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Quality Improvement of Sugarcane Top as Animal Feed with Biodelignification by *Phanerochaete Chrysosporium* Fungi on *In-vitro* Digestibility of NDF, ADF, Cellulose and Hemicellulose

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Abstract. The aims of this research were to improve the nutrients digestibility of sugarcane top fiber fractions (ADF, NDF, Cellulose and Hemicellulose) through biodelignification process using *Phanerochaete chrysosporium* fungi as forage feed. This study used a randomized block design (RBD) with 4 treatments and 5 rumen fluid sources as a group. The treatments consisted of: A=sugarcane top (without fermentation), B=sugarcane top fermented with 5% *Phanerochaete chrysosporium*, C=sugarcane top fermented with 10% *Phanerochaete chrysosporium*, D=sugarcane top fermented with 15% *Phanerochaete chrysosporium* with fermentation times is 21 days. The results of experiment showed that dose of 10% *Phanerochaete chrysosporium* with a fermentation time of 21 days could improve the digestibility of sugarcane top fiber fractions, as indicated by the higher digestibility of NDF (54.21%), ADF (53.06%), Cellulose (49.10%), and Hemicellulose. (57.73%).

1. Introduction

Indonesia was an agricultural country with varied agricultural by-products. Sugarcane is one of the strategic agricultural commodities that provides many side products from both on farm and off farm. The area of sugarcane planted in 2010 was 418,259 ha with a national sugarcane production of 34,218,549 tons. Sugarcane waste generated from this area will reach 17,793,645 tons^[1].

One of the by-products of the sugarcane plant is sugarcane top, which are very potential to be used for feed because of the large amount of them. Efforts to use sugarcane by-products have been done a lot, but they are still not optimal. This requires an appropriate technology approach to further increase the added value of sugarcane top. One strategy that can be done is to use it as fermented feed.

Sugarcane top contain nutrients that allow them to be used as animal feed. The chemical composition of sugarcane top is 39.9% dry matter, 7.42% ash, 42.30% crude fiber, 7.4% crude protein, 2.90% crude fat, and 40.00% BETN, 77.1% NDF, 48.9% ADF, Hemicellulose 28.2 %, Cellulose 32.0%, Lignin 13.6%, and Silica 6.7%^[2,3]

The main problems of sugarcane top using for animal feed are its low nutritional value and digestibility and high value of lignin. Lignin physically and chemically are the main factors causing the inability of livestock to digest feed ingredients. Lignin chemically binds to structural carbohydrate

components and physically acts as a barrier to the process of cell wall breakdown by rumen microbes^[4]. Some of the microbes that could act as decomposing agents in fermented feed are mold, yeast and bacteria.

Types of fungi that have the ability to degrade high lignin are fungi that are included in the white rot fungus. *Phanerochaete chrysosporium* is a fungus that belongs to this type, known to produce lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase enzymes. *Phanerochaete chrysosporium* is one of the fungi that can break down bonds and degradation lignin with the help of lignin-degrading enzymes. These molds also degrade cellulose, hemicellulose and lignin polymers with the help of extracellular enzymes. Biodegradation is a process of changing the substrate by microorganisms which involves a number of reactions into simpler products. The activity of overhauling the substrate components requires nutrients obtained from the overhaul.

The aim of this study was to reduce the lignin content and increase the nutritional content and digestibility of fiber fractions (NDF, ADF, Cellulose and Hemicellulose) of sugarcane top with *Phanerochaeta chrysosporium* fungi.

2. Material and Method

2.1. Sampling

Sugarcane tops are used as feed sample. The sample is dried using sunlight in a greenhouse at the collection site. Drying is continued using a 60 °C oven for 48 hours until the sample is dry enough to be cut and mashed using a grinding machine.

This study used a randomized block design (RBD) with 4 treatments and 5 rumen fluid sources as a group. The treatments consisted of: A=sugarcane top (without fermentation), B=sugarcane top fermented with 5% *Phanerochaete chrysosporium*, C=sugarcane top fermented with 10% *Phanerochaete chrysosporium*, D=sugarcane top fermented with 15% *Phanerochaete chrysosporium* and fermentation times was 21 days.

2.2. *Phanerochaeta chrysosporium* inoculum preparation

Isolate *Phanerochaete chrysosporium* was cultured on PDA media, incubated at 30 °C for 7 days. Sterilize 100 grams of sugarcane tops that have been added with distilled water to 60% water content. Autoclave for 30 minutes at 121 °C, after warming with 1 test tube isolate *Phanerochaete chrysosporium*. Incubation for seven days.

2.3. Biodelignification of sugarcane tops with *Phanerochaeta chrysosporium*

100 grams of sugarcane tops are added with water to a water content of 60%, sterilize with an autoclave for 30 minutes at 121 °C. After that, inoculate *Phanerochaeta chrysosporium* 0, 5, 10 and 15%. Biodelignification conducting for 21 days.

2.4. Chemical Analysis

Each sample was analyzed to determine the content of dry matter, organic matter, crude protein, crude fat, and crude fiber using proximate analysis^[5]. NDF, ADF, cellulose, hemicellulose, and lignin were analyzed using Van Soest analysis^[6]

2.5. In-vitro method

In vitro analysis was carried out to determine the digestibility^[2] of fermented sugarcane top using *Phanerochaeta chrysosporium* of each feed component and the rumen process was stopped by immersing the erlenmeyer tube in ice water to stop microbial activity, then measuring the pH using pH measuring device. The next step is to separate the supernatant from the residue. The mixture obtained from in-vitro analysis was put into a centrifuge tube then separated by a centrifuge for 30 minutes at a speed of 3000 rpm and a temperature of 4°C until there was separation between the supernatant and the residue. The residue was filtered using Whatman No.41 filter paper then dried in an oven at 60°C, before analyzing the fiber fraction digestibility using the Van Soest analysis method.

2.6. Statistical Analysis

Data obtained from this research were statistically analyzed using SPSS software version 21.0.

3. Results and Discussion

The results of the average nutritional content of fermented sugarcane top with *Phanerochaeta crysosporium* from each treatment can be seen in Table 1. In general, each treatment experienced an increase in nutrient content. It was clear that the delignification process occurs due to the fermentation process using *Phanerochaeta crysosporium*. The best treatment was to use 10% *Phanerochaeta crysosporium* inoculum with a fermentation time of 21 days. Crude protein content increased to 7.16% and lignin decreased to 9.89%.

Table 1. Nutrient composition of Sugarcane top fermented by *Phanerochaeta chrysosporium*

Nutrient Content (%)	Treatments			
	A (0%)	B (5%)	C (10%)	D (15%)
Dry matter	89,35 ± 0,123	94,29 ± 0,321	93,20 ± 0,101	94,08 ± 0,321
Organic matter	91,57 ± 0,219	94,29 ± 0,002	97,63 ± 0,171	95,99 ± 0,112
Crude Protein	5,68 ± 0,253	6,25 ± 0,125	7,16 ± 0,321	6,82 ± 0,311
NDF	57,13 ± 0,342	53,16 ± 0,189	49,01 ± 0,231	51,70 ± 0,021
ADF	45,71 ± 0,251	43,33 ± 0,326	39,26 ± 0,321	42,05 ± 0,003
Celullose	28,21 ± 0,235	26,15 ± 0,215	23,58 ± 0,126	25,12 ± 0,231
Hemicelullose	11,41 ± 0,251	9,82 ± 0,312	9,74 ± 0,312	9,64 ± 0,121
Lignin	15,05 ± 0,234	13,89 ± 0,153	9,89 ± 0,123	10,31 ± 0,312
Silica	4,81 ± 0,172	3,91 ± 0,132	3,11 ± 0,124	3,57 ± 0,121

Source: Ruminant Animal Laboratory, Animal Science Faculty, Andalas University (2019)

Lignin decomposition was associated with the metabolism of microorganisms. Among all microorganisms, white rot fungi degrade lignin more rapidly and extensively than other groups [7]. These microorganisms produce several ligninolytic enzymes (lacase, manganese peroxidase, and lignin peroxidase) which catalyze one-electron oxidation of lignin units, producing aromatic radicals [8]. Lignin degradation was mainly used by secondary metabolism, or limited availability of nitrogen, carbon, or sulfur, and is usually not degraded as the only source of carbon and energy, requiring additional substrates such as cellulose, hemicelulose or glucose [9]. Some white rot fungi attack lignin more easily than hemicelullose and cellulose. Many white rot fungi, however, show a simultaneous pattern of decay characterized by degradation of all cell wall components [10].

Table 2. NDF, ADF, Selulloce and Hemicelullose Digestibility of Sugarcane top Fermented by *Phanerochaeta chrysosporium*

Digestibility (%)	treatment				
	A	B	C	D	SE
NDF	46,74 ^b ± 0,032	48,21 ^{bc} ± 0,021	54,21 ^a ± 0,121	50,20 ^c ± 0,191	0,132

ADF	40,46 ^c ± 0,231	44,62 ^b ± 0,412	53,06 ^a ± 0,321	50,07 ^a ± 0,501	0,412
Cellulose	44,03 ^c ± 0,6721	46,23 ^{bc} ± 0,162	49,10 ^a ± 0,151	47,38 ^{ab} ± 0,512	0,124
Hemicellulose	48,24 ^c ± 0,351	51,09 ^{bc} ± 0,421	57,73 ^a ± 0,131	55,11 ^{ab} ± 0,152	0,231

^{a,b,c,ab,bc} superscript different means significantly different in a row (p<0.05)

3.1. Digestibility of Neural Detergent Fiber (NDF)

Based on Table 2, it could be seen that the average digestibility of fermented sugarcane top in each treatment ranges from 46.74% - 54.21%. The results of the analysis of diversity showed that the different doses of *Phanerochaete chrysosporium* had a very significant effect (P <0.01) on the digestibility of sugarcane top NDF. Judging (from Table 2) the best dose was found in treatment C, namely giving 10% *Phanerochaete chrysosporium* mold with an average NDF digestibility increased from 46.74% to 54.21%.

The high value of NDF digestibility in treatment C (10% *Phanerochaete chrysosporium* inoculum) was due to the *Phanerochaete chrysosporium* fungi producing high ligninase enzyme, so that in the process of lignin degradation by the ligninase enzyme in perfectly fermented sugarcane top, which could be seen from the low lignin content of 9.89% of treatment C (Table 1). The ligninase enzyme could break the lignocellulose and lignohemicellulose bonds, thereby facilitating the penetration of rumen microbial enzymes to degraded NDF. In treatment D, the digestibility of NDF decreased again, this is due to increase of use of organic matter by *Phanerochaete chrysosporium* fungi. The results of research by^[10] stated that using a dose of 6 -10% *Phanerochaete chrysosporium* fungi could increase the digestibility of fermented palm sludge. Increasing the use of *Phanerochaete chrysosporium* fungi dosage increases decomposition of the chemical components of oil palm fronds and causes decrease dry matter content^[11].

3.2. ADF (Acid Detergent Fiber) Digestibility

The digestibility of ADF of fermented sugarcane top in each treatment ranged from 40.46% - 53.06%. The results of the analysis showed that different doses of *Phanerochaete chrysosporium* had a very significant effect (P <0.01) on ADF digestibility of sugarcane tops. Indicated by Table 2 that the best dose was found in treatment C, namely giving 10% *Phanerochaete chrysosporium* fungi with an average ADF digestibility from 46.74% to 54.21%. Fermentation processing on sugarcane tops is able to break down ADF into simpler compounds that dissolve easily. This shows that the bond stretching occurs, so that the proportion of cell contents increases but the proportion of ADF decreases. The results of Wardani's research^[12] shown that fermentation process of oil palm fronds with *P. chrysosporium* can reduce the ADF content by 40.97% of the content before fermentation, namely 52.72% at an inoculum dose of 7.5% and fermentation time of 20 days, where lignin content in the study was also low at 19.27%. According^[13], the greater inoculum dose used in fermenting oil palm fronds, the greater the reduction in NDF and ADF content. ^[14] stated that during the bioconversion process of agricultural waste by *P.chrysosporium*, the peak production of lignin-degrading enzymes was on day 10 and day 21.

Digestibility of ADF in treatment D (15% *Phanerochaete chrysosporium*) was decreased, that happen because fermented sugarcane tops had a higher lignin content than treatment C (Table 1). ^[15] explained that the decrease in digestion was caused by the higher lignin content in the oil palm frond compared to other treatments, which could inhibit work of rumen microbes to digest feeds. The higher lignin and silica content of feed, more difficult it would be to digest it.

3.3. Cellulose Digestibility

The digestibility of cellulose of fermented sugarcane tops in each treatment ranged from 44.03% - 49.10%. The results of the analysis showed that the administration of different doses on the digestibility of cellulose fermented sugarcane tops with *Phanerochaete chrysosporium* had a significant effect ($P < 0.05$). From the results of statistical analysis, it was explained that the best treatment was seen in treatment C (10% *Phanerochaete chrysosporium*). This result was due to the higher the digestibility of NDF and ADF in treatment C compared to the other treatments. Digestibility of NDF and ADF is directly proportional to digestibility of cellulose. Cellulose is a substance contained in NDF and ADF, so the higher the NDF and ADF, the digestibility of cellulose would also increase.

The higher digestibility of cellulose in treatment C (10% *Phanerochaete chrysosporium*) was caused by the optimal fermentation process in this treatment, resulting in the complete release of lignocellulose and lignohemicellulose bonds which would facilitate the penetration of rumen microbes as a result, the digestibility of cellulose could increase.^[16] states that although cellulose is difficult to destroy in the digestive system, with the help of microbes that produce cellulase enzymes, it could digest and utilize cellulose components.

Digestibility of cellulose in treatment D has decreased, this occurs because of the dosage (15% *Phanerochaete chrysosporium*) and 21 days of fermentation, the content of food substances that are easily digested has been used up so that the components that are left are insoluble components such as lignin, because the content in high D treatment (Table 1), so that the digestibility of cellulose is low.^[17] states that lignin plays an important role in strengthening the structure of the cell wall of the feeds that binds cellulose and hemicellulose so that it is difficult to digest by microorganisms, and also the exhaustion of soluble feedstuffs which are thought to have been utilized by microorganisms for growth so that what is left is food ingredients that are in the form of lignin and silica.

3.4. Hemicellulose Digestibility

Hemicellulose digestibility of sugarcane tops fermented with *P. chrysosporium* in each treatment ranged from 48.24% - 57.73%. The results of the analysis showed that giving different doses to the digestibility of hemicellulose sugarcane tops fermented with *Phanerochaete chrysosporium* had a very significant effect ($P < 0.01$). The average hemicellulose digestibility increased from 48.24% to 57.73%. Increased digestibility of hemicellulose occurred in treatment C (10% *Phanerochaete chrysosporium* mold) which was 57.73%. This is caused by the breakdown of lignin and cellulose so that the degradation process of hemicellulose in the rumen increases. According^[18] stated that the higher inoculum level is used, the more microbes would produce enzymes to overhaul the enzymes for fermentation process.

Hemicellulose is the difference between the NDF content and the ADF content, from the *in-vitro* analysis results it was also found that the digestibility of hemicellulose was higher than digestibility of cellulose, even though the hemicellulose and cellulose components usually bind by lignin and silica according to the opinion of^[19]. The digestibility of hemicellulose was higher than that of cellulose, because hemicellulose is a fraction that is more easily digested by rumen microbes than cellulose. It was explained that the constituent components of hemicellulose consist of polymers that are less resistant to chemical and enzymatic solvents than cellulose.

4. Conclusion

The results of the research can be concluded that a dose of 10% *Phanerochaete chrysosporium* with a fermentation time of 21 days on sugarcane top increases the nutritional value and *in-vitro* nutrients digestibility, namely the digestibility of NDF (54.21%), ADF (53.06%), cellulose (49.10%) and hemicellulose (57.73%).

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