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Lignin Content, Ligninase Enzyme Activity and *in vitro* Digestability of Sugarcane Shoots using *Pleurotus ostreatus* and *Aspergillus oryzae* at Different Fermentation Times

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Abstract: This study aims to obtain the best lignin content, ligninase enzyme activity and *in vitro* digestibility value of fermented sugarcane shoots using *Pleurotus ostreatus* and *Aspergillus oryzae* at different fermentation times. The research process is divided into 2 stages. Stage 1 treatment samples include A1B1 = Sugarcane shoots fermented with *Pleurotus ostreatus* for 14 days; A1B2 = Sugarcane shoots fermented with *Pleurotus ostreatus* for 21 days; A1B3 = Sugarcane shoots fermented with *Pleurotus ostreatus* for 28 days; A2B1 = Sugarcane shoots fermented with *Aspergillus oryzae* for 14 days; A2B2 = Sugarcane shoots fermented with *Aspergillus oryzae* for 21 days; A2B3 = Sugarcane shoots fermented with *Aspergillus oryzae* for 28 days. The design used is a completely randomized design with a factorial pattern. Stage 2 treatment samples cover A = Sugarcane shoots fermented with *Pleorotus ostreatus* for 21 days; B = Sugarcane shoots fermented with *Pleorotus ostreatus* for 28 days; C = Sugarcane shoots fermented with *Aspergillus oryzae* for 21 days; D = Fermented sugarcane shoots with *Aspergillus oryzae* for 28 days. The design used was a randomized block design. The results showed that there was no interaction between the type of mold and the fermentation time on the lignin content ($P>0.05$), but there was an interaction with the enzyme activity of Laccase, LiP and MnP ($P<0.05$). Also, there were significant differences in the digestibility of protein, cellulose, hemicellulose, VFA and NH_3 ($P<005$), however the digestibility of DM, OM, ADF, NDF and rumen fluid pH had no significant difference ($P>0.05$). It was concluded that sugarcane shoots fermented with *Pleurotus ostreatus* mold for 28 days got the best results with the value as follow, lignin content (11.55%), CP digestibility (57.90%), cellulose digestibility (50.25%), Hemicellulose digestibility (62.65%), Laccase enzyme activity (2.68 U/mL), LiP enzyme activity (19.44 U/mL), VFA (111.67 mM) and NH_3 (10.48 mg/100 mL).

Keywords: *Aspergillus oryzae*, Fermentation, Lignin, *Pleurotus ostreatus*, Sugarcane Shoots

Introduction

Sugarcane shoots are not widely used by sugar producers. It usually wastes so that it has the potential as a potential ruminant feed provider. Sugarcane is harvested in the dry season. Therefore, it can be used as an alternative feed to replace grass, which in the dry season is very limited in availability. Sugarcane plantations in Indonesia cover an area of 453,328 hectares and produce sugar cane shoots of 30% (DJP, 2019).

The biggest obstacle in the utilization of sugarcane shoots as ruminant feed is the high lignin content (Susanti *et al*., 2020). Lignin is a wood substance in plants that cannot be digested and reduced the ability of livestock to consume food (Jamarun *et al*., 2018). Fermentation technology using microorganisms that produce lignin's enzymes (Laccase, Manganese peroxidase and lignin peroxidase) is one of the most effective solutions to overcome the problem (Jamarun *et al*., 2017a; Pazla *et al*., 2020).

Pleurotus ostreatus and *Aspergillus oryzae* molds are microorganisms that produce ligninase enzymes which are effective in degrading lignin (Zhang *et al*., 2015; Dimawarnita and Tripanji, 2018). The effectiveness of this molds are largely determined by the incubation time and the type of material being processed. Anita *et al*. (2011) reported that bagasse fermented using *Pleurotus ostreatus* and *Phanerochaete chrysosporium* for 28 days gave the best results with a lignin degradation value of 24%, while bagasse fermented using *Pleurotus ostreatus* and *Trametes versicolor* for 7 days managed to reduce lignin 17.48%. Lignin degradation and ligninase enzyme activity of *Aspergillus oryzae* were higher than *Phanerochyate chrysosporium* (Guo *et al*., 2014).

Fermentation of sugarcane shoots with different types of molds and duration of fermentation will certainly have varying effects on lignin content, ligninase enzyme activity and digestibility values in ruminants, but the extent of the effect is not certainly known. To prove the extent of this influence, it is necessary to conduct in-depth research on ruminants in vitro. This study aims to obtain the best lignin content, ligninase enzyme activity and *in vitro* digestibility value of fermented sugarcane shoots using *Pleurotus ostreatus* and *Aspergillus oryzae* at different fermentation times.

Materials and Methods

This research was divided into 2 experimental stages. The first stage is fermentation of sugarcane shoots with *Pleurotus ostreatus* and *Aspergillus oryzae* at different fermentation times to see the level of lignin content and ligninase enzyme activity. The second stage is the digestibility and fermentability test of the rumen fluid from the best fermented sugarcane shoots was carried out in stage 1 using *in vitro* techniques.

Experimental Site

This research was carried out in three laboratories which are at the ruminant nutrition laboratory, the feed industry technology laboratory and the biotechnology laboratory at the Faculty of Animal Husbandry andalas University, Padang, West Sumatra, Indonesia. Sugarcane shoots samples were taken from sugarcane plantations, Koto Lalang area, Kuranji, Padang.

Research Material

The materials used in this study covers *Pleurotus ostreatus* mold, *Aspergillus oryzae* mold, Potato Dextrose Agar (PDA) medium, sugarcane shoots, aquades, reagents (to measure the activity of ligninase enzymes such as ABTS), guaiacol and veratryl alcohol, rumen fluid, Mc. Dougalls, H_2SO_4, Neutral Detergent Fiber (NDF) solution, Acid Detergent Fiber (ADF) solution and rumen fluid from goat.

The equipment used is a set of equipment for rejuvenation of molds, which includes autoclave, glass beaker, cotton, aluminum foil, test tube, analytical balance, pH meter, plastic and pipette.

Research Methods

The research is divided into 2 stages as follow:

1. Research Stage I (fermentation of sugarcane shoots with *Pleurotus ostreatus* and *Aspergillus oryzae* at different fermentation times)

Parameters measured

The variables observed were lignin content after fermentation and ligninase enzyme activity consisting of laccase, Lignin Peroxidase (LiP) and manganese peroxidase (MnP).

b. Research Implementation

Samples of sugarcane shoots were taken as much as 15 kg. The sugarcane shoots were chopped 3-5 cm and dried in the sun for 2 days to reach 10% moisture content, then ground using a grinder into flour.

Pleurotus ostreatus and *Aspergillus oryzae* were rejuvenated on PDA media and incubated for 7 days at 30°C. Then, a total of 100 grams of crushed sugarcane shoots are put into plastic and add 50% aquadest until the water content reaches 60%. After the medium cooled, the *Pleurotus ostreatus* and *Aspergillus oryzae* were inoculated with test tube (5×10^6 cfu/ml) each which had been grown which then being incubated for 14, 21 and 28 days. After reaching the appropriate day for the treatment, the medium was ready to be harvested and the fresh weight was weighed, then put in the oven to dry at a temperature of 60°C. If it is dry, the medium is stored to be used as a test sample for the lignin contents, activity of the ligninase enzyme and material for the implementation of the second stage of research, namely *in vitro* Table 1 and 2.

Table 1: Chemical composition of sugarcane shoots before fermentation

Chemical composition	%DM
Dry matter	89.35±0.123
Ash	8.43±0.123
Organic matter	91.57±0.219
Crude protein	5.68±0.253
NDF	57.13±0.342
ADF	45.71±0.251
Cellulose	28.21±0.235
Hemicellulose	11.41±0.251
Lignin	15.05±0.234
Silica	4.81± 0.172

Table 2: Chemical composition of sugarcane shoots after fermentation of each treatment (% DM)

Chemical composition	Treatments A1B1	A1B2	A1B3	A2B1	A2B2	A2B3
Dry matter	92.77	91.75	91.15	93.27	92.24	91.49
Organic matter	90.17	89.64	90.10	90.51	90.39	89.94
Crude protein	7.90	8.10	8.56	7.73	8.43	7.84
ADF	62.28	56.20	50.20	62.94	52.19	61.70
Cellulose	44,98.00	40.72	35.01	45.01	36.28	39.68
NDF	71.63	69.94	67.93	72.75	69.18	71.06
Hemicellulose	9.35	13.74	17.72	9.81	16.99	9.36
Ash	9.49	9.69	9.75	10.08	10.09	10.15
Silica	96.64	96.55	96.61	96.64	96.58	96.71

The procedure from (Warishi *et al*., 1992) was used to test the activity of the MnP enzyme. Two stages of measurement occurred, namely, the measurement following the addition of Mn and the measurement without the addition of Mn. Measurement upon the addition of Mn: For this measurement, 0.1 buffer lactate (pH 4.5, 50 M), 0.1 mL guaiacol (4 mM), 0.2 mL $MnSO_4$, 0.3 mL Aquades (1 mm), 0.1 mL H_2O_2 (1 mm) and 0.2 mL filtrate enzyme were added to a 2 mL tube for a total volume of 1 mL. The cuvette tube was shaken slowly so that all ingredients were mixed. The enzyme activity reaction was carried out at a temperature of 20±1°C. Absorbance was measured at 0 and 30 min at wavelength 465 nm. Measurement without the addition of Mn: For this measurement, 0.1 mL buffer lactate (pH 4.5 50 M), 0.1 mL guaiacol (4 mm), 0.5 mL distilled water, 0.1 mL H_2O_2 (1 mm) and 0.2 mL filtrate enzyme were added to a 2-mL tube for a total volume of 1 mL. The cuvette tube was shaken slowly so that all the ingredients were mixed. The enzyme activity reaction was carried out at a temperature of 20±1°C. Absorbance was measured at 0 and 30 min at wavelength of 465 nm. The activity of MnP is the activity observed upon the addition of Mn minus the enzyme activity without the addition of Mn.

c. Research design

The design used is a completely randomized design with a factorial pattern (2×3×3), as follow:

Factor A is the type of mold:

1. *Pleurotus ostreatus* (A1)
2. *Aspergillus oryzae* (A2)

Factor B is fermentation time:

1. 14 days (B1)
2. 21 days (B2)
3. 28 days (B3)

This design follows the model of (Steel and Torrie 1960). Also, Duncan's Multiple Range Test (DMRT) was used due to the difference of mean treatment value from the analysis of variance.

From the results of the first stage of the research, it was found that the best 4 treatment combinations would be continued in the second phase of the research. The best selection was done based on the lowest lignin content and the highest crude protein after fermentation.

2. Research Stage 2 (The digestibility and fermentability test of the rumen fluid from the best fermented sugarcane shoots was carried out in stage 1 using *in vitro* techniques).

a. Parameters measured

The parameters measured were dry matter digestibility, organic matter digestibility, fiber fraction (NDF, ADF, Cellulose, Hemicellulose) digestibility, rumen pH, Volatile Fatty Acids (VFA) and NH_3 concentrations.

Formula:

$$DMD(\%) = \frac{SW \times DMS - (RW \times DMR - blank)}{SW \times DMS} \times 100\%$$

$$OMD(\%) = \frac{SW \times DMS \times OMS - (RW \times DMR \times OMR - blank)}{SW \times DMS \times OMS} \times 100\%$$

$$NDFD(\%) = \frac{SW \times DM \times NDFS - (RW \times DM \times \%NDFR)}{SW \times DM \times NDFS} \times 100\%$$

$$ADFD(\%) = \frac{SW \times DM \times ADFS - (RW \times DM \times \%ADFR)}{SW \times DM \times \%ADFS} \times 100\%$$

$$CelD(\%) = \frac{SW \times DM \times CelS - (RW \times DM \times \%lR)}{SW \times DM \times \%CelS} \times 100\%$$

Where, DMD is Dry Matter Digestibility, SW is Sample Weight, DMS is Dry Matter Sample, RW is Residual Weight, DMR is Dry Matter Residual, OMD is Organic Matter Digestibility, OMS is Organic Matter Sample, OMR is Organic Matter Residual, NDFD is NDF Digestibility, NDFS is NDF sample, NDFR is NDF residual, ADFD is ADF digestibility, ADFS is ADF sample, ADFR is ADF residual, CelD is cellulose digestibility, CelS is cellulose sample, CelR is cellulose residual, HemiD is hemicellulose digestibility, HemiS is hemicellulose sample, HemiR is hemicelluloce residual.

b. Research Procedure

Implementation of *in vitro* for the measurement of digestibility was carried out following the method of (Tilley and Terry, 1963). Furthermore, the Measurement of NH_3 and VFA concentrations was following the (Department of Dairy Science, 1966) procedure.

c. Research design

The design used was a randomized block design, which consisted of 4 treatments and 3 replications.
The treatments are as follow:

A: Sugarcane shoots fermented with *Pleurotus ostreatus* for 21 days
B: Sugarcane shoots fermented with *Pleurotus ostreatus* for 28 days
C: Sugarcane shoots fermented with *Aspergillus oryzae* for 21 days
D: Sugarcane shoots fermented with *Aspergillus oryzae* for 28 days

Analysis of variance was used to analyze data based on Steel and Torry (1960). DMRT test was used due to the difference of mean treatment value

Results and Discussion

Research Stage I

a. Lignin Content

The average lignin content of sugarcane shoots fermented with *Pleurotus ostreatus* and *Aspergillus oryzae* at different fermentation times is presented in Table 3.

Table 3 shows that there is no interaction (P>0.05) between the type of mold (A) and the length of fermentation (B) on the lignin content of sugarcane shoots, but the type of mold and the fermentation time have a significant effect (P<0.05) on the content of sugarcane shoots. The lignin content of sugarcane shoots fermented with *Aspergillus oryzae* was significantly (P<0.05) higher than that of sugarcane shoots fermented with *Pleurotus ostreatus*. This condition proves that sugarcane shoots fermented with *Pleurotus ostreatus* are better in reducing lignin content, which is 19.01%. The low lignin content in sugarcane shoots fermented with *Pleurotus ostreatus* (12.52%) showed that the lignin content in sugarcane shoots could be optimally reduced by *Pleurotus ostreatus.*

The ability of *Pleurotus ostreatus* and *Aspergillus oryzae* in degrading sugarcane shoot lignin was still below that of the *Phanerochyate chrysosporium* mold. Yanti *et al*. (2021) reported that sugarcane shoots fermented with *Phanerochate chrysosporium* for 21 days produced a lignin content of 9.89% with a degradation rate of 34.29%, while sugarcane shoots fermented for 20 days using *Pleurotus ostreatus* and *Aspergillus oryzae* only produced lignin content, 12.62 and 13.66% with lignin degradation rates of 16.15 and 9.24% respectively. This difference could be caused by the influence of the amount of mold inoculum used in the fermentation process. Similarly, Mirnawati *et al.* (2013) stated that the amount of inoculum dose added to the substrate will affect the level of degradation of crude fiber.

The duration of fermentation significantly affects the lignin content of sugarcane shoots. Fermentation time of 28 days for both types of mold showed the lowest lignin content of 12.66%. The longer the fermentation time, the higher the level of degradation of the substrate, because the mold needs a carbon source for its growth. The mold fulfills it through the reshuffle of crude fiber (lignin) with the help of the ligninase enzyme (Astuti *et al*., 2021).

b. Laccase Enzyme Activity

The average activity of the Laccase enzyme from fermented sugarcane shoots in each treatment is presented in Table 4.

Table 4 shows that there is a significant interaction (P<0.05) between the type of mold and the time of fermentation on laccase enzyme activity. The activity of the laccase enzyme in A1B3 treatment, namely sugarcane shoots fermented with *Pleurotus ostreatus* for 28 days was significantly (P<0.05) higher (2.68 u/mL) compared to other treatments. This is because the carbon and nitrogen sources in sugarcane shoots are still quite high, so the activity of the laccase enzyme is also high. Similarly, Majeau *et al*. (2010) stated that the activity of the laccase enzyme is influenced by the availability of carbon and nitrogen sources on the substrate.

c. Lignin Peroksidase Activity

The average activity of the lignin peroxidase enzyme from fermented sugarcane shoots in each treatment can be Table 5.

Table 3: The average lignin content of sugarcane shoots fermented with *Pleurotus ostreatus* and *Aspergillus oryzae* at different fermentation times (%)

Type of mold	Fermentation time B1	B2	B3	Average
A1	13.39	12.62	11.55	12.52^B
A2	14.43	13.66	13.78	13.96^A
Average	13.91^a	13.14^{ab}	12.66^a	

Note: Upper case (A, B) and lower case (a, b) are different on the same row and column which indicated significance (P<0.05)

Table 4: Laccase enzyme activity of fermented sugarcane shoots in each treatment (U/mL)

Type of mold	Fermentation time B1	B2	B3
A1	1.16^{aA}±0,55	1.62^{aA}±0,13	2.68^{aB}±0.83
A2	1.00^{aA}±0,01	1.18^{aA}±0,05	1.32^{bA}±0.08

Note: Upper case (A, B) and lower case (a, b) are different on the same row and column which indicated significance (P<0.05)

Table 5: Lignin peroxidase enzyme activity of fermented sugarcane shoots in each treatment (U/mL)

Type of mold	Fermentation time B1	B2	B3
A1	7.47^{aA}±0.88	14.34^{aB}±0.51	19.44^{aC}±1.45
A2	5.63^{bA}±0.30	12.68^{aB}±0.55	10.31^{bC}±0.15

Note: Upper case (A, B) and lower case (a, b) are different on the same row and column which indicated significance (P<0.05)

The treatment showed a significant interaction (P<0.05) between the type of mold and the time of fermentation on the activity of the LiP enzyme. Sugarcane shoots fermented with *Pleurotus ostreatus* for 28 days (A1B3) were significantly higher (19.44 U/mL) compared to treatments A1B2 (14.34 U/mL), A2B2 (12.68 U/mL), A2B3 (10.31 U/mL), A1B1 (7 .47 U/mL) and A2B1 (5.63 U/mL). This indicates that sugarcane shoots fermented with *Pleurotus ostreatus* mold increased as longer fermentation time. Likewise, Dimawarnita (2019) that the LiP enzyme activity of the *Pleurotus ostreatus* fungus continued to increase until the fifth month. Also, Gomes *et al*. (2009) added that LiP enzyme activity in *Lentinus sp* continued to increase in the fifth week.

The activity of the LiP enzyme in this study was higher than that of Puspita (2007), which found a LiP enzyme activity value of 0.430 U/mL in the wild *Pleurotus spp*. Cocoa pods fermented with *phanerochayte chrysosporium* only produced a LiP activity of 0.527 U/mL (Yakin *et al*., 2017).

d. Manganese Peroksidase Activity

The average activity of the manganese peroxidase enzyme from fermented sugarcane shoots in each treatment can be seen in Table 6.

Table 6 shows that there is a significant interaction (P<0.05) between the type of mold and the time of fermentation on the activity of the MnP enzyme. MnP enzyme activity in the A2B1 treatment, namely sugarcane shoots fermented with *Aspergillus oryzae* for 14 days showed the highest MnP activity at 4.60 U/mL and was not different (P>0.05) from the MnP activity produced by *Pleurotus ostreatus* for 14 days of fermentation (A1B1), which was 3.60 U/mL. Optimum MnP activity in this study was found on day 14. Furthermore, Nurika *et al*. (2019) reported that MnP enzyme activity was also optimum on the 14th day (0.605 U/mL) of bagasse fermentation by the fungus *Phlebia sp.MG-60* and decreased until the 28th day.

MnP enzyme activity in molds is influenced by availability of nutrients from lignocellulosic degradation (Giardina *et al*., 2000). The lignocellulose content in the substrate is known to act as an inducer in enzyme production. Inducers are compounds or elements that support reactions in enzymes and support enzyme secretion (Acevedo *et al*., 2011). Table 6 shows that the longer the fermentation time, the lower the activity of the MnP enzyme produced. It is suspected that the longer the fermentation time, the nutrients needed by the mold in producing MnP enzymes will decrease so that it has an impact on MnP activity. The same happened to *Trametes villosa*. On the 15th day the fungus showed an increase in MnP activity, while after the 15th day the activity decreased until it reached 0 on the 30th day (Lordêlo *et al*., 2014).

Research Stage 2

a. In vitro digestibility

The effect of fermented sugarcane shoots with different types of mold and time on *in vitro* digestibility is presented in Table 7.

Table 7 shows that the treatment had no significant effect (P>0.05) on DMD, OMD, NDFD and ADFD. The digestibility value of feed is strongly influenced by the lignin content in the feed ingredient. Lignin is the substance that cannot be digested by rumen microbes and blocks the penetration of rumen microbial enzymes to degrade food substances into simple molecules. The lignin content after fermentation in treatments A, B, C and D was almost the same, which are 12.62, 11.55, 13.66 and 13.78%, so that the ability of rumen microbes to degrade feed was also the same. The higher the lignin content in the feed, the lower the digestibility value of the feed.

The same dose of inoculum in each treatment was suspected to be the cause of dry matter digestibility not significantly different between treatments (P>0.05). Differences in inoculum doses result in differences in the amount of mycelium that was formed so that the degradation of lignin was to be certainly different. A high dose of inoculum allowed more mycelium to be formed so that the mold need more energy. This energy is obtained from the breakdown of cellulose, hemicellulose and lignin substrates as carbon sources (Suharnowo *et al*., 2012).

Dry matter digestibility in treatment B was higher than the other treatments, although not significantly different (P>0.05) statistically. Dry matter digestibility of sugarcane buds fermented with *Pleurotus ostreatus* mold for 28 days was 34.16% higher than treatments A (33.57%), D (33.09%) and C (32.71%). The high dry matter digestibility is due to the accumulation of enzymes produced during the fermentation process. Enzymes increased the crude protein content in feed ingredients and dry matter digestibility is also affected by protein levels in feed ingredients. Also, Routa *et al*. (2015) reported that there is an increase in dry matter digestibility in fermented young coconut coir due to the accumulation of enzymes from white oyster mushrooms. The organic matter digestibility pattern in this study was the same as the dry matter digestibility pattern. The more dry matter digestibility increases, the higher organic matter digestibility improved (Pazla *et al*., 2018a; Suyitman *et al*., 2020).

The dry matter digestibility and organic matter of fermented sugarcane shoots in this study were lower than the research by Ay *et al*. (2018) that obtained DMD and OMD values of 60.2 and 58.2%. However, the result was higher than Elmi (2017) with values of 21.12 and 12.71%. The different value is caused by the difference in the fermentation materials used.

Digestibility of crude protein, cellulose and hemicellulose was significantly affected by treatment ($P<0.05$). Treatment B showed the highest digestibility values for CPD, cellulose digestibility and hemicellulose digestibility was 57.90, 50.25, 62.65% respectively. The high crude protein content in treatment B caused the effectiveness of rumen microbes to utilize protein to form microbial protein to be optimal so that it would increase the rumen microbial population, especially proteolytic bacteria. Pazla *et al*. (2018b) explained that an increase in the microbial population would increase feed digestibility. The increase in crude protein content was caused by an increase in the mass of mold cells. In addition, Shaba and Baba (2012) stated that the fungus *Pleurotus ostreatus* also secretes protease enzymes. The secretion of protease enzymes by the fungus *Pleurotus ostreatus* also plays a role in increasing the protein content of fermented sugarcane shoots so that the absorption of amino acids as the simplest form of protein will be more easily absorbed. According to Mirnawati *et al*. (2010) mold can increase the protein content of fermented substrate biomass by secreting extracellular enzymes.

The low digestibility of crude protein in sugarcane shoots fermented with *Aspergillus oryzae* for 21 days (Treatment C) was due to the fact that *Aspergillus oryzae* with a fermentation period of 21 days had not been able to optimize the growth of mold mycelia so that the enzymes produced were few and caused the process of decreasing lignin not optimally.

NDF and ADF are fiber fractions from feed consisting of lignin. The digestibility of NDF is higher than the digestibility of ADF because the NDF fraction still contains nutrients that are easily utilized by rumen microbes such as hemicellulose and cell wall proteins, while ADF contains many fractions that are difficult to digest, namely lignin and silica (Jamarun *et al*., 2017b); (Pazla and Jamarun 2021a). Similarly, Hambakodu *et al*. (2020) highlighted that lignin levels have a negative correlation with ADF digestibility. The lower the lignin content, the higher the digestibility of ADF. The digestibility of NDF and ADF which did not differ between treatments in this study was also in line with the results of Samadi *et al*. (2016) who reported that fermentation of sugarcane bagasse for up to 28 days had no significant effect on crude fiber content.

Cellulose and hemicellulose are food substances that was be converted into energy (VFA) by rumen microbes. Low lignin in treatment B caused rumen microbes, especially cellulolytic bacteria and hemicellulolytic bacteria, to degrade cellulose and hemicellulose more than in other treatments. Hemicellulose was more degraded by rumen microbes than cellulose because cellulose-digesting bacteria also play a role in degrading hemicellulose which causing the digestibility value of hemicellulose to be higher than cellulose.

b. Rumen Fluid Characteristics

The effect of fermented sugarcane shoots with different types of mold and time on Rumen fluid characteristics is presented in Table 8. The Table 8. Shows that the treatment had no significant effect on the pH of the rumen fluid ($P>0.05$). The average value of rumen pH in this study was still within the normal range which was able to support rumen microbial growth. The ideal rumen pH range to maintain normal rumen metabolic processes reported by several research results is 6.0-7.0 (Jamarun *et al*., 2017c; Jamarun *et al*., 2020).

Fermentation time and different types of molds had a significant effect ($P<0.05$) on the total VFA concentration. Sugarcane shoots fermented with *pleurotus ostreatus* with 28 days of fermentation (Treatment B) showed the highest VFA concentration. VFA concentration decreased in sugarcane shoot fermentation using *Aspergillus oryzae* on 21 and 28 days ($P<0.05$) and there was no significant difference in VFA production in 21-day fermentation using *Pleurotus ostreatus* (Treatment A).

Table 6: Manganese peroxidase enzyme activity of fermented sugarcane shoots in each treatment (U/mL)

	Fermentation time		
Type of mold	B1	B2	B3
A1	$3{,}60^{aA}\pm0{,}06$	$2{,}96^{aA}\pm0{,}90$	$2{,}64^{aA}\pm0{,}84$
A2	$4{,}60^{aA}\pm0{,}34$	$2{,}00^{aB}\pm0{,}32$	$3{,}74^{aA}\pm0{,}39$

Note: Upper case (A,B) and lower case (a,b) are different on the same row and column which indicated significance ($P<0.05$)

Table 7: *In vitro* digestibility of fermented sugarcane shoots in each treatment (%)

	Treatments				
Parameters	A	B	C	D	SEM
Dry Matter	33.57	34.16	32.71	33.09	0.76
Organic Matter	35.21	35.49	34.00	34.45	0.75
Crude Protein	46.54^{b}	57.90^{a}	35.86^{d}	42.16^{c}	0.98
NDF	39.81	43.72	41.13	37.20	0.63
ADF	34.42	37.03	34.39	34.78	0.71
Cellulose	45.32^{b}	50.25^{a}	40.88^{c}	33.11^{d}	0.97
Hemicellulose	61.89^{a}	62.65^{a}	61.86^{a}	53.18^{b}	1.28

Note: Means in the same row with different letters (a,b,c,d) are significant ($P<0.05$)

Table 8: Rumen fluid characteristics of fermented sugarcane shoots in each treatment (%)

	Parameters		
Treatments	pH	VFA (mM)	NH_3 (mg/100 mL)
A	6.83	106.67^{a}	9.27^{c}
B	6.86	111.67^{a}	10.48^{d}
C	6.98	86.67^{b}	8.08^{b}
D	6.92	80.00^{b}	7.08^{a}
SEM	0.04	4.33	0.13

Note: Means in the same column with different letters (a,b,c,d) are significance ($P<0.05$)

The decrease in VFA production in treatments A, C and D was due to the high content of lignin so that it could not be properly degraded by rumen microbes. In addition, Roni (2018) reported that the high lignin content in the feed even after fermentation would reduce the VFA concentration. The concentration of VFA obtained in this study is still in the normal range according to (McDonald *et al*., 2010) and (Riestianti *et al*., 2021), which are 80 -160 mM and 107.78 – 127.14 mM.

Ammonia (NH_3) is the main product of the fermentation of feed protein in the rumen by rumen microbes, where the higher the NH_3 concentration, the higher the feed protein undergoes fermentation in the rumen. The NH_3 value of the rumen fluid in this study was significantly ($P>0.05$) influenced by the treatment. Treatment B showed the highest concentration of NH_3 (10.48 mg/100 mL). The concentration of NH_3 in treatment B was in line with the high crude protein content which was also the highest (8.56%) among other treatments. In addition, the lowest lignin content in treatment B made the protein able to be degraded more by rumen microbes. Liklewise, Pazla and Jamarun (2021b) stated that the high and low concentrations of NH_3 are determined by the level of protein in the feed and the degree of degradability of the feed in the rumen. The range of NH_3 values obtained in this study was still within the normal range for rumen microbial activity and growth. The minimum concentration of NH_3 required for the formation of microbial protein is 5 mg/100 mL (Satter and Slyter, 1974). NH_3 concentration 3.51 -4.57 mg/100 mL was still able to maintain the performance of rumen microbes in degrading feed (Jamarun *et al*., 2021).

Conclusion

The conclusion of this study was that sugarcane shoots fermented with *Pleurotus ostreatus* for 28 days produced the lowest lignin content with the highest ligninase enzyme activity, the best *in vitro* digestibility and optimum rumen fluid characteristics for microbial growth.

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Author's Contributions

Roni Pazla: Supervised the conduct of the research, analyzed the data and wrote articles.

Novirman Jamarun: Designed the study.

Lili Warly: edited the article.

Gusri Yanti and Nur Azijah Nasutian: Carried out fermentation and *in vitro* tests in the laboratory.

Ethics

This article was written from data from the latest author's research and is original. Corresponding author has ensured that all authors involved in this article have read and approved this article for publication. There are no ethical issues in this study.

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