

STUDY OF FERMENTATION OF LEMONGRASS WASTE (*Cymbopogon nardus*), RICE BRAN AND CORN FRACTION WITH *Pleurotus ostreatus* ON FIBER FRACTION AND DIGESTION OF DRY MATTER, ORGANIC MATTER, AND CRUDE PROTEIN

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Abstract

Lemongrass waste is an alternate feed to replace grass for ruminants' diet, but it has a high crude fiber content. Therefore, it is necessary to handle and process lemongrass waste. This study examines the effect of inoculum dose and fermentation time of lemongrass waste (*Cymbopogon nardus*) with *Pleurotus ostreatus* on fiber fraction, dry matter digestibility, organic matter, and crude protein. A randomized block design was employed, consisting of six treatments of lemongrass waste with rice bran and corn shards and three replications as a group. The inoculum doses were 6%, 9%, and 12%, and the fermentation times were 14 and 21 days. The observed parameters were fiber fraction (NDF, ADF, Hemicellulose, Cellulose, dry matter digestibility of organic matter, crude protein. Data were processed using Analysis of Variance (ANOVA), and differences between treatments were tested using Duncan Multiple Range Test (DMRT). The results of the analysis of variance showed that the treatment had a very significant effect ($P < 0.01$) on NDF, ADF, and Hemicellulose and a significant effect ($P < 0.05$) on dry matter digestibility and organic matter digestibility. However, no significant effect ($P > 0.05$) was found on crude protein digestibility. It was concluded that the best dose in this study was 12% and a fermentation time of 21 days with NDF content of 66.11%, ADF 48.16%, cellulose 30.38%, hemicellulose 14.16%, DMD 58.01%, OMD 58.24%, and CPD 50.16% in vitro.

Keywords: digestibility nutrient, fiber fraction, Lemongrass Waste, *Pleurotus ostreatus*

KAJIAN FERMENTASI LIMBAH LEMONGRASS (CYMBOPOGON NARDUS), DEDAK PADI DAN PECAHAN JAGUNG DENGAN PLEUROTUS OSTREATUS TERHADAP FRAKSI SERAT DAN KECERNAAN DARI BAHAN KERING, BAHAN ORGANIK DAN PROTEIN KASAR.

Abstrak

Limbah serai wangi dapat dijadikan pakan alternatif pengganti rumput untuk ternak ruminansia, tetapi mempunyai kandungan serat kasar yang tinggi. Untuk itu perlu penanganan dan pengolahan terhadap limbah serai wangi. Penelitian ini mengkaji pengaruh dosis inokulum dan lama fermentasi limbah serai wangi (*Cymbopogon nardus*) dengan *Pleurotus ostreatus* terhadap fraksi serat, pencernaan bahan kering, bahan organik, protein kasar. Metode penelitian adalah rancangan acak kelompok yang terdiri dari 6 perlakuan limbah serai wangi dengan dedak padi dan pecahan jagung dan 3 ulangan sebagai kelompok. Dosis inokulum adalah 6%, 9% dan 12% dan lama fermentasi 14 dan 21 hari. Parameternya adalah fraksi serat (NDF, ADF, Hemicelulosa, Selulosa, pencernaan bahan kering bahan organik, protein kasar. Data diolah menggunakan Analisis of Variance (ANOVA) dan perbedaan antar perlakuan diuji dengan Duncan Multiple Range Test (DMRT). Hasil analisa ragam menunjukkan bahwa perlakuan memberikan pengaruh berbeda sangat nyata ($P < 0,01$) terhadap NDF, ADF dan Hemicelulosa serta pengaruh berbeda nyata ($P < 0,05$) terhadap pencernaan bahan kering dan pencernaan bahan organik tetapi berbeda tidak nyata ($P > 0,05$) terhadap pencernaan protein kasar. Disimpulkan dosis terbaik pada penelitian ini adalah 12% dan lama waktu fermentasi 21 hari dengan kandungan bahan NDF 66,11%, ADF 48,16%, selulosa 30,38%, hemicelulosa 14,16%, KcBK 58,01%, KcBO 58,24% dan KcPK 50,16% secara In-vitro.

Keywords: fraksi serat, pencernaan nutrisi, limbah serai, *Pleurotus ostreatus*

INTRODUCTION

Animal husbandry is part of the agricultural subsector that continues to be developed to meet the growing need for animal protein. As the livestock population increases, the demand for good animal feed

will increase as well. A feed is considered of good quality if it has complete nutrients, such as carbohydrates, protein, vitamins, and minerals. The availability of animal feed ingredients must also be effective, not competing with human food needs. One

promising feed source is plantation waste, such as lemongrass.

Lemongrass (*Cymbopogon nardus*) is one of the most important essential oil-producing plants that has long been cultivated in Indonesia. It is characterized by wide leaf shape and a darker leaf color (dark green) than the usual lemongrass leaf shape. The growth of lemongrass is influenced by soil fertility, climate, and altitude. Lemongrass can grow in various types of soil, both lowlands and highlands, up to an altitude of 1,200 m above sea level, with an optimum altitude of 250 m above sea level. In West Sumatra, lemongrass is cultivated in areas such as Solok, Sawahlunto, Pasaman, and Tanah Datar.

Solok City, West Sumatra, lemongrass cultivation covered a total land area of 25.3 hectares in 2017, yielding 70.5 tons/year of fresh product and 1.93 tons/ha/year of lemongrass distillation waste, amounting to 48,829 tons / 25.3 hectares of lemongrass waste is (Solok City Statistics Agency, 2017). By 2018, cultivation expanded to 40.2 hectares, producing 110.14 tons/year fresh product and a total waste of 2.74 tons/ha/year, resulting in 110.148 tons/ 40.2 hectares waste. Lemongrass has a very high potential to grow in Solok city, because of its ideal geographical location at an altitude of 390 - 1458 meters above sea level.

Lemongrass waste has a disadvantage from its high-water content of 74.70%, and distillation results ranging from 24.01%-62.02%, making it prone to spoilage and molds. It also has a high crude fiber content of 35.14% and lignin content of 14.14%, which greatly affects the content of cellulose and hemicellulose, especially the formation of complex bonds of lignocellulose and lignohemicellulose (Kamoga et al., 2013). Distillation increased the lemongrass's lignin content to 27.38% (Kamoga et al., 2013). Because of its low digestibility, the high lignin content is a big problem when used as a substitute for grass for ruminants, as it decreases the animals' productivity and performance.

To overcome these obstacles, lemongrass waste must be processed before it is fed to livestock. Lignin content can be hydrolyzed using fungi that contain lignase enzymes in the fermentation process, such as *Pleurotus ostreatus* or oyster mushroom. Oyster mushrooms are white weathering fungi

that grow quickly in lignocellulosic waste, which belongs to the Basidiomycetes group that produces extracellular lignocellulolytic enzymes such as lignin peroxidase. This type of fungus is the only group of microorganisms that have the ability to break down lignin into carbon dioxide and water extensively, producing a group of enzymes that are directly involved in the breakdown of lignin, including a type of phenol-oxidase called laccase, lignin peroxidase (LiP) and manganese peroxidase (MnP) (Fitria, 2008). Ligninolytic enzymes work on several substrates, especially phenolic compounds, which is one of this enzyme's advantages (Verma & Madamwar, 2002).

Fermentation with oyster mushroom (*Pleurotus ostreatus*) has been shown to reduce crude fiber content, cellulose content, lignin content and increase crude protein content. Fermenting *Azolla microphylla* with 3%, 6%, and 9% *Pleurotus ostreatus* inoculum for 14 and 21 days improved its nutritional profile (Noferdiman et al., 2014). Oyster mushrooms are also rich in protein, twice as high as that of asparagus, cabbage and potatoes, four times as high as that of tomatoes and carrots, and six times as high as that of citrus fruits.

Research conducted by Sukamto and Djazuli (2011) proved that lemongrass waste is a potential feed alternative based on its nutritional content, with 7.00% protein, higher than rice straw, which is only 3.93%. It also has 2.3% fat, 3353.00 kcal/GE/kg energy, 25.73% crude fiber, 0.35% calcium, 0.14% phosphorus and 7.91% ash. Oyster mushroom contains 10.5-30.4% protein, 56.6% carbohydrates, 1.7-2.2% fat, and 7.5-8.7% fiber (Sumarmi, 2006).

A substrate is needed to accelerate the work of ligninolytic enzymes, such as rice bran. Rice bran enhances porosity under aerobically and anaerobic conditions, so that the fungus can develop properly. Although the bran contains high fiber, the crystallization has not occurred, so it is still at an amorphous stage, making it easier to digest. Rice bran is also inexpensive and widely available. In addition, corn flour can also be used as a substrate for its high enough carbohydrates and contains sugar (monosaccharides) which is a carbon source for fungal growth.

Research on lemongrass waste has been carried out to increase its nutritional content. According to Rahmadiani (2021), a mixture of

lemongrass waste and tofu pulp can maintain pH value and increase volatile fatty acid (VFA) and ammonia (NH₃) concentrations. The fermentation mixture of lemongrass waste, rice bran and corn flour with oyster mushroom is expected to show the best results on rumen fluid pH, NH₃ production, and VFA.

For rumen microbes, VFA has two important roles, namely as a source of energy and carbon skeleton for the formation of microbial protein and NH₃. Carbohydrates and fiber contained in lemongrass waste fermented in the rumen by rumen microbes will produce VFA. The process runs smoothly if the pH is normal or balanced. The novelty of this research lies in the use of rice bran mixed with lemongrass waste to create good fermentation porosity and corn flakes function as nutrients for the growth of *Pleurotus ostreatus* (Zulkarnain and Siswanti, 2022).

MATERIALS AND METHODS

The research was conducted from July 2022 to December 2022 at the Feed Industry Technology Laboratory (TIP), Faculty of Animal Husbandry, Ruminant Nutrition Laboratory and Animal Product Technology Laboratory (THT), Universitas Andalas, Padang.

The lemongrass waste used in this study was sourced from Jl. Kapten Marah Yulius, Tanah Garam, Lubuk Sikarah, Solok City, West Sumatra. Rice bran was used to facilitate aeration, as its porous structure allows fungal mycelium to penetrate the substrate easily. Corn fractions were also used as a substrate because of their carbohydrate and monosaccharide content, which are a source of carbon for fungal growth. The lemongrass waste was air-dried before used.

The inoculum used in this study was *Pleurotus ostreatus* (white oyster mushroom). Materials used for the *in vitro* experiments were rumen fluid, McDougall solution, and reagents for proximate analysis, according to the proximate analysis methods by AOAC (2010). Fiber fraction analysis was conducted using the VanSoest method, with results presented in Table 1.

The equipment used was tools for testing the characteristics of in-vitro rumen fluid and tools for analyzing dry matter, organic matter, crude protein, Erlemeyer,

centrifuge, analytical balance, electric oven, furnace, desiccator cup, aluminium foil, filter paper, glass cup, gauze, and shaker water bath.

Lemongrass waste was chopped into 2-3 cm long pieces, and aerated for about 8 hours to reduce its water content. It was then mixed with corn fractions and rice bran in a ratio of 70%: 10%: 20%. For fermentation, the *Pleurotus Ostreatus* inoculum was added to the substrate fermentation mixture (Lemongrass Waste + Corn + Rice Bran) as much as 6%, 9%, and 12% of the total substrate weight.

The lemongrass waste mixture was put into plastic bags with a 2 kg capacity. Each bag received 100 ml of distilled water and was then homogenized. The samples were then autoclaved at 121 °C for 30 minutes. Samples that have been autoclaved were aerated under laminar for 5 minutes, then inoculated with *Pleurotus Ostreatus* (6%, 9%, 12%), covered, and punctured on the top and bottom surfaces with the same number of punctures. Then, the plastic was incubated on the fermentation rack according to the treatment for 14 and 21 days. After incubation, the fermented lemongrass waste and rice bran were harvested, air dried for 2 hours, then oven-dried at 600 °C for 24 hours to obtain Air Dry Weight data. The material was ground, and chemical analysis was carried out according to the parameters. Table 2 presents the nutrient content of lemongrass waste after fermentation (%DM).

All ingredients were dissolved in 1 liter of distilled water, while the buffer solution was prepared the day before fermentation. Before use, the Mc Dougalls solution was placed in a shaker water bath at 39°C and CO₂ gas flowed for 30-60 seconds to maintain anaerobic conditions. The pH was adjusted to be near 7 using 20% NaOH or 20% H₂PO₄.

This experimental study was conducted using a completely randomized design (CRD) in a 3 x 2 factorial pattern with three replicates. The treatment factors were as follows:

Factor A = Inoculum level of *Pleurotus ostreatus*
 A1: 6%
 A2: 9%
 A3: 12%

Factor B = Duration of fermentation
 B1: 14 days
 B2: 21 days

Table 1. Nutrient Content of Lemongrass Waste Before Fermentation (%DM)

Nutrient Content	Lemongrass Waste
Water Content	64,58
Dry Material	35,42
Ash	16,44
Organic Material	83,56
Crude Protein	5,43
Crude Fiber	35,4
Crude Fat	2,83
Non-Nitrogen Free Extract (NNFE)	39,90
Total Digestible Nutrient (TDN)	51,6
Neutral Detergent Fiber (NDF)	74,46
Acid Detergent Fiber (ADF)	65,09
Cellulose	41,23
Hemicellulose	9,37
Lignin	14,14

Source: Ruminant Laboratory, Faculty of Animal Science, 2022.

Table 2. Nutrient Content of Lemongrass Waste after Fermentation (%DM)

Nutrient	A1B1	A2B1	A3B1	A1B2	A2B2	A3B2
Content						
Total Water Content	63,93	62,27	61,90	61,42	60,35	58,91
Total Dry Ingredients	36,07	37,73	38,10	38,58	39,65	41,09
Ash	9,41	9,25	8,61	8,36	7,35	7,17
Organic Material	90,58	90,75	91,39	91,64	92,65	92,83
Crude Protein	8,47	9,26	9,97	10,82	12,34	12,46
Crude Fiber	31,41	29,00	27,43	26,89	22,54	20,39
Crude Fat	1,96	1,89	1,83	1,79	1,70	1,677
Nitrogen Free- Extract (NFE)	50,41	52,20	53,70	53,65	57,49	59,71
Total digestible nutrition (TDN)	55,35	57,63	59,09	59,68	63,65	65,48
Neutral detergent fiber (NDF)	71,66	70,57	69,90	69,21	67,29	66,11
Acid detergent fiber (ADF)	61,97	59,73	58,24	54,88	52,43	48,16
Cellulose	37,09	36,29	35,44	34,05	32,53	30,38
Hemicellulose	9,69	10,84	11,66	11,87	11,58	14,16
Lignin	12,59	10,70	10,19	9,80	9,11	8,02
NH ₃	6,80	6,94	7,51	7,65	7,79	7,93

Source: Ruminant laboratory Faculty of Animal Science, 2022

Table 3. Preparation of McDougall's solution

Chemicals	Quantity (grams)
NaHCO ₃	9,80
Na ₂ HPO ₄ H ₂	4,62
KCl	0,57
MgSO ₄ 7H ₂ O	0,12
NaCl	0,47
CaCl ₂ 2H ₂ O	0,05

Source: Tilley and Terry (1963)

Table 4. Content of NDF, ADF, Hemicellulose, Cellulose, Fermented Lemongrass Waste with *Pleurotus ostreatus* (%DM)

Inoculum dose (%)	Fermentation Duration (days)	NDF (%)	ADF (%)	Hemicellulose (%)	Cellulose (%)
6	14	71,66 ^a	61,97 ^a	9,69 ^c	37,09 ^a
9	14	70,57 ^b	59,73 ^b	10,84 ^c	36,29 ^a
12	14	69,90 ^{bc}	58,24 ^{bc}	11,66 ^b	35,44 ^a
6	21	69,21 ^d	54,88 ^d	11,87 ^b	34,05 ^a
9	21	67,29 ^c	52,43 ^c	11,58 ^b	32,53 ^b
12	21	66,11 ^f	48,16 ^f	14,16 ^a	30,38 ^c

Description: Different superscripts on NDF, ADF, and Hemicellulose columns indicate highly significant differences (P<0.01). Meanwhile, different superscripts on the Cellulose column indicate significant differences (P<0.05).

Table 5. DMD, OMD, CPD of Fermented Lemongrass Waste with *Pleurotus ostreatus* (%DM)

Inoculum dose (%)	Fermentation Time (days)	DMD (%)	OMD (%)	CPD (%)
6	14	55.71 ^d	55.90 ^c	45.91
9	14	55.95 ^{dc}	56.37 ^{bc}	47.35
12	14	56.00 ^{bc}	56.57 ^{bc}	48.12
6	21	56.20 ^b	56.80 ^b	48.70
9	21	57.54 ^{ab}	57.82 ^a	49.99
12	21	58.01 ^a	58.24 ^a	50.16

Description: DMD = Dry matter digestibility, OMD = Organic matter digestibility, CPD = Crude protein digestibility. Different superscripts in DMD, and OMD indicate highly significant differences (P<0.01).

In-vitro experiment followed the Tilley and Terry (1963) method, namely Randomized Group Design (RAK) with six treatments and three groups of rumen fluid collection as replicates. Grouping was based on different rumen fluids. The treatment in this study was fermented lemongrass distillation waste consisting of 70% lemongrass waste (LW) and 20% rice bran (RB) and 10% corn fractions (CF), (Rahmadiani, 2021; modified). The treatment applied were combinations of different *Pleurotus ostreatus* inoculum levels and fermentation durations, as outlined below:

A1B1 = LW + RB+CF + 6% inoculum for 14 days

A1B2 = LW + RB +CF+ 6% inoculum for 21 days

A2B1 = LW + RB +CF+ 9% inoculum for 14 days

A2B2 = LW + RB +CF+ 9% inoculum for 21 days

A3B1 = LW + RB +CF+ 12% inoculum for 14 days

A3B2 = LW +RB +CF+ 12% inoculum for 21 days

Parameters observed included: Content of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Cellulose, Hemicellulose (Van Soest 1994), Digestibility of dry matter and organic matter (Tilley & Terry Method 1963). The data were analyzed using analysis of variance according to the Randomized Group Design. The mathematical model of the design used was according to Steel and Torrie (1991).

RESULTS AND DISCUSSION

The crude protein increased from 5.43% before fermentation to 12.46% post-fermentation, while the nitrogen-free extract (NFE) rose from 39.90% to 59.71%. Conversely, crude fiber diminished from 35.40% before fermentation to 20.39% after fermentation (Tables 1 and 2).

This increase in proteins was attributed to the Lemongrass residue, rice bran, and corn mixture. The fungi utilize flakes for reproduction, increasing the crude protein content of the fermented product.

Neutral Detergent Fiber (NDF)

In this study, the NDF content across treatments ranged from 66.11% to 71.66%. The analysis of variance results showed a very significant interaction ($P < 0.01$) between inoculum dosage and fermentation duration. The higher the dose and the longer the fermentation, the greater the decrease was observed in NDF, ADF, and Hemicellulose. This reduction was due to the activity of *Pleurotus ostreatus* inoculum containing ligninase enzymes that break down fiber (Noferdian et al., 2014).

Table 4 shows that the highest value of NDF (71.66%) was recorded in the treatment of 6% inoculum for 14 days. This suggests that the lignin and hemicellulose hydrolysis process was not optimal at this inoculum level and fermentation duration. The lowest NDF content (66.11%) was observed in the treatment with 12% inoculum and 21 days of fermentation time, indicating that this treatment is more effective in degrading fiber.

NDF is composed of cellulose, hemicellulose, and lignin components. The higher dosage and longer duration result in more activity of ligninase enzymes, a lignin-breaking enzyme produced by microorganisms with lignophilic properties that can break bonds in the cell wall and NDF components (Noferdian et al., 2014). Thus, the fermentation process using *Pleurotus ostreatus* at higher inoculum doses and longer durations is more effective in improving the nutritional quality of lemongrass waste.

Acid Detergent Fiber (ADF)

Acid detergent fiber (ADF) is a food substance that is insoluble in acid detergent, consisting of cellulose, lignin and silica (Van Soest 1994). In this study, the ADF content ranged from 48.16% to 61.97%. Analysis of variance showed significant interaction ($P < 0.05$) between factor A (inoculum dosage) and factor B (duration). This interaction occurs because both factors affect fermentation. Similar to NDF, ADF is also a cell wall component so the interaction between factors A and B may occur. The results of the analysis of variance showed significantly different results so the DMRT test was required.

The treatment with 6% inoculum and 14 days fermentation resulted in the highest ADF content (61.97%). The inoculum in this

treatment using *Pleurotus ostreatus* which functions to hydrolyze may not have not been able to break down lignocellulosic bonds. Crude protein in this treatment was also lower than the other treatments at 8.47%. This is because the content of microorganisms in this treatment tends to be lower which causes the breakdown of lignocellulose bonds to not be maximized. According to Sandi, et al. (2010), the high protein content in fermented feed is due to the contribution of microorganisms. According to Ibrahim (2017), the more the number of microbes, the lower the ADF content. Another factor causing the high ADF content in the 6% dose treatment and 14 days fermentation time can also be influenced by the content of lignin and cellulose. By the statement of Usman et al. (2019) ADF consists of lignin and cellulose in plant cell walls.

The treatment of a 12% dose and 21 days fermentation time has an ADF content of 48.16%. The work of the cellulase enzyme in *Pleurotus osteratus* in this treatment causes the cell wall to hydrolyze so that the lignocellulose and lignohemicellulose bonds are stretched. The breakdown of cell walls converts complex compounds into simpler components during the fermentation process, causing changes in ADF levels (Anam et al., 2012). *Pleurotus ostreatus* contains ligninase, lignocellulose, and lignohemicellulose enzymes that can degrade lignin, cellulose, and hemicellulose. This is according to the opinion of Verma and Madamwar (2002), which states that oyster mushrooms are also one of the white weathering fungi that grow quickly in lignocellulosic waste. The advantage of this ligninolytic enzyme is that it can hydrolyze several substrates, especially phenolic compounds.

Hemicellulose

In this study, the hemicellulose content ranged from 9.69% to 14.16%. Based on the results obtained, the analysis of variance showed that the interaction gave a very significant effect ($P < 0.01$), so a further DMRT test was needed. The dose of inoculum and the length of fermentation on hemicellulose content showed a significantly different effect ($P < 0.01$) on fermentation products. Based on the results of this study, it can be known that inoculum doses of 6%, 9%, and 12% showed different abilities in

hemicellulose hydrolysis. This happens because hemicellulose has a simpler molecular weight than cellulose, so it is easier to degrade. Factor B (length of fermentation) based on the DMRT test showed a significantly different effect ($P < 0.01$). This is because the longer the fermentation time, the more significantly the hemicellulose content differs because the level of hemicellulose degradation is easier than that of cellulose.

In this study, there was an increase in hemicellulose from 9.37% (Table 1) to 14.16% (Table 2). This revealed the ability of the *Pleurotus ostreatus* fungus to produce ligninase and cellulase enzymes in hydrolyzing lignocellulose and lignohemicellulose in lemongrass waste.

The treatment of 12% dose and 21-day fermentation time has a hemicellulose content of 14.16%, which is due to the low NDF content in the treatment. Hemicellulose in the cell wall binds with lignin to produce lignohemicellulose bonds. Lignohemicellulose bonds cause digestibility to decrease so that hemicellulose content increases and NDF content decreases. The treatment of a 6% dose and 14 days fermentation time has a hemicellulose content of 9.69%. Hemicellulose is one of the most easily digested fractions by rumen microbes, this happens because in the rumen, there are cellulolytic bacteria that can digest hemicellulose.

Cellulosari ta

Cellulose is the largest component of plant stems and forms the basic structure of plant cell walls (Prayoga and Aziz, 2023). In this study, cellulose content was obtained from 30.38% to 37.09%. The results of the analysis of variance showed that the interaction gave a significant effect ($P < 0.05$) between the dose of inoculum and the length of fermentation. Cellulose is a component of the cell wall that is strongly bound with lignin to form lignocellulose. The treatment of inoculum dose of 6% and fermentation duration of 14 days showed a cellulose content of 37.09%, which is because the dose produces a cellulase enzyme that hydrolyzes fiber, which is still small in helping to hydrolyze cellulose. Cellulose is a mixed polymer of sugar compounds, so that with the help of cellulase enzymes, cellulose can be hydrolyzed into

simple sugars. According to Sastrohamidjojo (2005), cellulose includes non-starch polysaccharides that require enzymes to be digested, the fermentation process will help hydrolyze cellulose. The results of research by Sangadji et al. (2008) reported that the longer the fermentation, the more the cellulose content decreased (36.32% at 0 days incubation to 18.95% at 70-80 days incubation) in sago pulp fermented with *P. ostreatus*.

The treatment of 12% inoculum and 14 days of fermentation showed a cellulose content of 30.38%, which is because fermentation with *Pleurotus ostreatus* is one of the microorganisms that produce lignocellulase and cellulase enzymes capable of degrading cellulose. This is by the opinion (Noferdian et al., 2014) that one of the lignocellulolytic microbes capable of degrading cellulose and lignin is *Pleurotus ostreatus*. In the biodegradation process, enzymes break down fiber components such as cellulose, hemicellulose, lignin, and other polymers into simpler ones so that the biodegraded materials have better quality and digestibility than the original material. In addition to being a ligninolytic fungus, *Pleurotus ostreatus* can also produce the enzyme endocellulase (Noferdian et al., 2014). According to Achmad et al. (2011), *Pleurotus ostreatus* is one of the active lignin-degrading fungi that lives saprophytically.

Dry matter digestibility (DMD)

In Table 5, it can be seen that the average dry matter digestibility of Lemongrass waste fermented with *Pleurotus ostreatus* ranged from 55.71% - 58.01%. The results of the analysis of variance showed that the treatment gave a very significant effect ($P < 0.01$) on the digestibility of dry matter (DMD). Digestibility at a dose of 12% and 21 days of fermentation differed significantly ($P < 0.01$) from the treatment of 6% dose and 14 days of fermentation. This difference was caused by a decrease in lignin content and an increase in CP and TDN content after fermentation in Table 5, causing digestibility to increase. The results of this study demonstrate the ability of *Pleurotus ostreatus* as a fiber degrading fungus (Arnol et al., 2022).

Digestibility of organic matter (OMD)

The digestibility of organic matter of citronella waste fermented with *Pleurotus ostreatus* ranged from 55.90% - 58.24%. The results showed that the digestibility of organic matter tends to follow the digestibility of feed dry matter, because organic matter is dry matter that has been removed from the ash content. According to Fathul and Wajizah (2010), organic matter is part of dry matter, so if dry matter increases, organic matter will increase and vice versa. Organic matter is the material lost when heated in the furnace; in other words, this material is dry matter subtracted by ash. Therefore, if the digestibility of dry matter increases, the digestibility of organic matter also increases. In accordance with the opinion of Febrina et al., (2017) that a decrease in dry matter digestibility results in decreased organic matter digestibility or vice versa.

According to Lestari et al. (2012), soluble feed components are found in feed organic matter; the higher the content of feed organic matter in the ration, the higher the degraded organic matter components. Increasing the number of rumen bacteria will have a positive impact on digesting and utilizing organic matter. This statement is supported by Jayanegara et al. (2009), who found that the lignin content contained in low-quality rations can increase the digestibility value of organic matter. Jamarun and Zain (2013) stated that the digestibility of organic matter is strongly influenced by the chemical content of feed ingredients. In this study, after being fermented with *Pleurotus ostreatus*, the lignin content of lemongrass waste decreased from 14.14% to 8.02%. Lignin can inhibit the digestion process, especially in ruminant animals. Lignin is a complex polymer that cannot be digested by animal digestive enzymes, and can also inhibit the action of rumen microbes on digestible cellulose and hemicellulose. According to Ye et al. (2024), lignin inhibits the access of digestive enzymes to cellulose and hemicellulose, two types of fiber that can actually be fermented and digested by microbes in the ruminant digestive system.

Crude Protein Digestibility (CPD)

Different crude protein digestibility is also evidenced by different NH₃ production

during feed fermentation in the rumen of ruminants. In this study, the NH₃ concentration of rumen fluid was 6,8-7.93 mMol. Nasri (2023) stated that the average NH₃ concentration was 6.8%-8.7 mMol. Rumen microbes will use NH₃ to form body protein with the availability of energy from VFA, whose value also increases with increasing NH₃. The rumen microbial protein synthesis process is strongly influenced by the availability of ammonia (NH₃) in the rumen (Widyobroto et al., 2007). Reduced crude protein level in feed will lead to diminished NH₃ concentration. Rumen bacteria need NH₃ to work properly; when NH₃ levels are low, the bacteria can't do their job well, which means food isn't broken down as effectively. The increased levels of NH₃ indicate a substantial protein content in the meal, and vice versa (Table 2).

CONCLUSIONS

Based on the results, it can be concluded that the best dose in this study is 12%, and the length of fermentation time is 21 days with *Pleurotus ostreatus*, which can diminish NDF, ADF, and cellulose while augmenting hemicellulose, DMO, and CPD. In vitro analysis yielded NDF 62.33%, ADF 48.16%, Cellulose 30.38%, Hemicellulose 14.16%, DMD 58.01%, DMO 58.24%, and CPD 50.16% under these circumstances.

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