of the cocoa pod. In contrast test shows that isolate B (Chantharellus friesii) higher levels than isolate C (Lentinus torulosus) and A (Coprinus comatus). Hemicellulose content of the cocoa pod declined in the incubation period of 15 days but the incubation period of 30 days hemicellulose content increased. Although not the same as the hemicellulose content of the treatment is not white rot fungi were inoculated (control). This is caused by the ability of white rot fungi isolates to degrade hemicellulose, lignin makes increases with the length of incubation. The amount of lignin is degraded in the fermentation process causes hemicellulose content increased. According to Nelson and Suparjo [10], that the degradation of lignin will open up access to reshuffle the cellulose and hemicellulose. Results overhaul of cellulose to produce cellulase enzymes remodel simple sugars limit the production of most of the enzymes that degrade hemicellulose by white rot fungi [14].

In research with an incubation period of 15 and 30-day fermentation of the cocoa pod with white rot fungi can improve the hemicellulose content of only 6.07% by isolates B (Cantharellus friesii). The increase in value is higher compared to studies conducted by hemicellulose content of cocoa pod fermented by using Phanerochaete chrysosporium decreased by 4.41% by [10]. Results of analysis of variance showed that highly significant on the in vitro digestibility of dry matter and organic matter. Contrast test showed that cocoa pod fermented by white rot fungi is higher than unfermented cocoa pods (Control Vs A, B, C). This suggests that each inoculation of white rot fungi can improve in vitro digestibility of dry and organic matter cocoa pod.
Figure 2. Effect of the fermented cocoa pod with some white rot fungi with an incubation period of 15 and 30 days on the dry and organic matter in vitro.

In figure 2 shows that the incubation period of 15 and 30 days, the highest dry matter of cocoa pod digestibility was in B isolates (C. friesii), isolates C (L. torulosus), and then isolate A (C. comatus). Unfermented cocoa pod (control) has very low dry matter digestibility of 3.71% in contrast to research of Afrijon [15], the dry matter content cocoa pod are used 40.02%. In this study, dry matter digestibility values increased 80 to 86%. It is higher when compared with the results of research on in vitro dry matter digestibility cocoa pod that amoiasi with urea, an increase in dry matter ranges from 22 to 26% [15]. Similarly, studies in vitro dry matter digestibility rind fermented cocoa EM-4 conducted by Abidah [16], increasing the dry matter of 18 to 19%.

The results of this study showed that white rot fungi are fermented with the cacao pod able to increase the value of in vitro dry matter digestibility of 86% allegedly in the process of fermentation produce an enzyme that is able to reduce levels of fiber in the cocoa pod. This is in accordance with the opinion of Winarno et al. [17], the fermentation process feed material by microorganisms causes beneficial changes such as improving the quality of feed ingredients of both aspects of nutrition and digestibility and increases the shelf. Fermentation products usually have a higher nutritional value than the original material for their enzyme produced from the microbe itself. Contrast test conducted in this study shows no significant differences were found between the three isolates (A, B and C). While the incubation period finds a difference between 15 vs. 30 days on organic matter digestibility cocoa pod were inoculated isolate A (C. comatus), different from the inoculation isolate B and C.

In figure 3 shows that the digestibility of organic matter in the highest 15-day incubation A isolates (C. comatus), then isolate B (C. friesii) and isolate C (L. torulosus). By contrast, the incubation period of 30 days which is the highest organic matter digestibility was inoculated isolate B (C. friesii), but the
A isolates (C. comatus) and isolate C (L. torulosus) experienced a decline in value of 15 to 30 days. Organic matter digestibility cocoa pod used is very low at 3.14% in contrast to research [15], a digestible organic matter that is 46.37%. In this study, organic matter digestibility ranged between 70 to 83%. This value is higher than the in vitro digestibility of organic matter cocoa pod that amoniasi with urea, organic matter digestibility ranged from 18 to 27% [15].

The results showed that the fermentation of the cocoa pod with white rot fungi is able to increase the in vitro digestibility of organic matter by 83%, presumably because the white rot fungi produce enzymes that can degrade crude fiber fermentation process. This is in accordance with the opinion of [18], factors affecting the digestibility of organic material is crude fiber and mineral content of feedstuffs.

4. Conclusion

*Lentinus torulosus* is the most active isolate in degrading lignin and lacking its ability to degrade cellulose and hemicellulose. Fermented cocoa pod using three white rot fungi isolates namely (*Coprinus comatus*, *Cantharellus friesi*, and *Lentinus torulosus*), is able to improve the digestibility of dry matter and organic matter in vitro.

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