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Research Paper

The *In-vitro* Fiber Fractions Digestibility of Sugarcane Top by *Pleurotus ostreatus* and *Aspergillus oryzae* at Different Time of Incubation

Nur Azijah Nasution[1], Novirman Jamarun[2], Lili Warly[2], and Gusri Yanti[1]

[1]*Postgraduate Program Faculty of Animal Science, Andalas University, Padang, Indonesia*
[2]*Lecturer of Faculty of Animal Science, Andalas University, Padang, Indonesia*
Corresponding Author: novirman55@gmail.com

ABSTRACT: *This research aimed to find the best in-vitro fiber fractions (NDF, ADF, cellulose, and hemicellulose) digestibility of sugarcane top-fermented by Pleurotus ostreatus and Aspergillus oryzae. This research was conducted with four treatments such as A: Sugarcane top-fermented with Pleurotus ostreatus for 21 days, B: Sugarcane top-fermented with Pleurotus ostreatus for 28 days., C: Sugarcane top-fermented with Aspergillus oryzae for 21 days, and D: Sugarcane top-fermented with Aspergillus oryzae for 28 days. The in-vitro digestibility of fiber fractions was conducted for 48 hours incubation time in rumen fluid. The data were analyzed using randomized block design and continued by Duncan's test. The result of the experiment showed that the in-vitro digestibility of cellulose and hemicellulose were significantly different (P<0,05). Moreover, the in-vitro digestibility of ADF and NDF were not significant differences. Therefore, based on the data, it can be concluded that the in-vitro digestibility of fiber fractions of sugarcane top-fermented with Pleurotus ostreatus for 28 days (Treatment B) showed the best compared to other treatments.*
KEYWORDS: *Sugarcane top, Pleurotus ostreatus, Aspergillus oryzae, In-vitro*

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I. INTRODUCTION

The development of ruminant farm in tropical countries is generally more emphasis on the farm system that do not appear a competition in land use and the need of a feed. Therefore, the ruminant farm has an important role because of its ability to converted the feed ingredients into meat and milk. However, the obstacles faced during the dry season is the alternative feed ingredients are needed as a substitute for forages. One of the potential alternative feed is agricultural waste such as sugarcane top. The sugarcane top is plantation crop waste that has a great potential as animal feed because the large of quantity and it doesn't compete with human needs. According to [1] the sugarcane plantation area in Indonesia was 453.238 Ha, which is the production reach to 2.450.000 tons. As a producer of brown sugar, West Sumatra has sugarcane farmland of 7.305 ha in 2019 which the production reach 10.825 tons. The nutrition composition of sugarcane top was Dry matter 27,29%; Crude Protein 7,59%; Crude Fiber 40,39%; and Nitrogen Free Extract 40,67% [2]. As stated by [3] the sugarcane top is contains Neutral Detergent Fiber (NDF) 77,1%; Acid detergent fiber (ADF) 52,3%; hemicellulose 28,2%; cellulose 32,0%; silica 6,7% and lignin 14% [4].

The utilization of sugarcane top in Indonesia is still limited and it has not been widely used for animal feed. The limitations of the utilization of sugarcane top from the sugar industry because of its low digestibility and feed consumption compared to the grass. The use of sugarcane top as an animal feed requires a technology touch because it has high in crude fiber and lignin content and also low crude protein content and digestibility. One alternative that can be used to improve the digestibility of sugarcane top was by used microbes to change it to become a good feed for ruminants. As claimed [5] is one of the efforts to increase the nutritional value and the digestibility of agriculture waste was by utilizing microbes in the fermentation process of feed ingredients.

In the fermentation process can be used various microorganisms included the molds of Pleurotus ostreatus and Aspergillus oryzae. The mold of Pleurotus ostreatus can produce ligninase enzymes that have the

potential to change lignocellulolytic structures into glucose [6]. Furthermore, based on [7] that the mold of Aspergillus oryzae is the mold that produces most enzymes. The utilization of Pleurotus ostreatus and Aspergillus oryzae for fermentation sugarcane top was expected to improve the nutritional value and the digestibility of sugarcane top.

The one way to test the digestibility quality of feed ingredients was by using the In-vitro method. In-vitro technique [8] is an animal feed evaluation method that uses laboratory chemical analysis. [9], which is used to predict what happens the actual process of digestion in ruminants. [10] stated that the in-vitro is a laboratory-scale indirect method of estimating digestion by imitating the process that occurs in the digestive tract of ruminant animals.

Based on these conditions, this study is carried out to increasing the digestibility and utilization of sugarcane top through a fermentation process and seeing the effect of fermentation by using the Pleurotus ostreatus and Aspergillus oryzae to determine the in-vitro digestibility of NDF, ADF, Cellulose, and Hemicellulose.

II. MATERIALS AND METHODS

The materials used in the experiment were sugarcane tops that had been smoothed. Then it fermented with Pleurotus ostreatus and Aspergillus oryzae. The equipment used in this research was a set of tools for fermentation, a set of tools for proximate analysis, autoclave, analytical scales, beaker glass, measuring flask, magnetic stirrer, Erlenmeyer, pH meter, dropper puppets, and a set of tools for Van Soest analysis, etc.

This research was conducted at the Biotechnology Laboratory and the Technology Feed Industry Laboratory, Faculty of Animal Science, Andalas University, Padang. The design of the research used a Randomized Block Design (RAK) with 4 treatments and 4 replications. The sugarcane top samples were mashed, mixed, and fermented with the Pleurotus ostreatus and Aspergillus oryzae and put in a basket, then wrapped in a plastic facultative anaerobically. The fermentation was conducted for 21 to 28 days. After the sugarcane top has been harvested, then the sugarcane tops were milled and dried. The fermentation treatments were: A: Sugarcane top + Pleurotus ostreatus for 21 days, B: Sugarcane top + Pleurotus ostreatus for 28 days, C: Sugarcane top + Aspergillus oryzae for 21 days, and D: Sugarcane top + Aspergillus oryzae for 28 days.

The measurement of sugarcane top digestibility was conveyed with an in-vitro technique based on [11]. The sugarcane top sample for fermentation weighed as much as 2,5 grams and put into Erlenmeyer filled with 200 ml of goat rumen fluid and 50 ml of Mc Doughal's solution. Then, put it in a shaker water bath that had been filled the water with 39°C temperature and incubated for 48 hours. Then after the incubation, all the samples were analyzed to determine the digestibility of nutrients

The chemical analyses were conducted to find the digestibility of NDF, ADF, cellulose, and hemicellulose from the sugarcane top. The statistical analysis is to know the effect of the treatments towards the observed variables was analyzed by using the method of variance and continued with the Duncan test [12].

III. RESULTS AND DISCUSSION

Based on the statistical analyses, it was found that the *in-vitro* digestibility of fermented sugarcane top with *pleurotus ostreatus* and *Aspergilus oryzae* at different times of incubation can be seen in the Table 1.

Table 1. *In-vitro* nutrients digestibility of fermented sugarcane top (%)

Digestibility	A	B	C	D	SE
ADF	34,42±1,24	37,03±1,22	34,39±0,89	34,78±0,64	0,71
NDF	39,81±1,40	43,72±0,86	41,13±0,29	37,20±0,77	0,63
CELLULOSE	$45,32^{b}$±1,48	$50,25^{a}$±1,86	$40,88^{c}$±0,77	$33,11^{d}$±1,30	0,97
HEMICELLULOSE	$61,89^{a}$±2,05	$62,65^{a}$±1,69	$61,86^{a}$±1,56	$53,18^{b}$±1,71	1,28

Different superscript in the same rows were significantly different (P<0,05)

The result of the in-vitro nutrients digestibility of fermented sugarcane top is shown in table 1. The results of Duncan's tests showed that the digestibility of cellulose and hemicellulose on the sample was significantly different (P<0,05), where the in-vitro digestibility of ADF and NDF were not significantly different (P>0,05).

The best result of in-vitro cellulose digestibility of sugarcane top showed at fermented with the Pleurotus ostreatus for 28 days (50,25%) compared to fermented with Aspergillus oryzae for 21 days (45,32%), with the Pleurotus ostreatus for 21 days (40,88%) and with the Aspergillus oryzae for 28 days (33,11%).

On the other hand, the hemicellulose digestibility showed the best results were in the treatment with Pleurotus ostreatus for 28 days (62.65%), compared to treatment fermented with Pleurotus ostreatus for 21 days (61.89%), fermented with Aspergillus oryzae for 21 days (61.86%), and fermented with Aspergillus oryzae for 28 days (53.18%).

*Corresponding Author: novirman55@gmail.com

In this research, the in-vitro digestibility of ADF was not significantly different (P> 0.05) among the treatment means. The ADF digestibility has been tended to increase with the length of fermentation time. The highest in-vitro ADF digestibility was obtained in treatment fermented with Pleurotus ostreatus for 28 days, followed by the treatment with the Aspergillus oryzae for 28 days, Pleurotus ostreatus for 21 days, and the lowest digestibility at the treatment with the Aspergillus oryzae for 21 days.

The results of statistical analysis on the in-vitro NDF digestibility of the four treatments showed that there was no significant difference (P> 0.05) among the treatment means. The highest NDF digestibility was found in sugarcane top-fermented with Aspergillus oryzae for 28 days (41.93%) compared to the other treatments. The treatment has a significant effect on the digestibility of NDF, it caused the lignocellulose bonds were very strong and very complex to digest. The factors that influenced the NDF value were cellulose, hemicellulose, lignin, silica content and also, age and plant parts. The comparison of these results is a tendency that when the time of fermentation of sugarcane top increases, it has more increased yield, but it has low in digestibility value. By the research results of [13] that bagasse fermented with Trichoderma harzianum for 28 days had no significant effect (P> 0.05). The low digestibility value is also caused by the amount of mold used. If the amount of mold too little, so the work of mold is still very slow and has not been able to improve the digestibility of NDF of sugarcane top. [14] state that a dose of 25 grams of Pleurotus ostreatus mold is a very good way. These results indicated that the samples of sugarcane top-fermented with Pleurotus ostreatus for 28 days improved the digestibility of the sugarcane top.

The in-vitro digestibility of sugarcane top cellulose fermented with Pleurotus ostreatus for 28 days showed the significant effect and highest value compared to the other treatments. It causes the fermentation process to induced the apart of lignin, cellulose, and hemicellulose, because of that the delignification and depolimeryzation of the cellulose process were easier. The delignification and depolimeryzation of cellulose will make it easier for rumen microorganisms to improve the digestibility of cellulose. The research indicated that the highest digestibility of cellulose was in fermented sugarcane top with Pleurotus ostreatus for 28 days. It was due to the increased activity of the ligninase enzyme, especially for the laccase and lignin peroxidases (LiP) enzyme such as 1.99 U / mL and 13.51 U/ mL respectively, so that it caused an increase in the digestibility of cellulose.

Duncan's test showed that the hemicellulose digestibility of sugarcane top-fermented with Aspergillus ostreatus for 28 days was significantly different compared to Pleurotus ostreatus. Moreover, the sugarcane top-fermented with Pleurotus ostreatus for 21 days was not significantly different with Pleurotus ostreatus for 28 days. Also, the sugarcane top-fermented with Aspergillus oryzae for 21 days was not significantly different with Aspergillus oryzae for 28 days. The Different hemicellulose digestibility was influenced by different levels of NDF and ADF contents. The content of ADF and NDF was high so that the digestibility of hemicellulose was also high.

The increased digestibility of hemicellulose was also influenced by the lignin content of sugarcane top-fermented with Pleurotus ostreatus for 28 days, which was quite low so that it was supported the digestibility in the rumen effectively. The lignin content in plant cell walls binds the cellulose and hemicellulose so that the high lignin content will cause low digestibility. As stated by [15], the presence of lignin and anti-nutritional compounds in the feed will be an obstacle for rumen microbes to digest the feed. [16] also claimed that the lignin in the plant cell walls will be a barrier to the digestibility of ruminants. The lignin content in the feed will cause a low digestibility value in the rumen because the digestive enzymes will find it difficult to break down the lignin that binds to cellulose and hemicellulose [10].

IV. CONCLUSION

Based on the results of this study it can be concluded that the treatment has a significant effect on the digestibility of cellulose and hemicellulose. On the otherhand the treatment has no significant effect on the *in-vitro* digestibility of ADF and NDF. The highest digestibility value of ADF, NDF, Cellulose and Hemicellulose is 37.26%; 43.72%; 50.25%; and 62.65% respectively was found in the treatment B. The treatment B was sugarcane top fermented with *Pleurotus ostreatu*s for 28 days of incubation time.

V. RECOMMENDATION

Based on the research findings, the research offers some recommendations that for further research needs to make complete rations for goats based on the sugarcane top fermented with *Pleurotus ostreatus* for 28 days in *in-vivo* method.

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*Corresponding Author: novirman55@gmail.com

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*Corresponding Author: novirman55@gmail.com