

The effect of urea supplementation

by Agustin Dkk

Submission date: 27-Apr-2023 01:47PM (UTC+0800)

Submission ID: 2076921411

File name: stin_2019_IOP_Conf._Ser._The_effect_of_urea_supplementation.pdf (1.02M)

Word count: 6580

Character count: 34109

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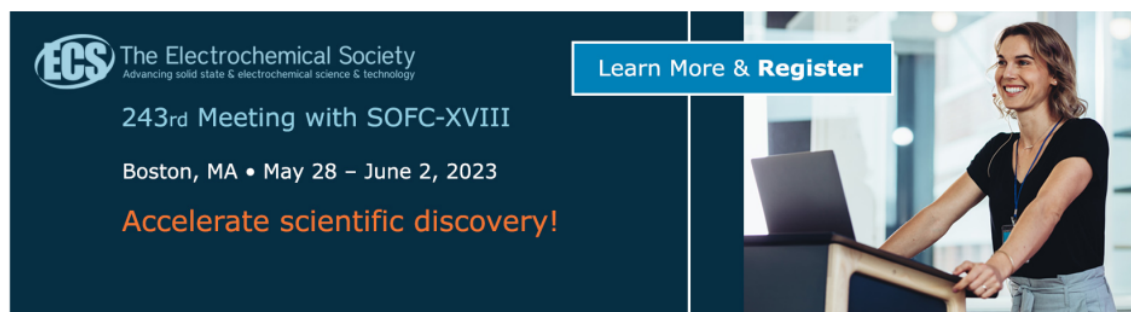
The effect of urea supplementation and incubation time in fermentation process of bagasse by using *Ganoderma lucidum* on the growth of *G. lucidum* and the nutritive value of bagasse

To cite this article: Fauzia Agustin *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **287** 012016

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The effect of urea supplementation and incubation time in fermentation process of bagasse by using *Ganoderma lucidum* on the growth of *G. lucidum* and the nutritive value of bagasse

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Abstract. Fermentation of bagasse with fungi *Ganoderma lucidum* was designed to determine the effect of urea and optimum time to get the best growth of *G. lucidum* and the nutritive value of bagasse and the optimum laccase activity of *G. lucidum*. Treatments were combination of nitrogen dose (0% urea and 0.3% urea) and incubation time (0, 10, 20, 30, 40, 50, 60 days). The treatments were arranged in factorial 2x7 and allocated in completely randomized design with three replications. The result showed that there was no interaction of urea supplementation and incubation time ($P>0.05$) on laccase activity, but each factor was significant different. The laccase activity in bagasse which supplemented with 0.3% urea was higher than bagasse without supplemented. Dry matter content of the substrate decreased as much as 27.45% in urea treatment, was higher than non urea supplementation (15.45%). Organic matter content of fermented bagasse decreased as much as 31.64%, was higher than non urea supplementation (21.86%). It can be concluded that (1) urea can be used 0.3% as nitrogen source in fermentation process using *G. lucidum*, (2) the highest VFA content of fermented bagasse was 98.25 mM in the length of fermentation up to 60 days with 0.3% urea with the NH_3 value was 15.99 mg%, (3) The highest dry matter and organic digestibility occurs in bagasse which is fermented with the addition of using *Ganoderma lucidum* which is at 40 days fermentation time with dry matter digestibility value 43.39% and organic matter digestibility value 40.97%.

Keywords – urea, laccase, *Ganoderma lucidum*, bagasse, fermentation.

1. Introduction

Bagasse is one of feedstuffs which have potential to be used as a source of energy for ruminant. However, it contains a high-lignin (11-22%), low protein and very low digestibility (16-22%). Lignin is a limiting factor for digestibility of fibrous feed. Its nutritive value can be improved through fermentation process. Solid state fermentation is one method of fermentation which can be applied to bagasse. Solid state fermentation is a process in which microorganism is grown on solid substrate.

Ganoderma lucidum is a white-rot fungi, a species of the class Basidiomycetes, which can degrade substances which contain lignin by producing ligninolytic enzyme, such as laccase [9] [27]. Previous studies have shown that *G. lucidum* can degrade raw material such as rice straw [26], palm by-product [11], bagasse [27]. The ability of *G. lucidum* to grow in fibrous feed are depended on the nutrients content of substrate and incubation time.



Plant cells are mainly composed by lignocellulosic material, which includes cellulose, hemicellulose, pectin and lignin (lignocellulosic complex) [12]. In nature, microorganisms, especially fungi, are able to degrade the plant cell wall through a set of acting synergistically enzymes. This phenomenon leads to glucose being released in a free form, which can enter the metabolism of the microorganism, providing its energy [12]. The main groups of fungal ligninolytic enzymes (ligninases) are lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac). Among the ligninases, Lac are the mostly studied [13] [14].

Laccase (EC1.10.3.2, p-diphenol oxidase) is one of enzyme that most widely distributed in white rot fungi [15] such as *G. Lucidum*. Fungal enzyme is responsible in mechanism for removing toxic phenols from the medium in which this fungi grow under natural conditions. Laccase only attack the phenolic subunits of lignin [16]. Laccases are oxidative enzymes related to the degradation of phenolic compounds, including lignin units [17]. Laccases are distributed among some plants [18], fungi [18] [19] [20], and bacteria [21] [22]. Laccase produced by some wood-rotting fungi from the genus Basidiomycetes play a major role in the biodegradation of lignin. Laccase produced by *G. lucium* can be used to improve feed quality of sugarcane bagasse.

There is still little information available concerning the use of urea in fermentation techniques for obtaining *Ganoderma lucidum* mycelium and its valuable components. Therefore, this study was conducted in order to determine the effect of urea and optimum time to get the best growth of *G. lucidum* and the nutritive value of bagasse. The ability of *G. lucidum* to degrade lignin is depended on the nutrients content of substrate and the ability of *G. lucidum* to produce ligninolytic enzyme i.e laccase; and it is also affected by incubation time. Moreover, the effect of incubation time and nutrients content of the substrate on the development of *G. lucidum* and optimum condition need to increase enzyme activity, have not available yet. Dry matter content and organic matter content of the substrate supplemented with urea in different incubation time is also determine in order to evaluate the growth of *G. lucidum*.

2. Material and method

2.1. Microorganism and starter

Ganoderma lucidum was obtained from the collection of Indonesian Biotechnology Research Institute for Estate Crops (IBRI), Bogor. Starter was obtained by rejuvenating inoculums stock from IBRI at Ruminant Nutrition Laboratory, Faculty of Animal Science, Andalas University. The mycelia of *G. lucidum* was grown in sterile petri dishes containing potato dextrose agar (PDA).

2.2. Making process of *Ganoderma lucidum* inoculum

Inoculums of *G. lucidum* was made by using the medium of rice bran and corn that has been autoclaved and then was inoculated with starter of *G. lucidum* that has grown in petri dishes with PDA medium until the mycelium of *G.lucidum* meet medium of rice bran and corn.

2.3. Substrate preparation and inoculation

The main material used in this search was sugarcane bagasse. It was chopped to the size of 2-3 cm and was added with water up to 70% moisture content. Mixed substrate was inserted into the bottle 300 ml, covered with aluminum foil, and then was sterilized by using autoclave for 30 min with a pressure of 1.2 and a temperature of 121°C. After cooling, sterilized bottle were inoculated with 4% inoculums aseptically in the laminar flow cabinet. The inoculated substrates was incubated at 27-28°C for 0, 10, 30, 40, 50 and 60 days. Fermentation products were harvested according to the time of incubation.

2.4. Experimental design

The experiment was arranged in a completely randomized design (CRD) 2 x 7 factorial with 3 replications [28]. The first factor was urea supplementation (0%, 0.3%) and the second factor was

1 incubation time (0, 10, 20, 30, 40, 50, 60 days) The combination between factors was decided as treatment. Parameter:

- I. The growth of *G. lucidum*: 1. pH value of fermentation product of *G. lucidum*. 2. dry matter content of fermented bagasse. 3. organic matter content of fermented bagasse,
- II. The nutritive value of fermented bagasse: 1. Rumen Characteristic: pH, VFA, NH₃. 2. Invitro Nutrient Digestibility of Bagasse Fermentation Product Using *Ganoderma lucidum*.

4 2.5. Sample and data analysis

Percentage of dry matter (DM) content and organic matter (OM) content in each incubation time were calculated by drying it in oven. The data were statistically processed by using Analysis of Variance (ANOVA). The difference between treatments was tested by orthogonal contrasts [28].

3. Result and discussion

3.1. The growth of *Ganoderma lucidum*

3.1.1. Growing percentage of *Ganoderma lucidum*

Based on the results of investigation was known that *G. lucidum* can grow 100%. The growing of *G. lucidum* is determined by internal and external factors, namely the nutrient content in the substrate, the substrate moisture content, pH, temperature and humidity [8]. The water content of the substrat, sugarcane bagasse in this study was 70%, with a ± 5.5 pH. Fermentations with *G. lucidum* in this research have been done at 28 °C. The effect of temperature on growth and product formation has not been systematically studied, and a temperature of 28°C is suitable for maximum growth for *G. lucidum*, in this research.



Figure 1. Starter of *Ganoderma lucidum*.

The substrate used for the growth of fungi *G. lucidum* is sugarcane bagasse. *Ganoderma lucidum* grows well on substrates with a moisture content of 70%. There were no contamination during the fermentation process until the end of the incubation period of 60 days. Artificial cultivation of *G. lucidum* has been achieved using substrates such as grain, sawdust, wood logs [30, 31, 32], Lignocellulose is a common substrate for laccase and the laccase ability to break down nonphenolic ligno-cellulose is provided by certain phenolic compounds acting as mediators. The production of the inoculum is a crucial step that often receives only minor attention. The size, viability and homogeneity of the inoculum can affect the performance of the subsequent fermentation significantly [33] and therefore must be standardized in order to obtain a reproducible process. The optimum fermentation conditions depend on its biomass. Various factors interact to influence the relative productivity of these products.

3.1.2. pH of fermentation

Medium pH is an important parameter affecting growth and product formation in fermentation process. The fermentation process of bagasse supplemented with urea using *G. lucidum* was strongly

influenced by the degree of acidity (pH). Prior to fermentation, pH of bagasse supplemented with urea showed a higher value than without urea. This is obviously due to urea is alkaline, so that with the addition of 0.3% urea in the substrate before fermentation, it increases the pH value of the bagasse from 5:22 became 5:58. This initial substrate pH conditions also influence the growth of *G. lucidum*. Mycelial growth is good for *G. lucidum* is at a pH value 5.0-5.5 [8]; and based on these recommendations, the bagasse substrate, both plus urea or have no added urea, can be covered by *G. lucidum*; but based on observations during the study, it was noted that the faster growth of *G. lucidum* on the bagasse substrate is substrate with plus urea. It is proved that *G. lucidum* also needed a source of nitrogen for its growth. Initial pH of substrate also influence the growth of mycelial. *Ganoderma* adapts during the early stages of the fermentation. Different culture conditions and medium compositions have also been reported to strongly influence mycelial growth and the production of biopolymers [34]. Culture pH had a significant effect on exopolysaccharides yield, chemical composition and molecular weight, and mycelial morphology [35]. The pH and temperature optimal for laccases were found to be pH 5.0 and 30°C, respectively [36] Laccase was active in a wide range of pH values. At pH values above pH 5.0, the enzyme activity decreased gradually by 50% at pH 8 [36]. Laccase activity was relatively stable in the range of pH 3.0–7.0. Microorganisms grow best at their optimum growth pH. Growth occurs slowly or not at all below the minimum growth pH and above the maximum growth pH. The enzyme activity declined when the temperature was increased from 30 to 80°C [36].

Based on the results revealed that there is no interaction ($P > 0.05$) between the addition of urea with incubation time, as well as each factor was not significant different the pH value. At 10 and 20 days of fermentation, there is a decrease of pH value, this indicates that there has been a degradation of nutrients that produce organic acids that caused a decrease in the pH value. The pH value of the fermentation period of 30 days had no significant difference with fermentation time of 40 days ($P > 0.05$). The highest pH values after fermentation sugarcane bagasse is at long fermentation 40 days. In the fermentation period of 30 days is visible growth is also very good, and this can be seen from the amount of mycelium increasingly meet all of bottle fermentation. After 40 days of fermentation, the pH value declined. (Figure 2) and this also shows that the fermentation process is still continue until 60 days because they still form organic acids produced from fermentation by *G. lucidum*. During a fermentation the pH has a tendency to change for several reasons.

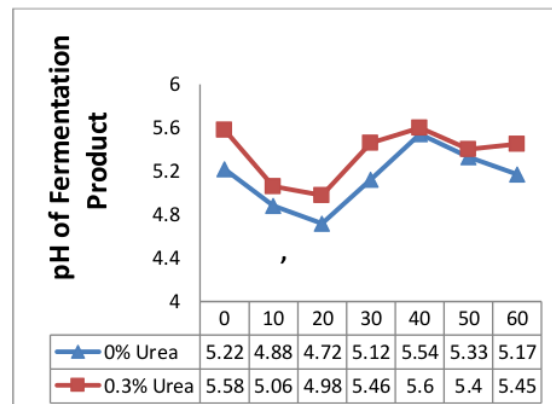


Figure 2. pH value of fermentation product of *G. lucidum*.

3.1.3. Dry matter and organic matter content of fermentation product of *G. lucidum*

The effect of urea supplementation in different fermentation time on dry matter content was also evaluated at concentration 0.3%. The content of dry matter (DM) of fermented sugarcane bagasse

(Figure 3) was highly significant difference ($P < 0.01$). This result indicates that the nutrients contained in the sugarcane bagasse have been used by *G. lucidum*. The period during which the dry matter content decreased corresponded with the period in which the mycelial growth was increasing rapidly at day 20. Dry matter content continues to decrease with the length of fermentation up to day 60. Dry matter contained in the sugarcane bagasse is used by *G. lucidum*. We know that dry matter consists of inorganic and organic matter, in which minerals such as calcium, phosphorus and magnesium required for the mycelial growth are components of inorganic matter. Urea can be used as a nitrogen source for the mycelial growth of *G. lucidum*. Nitrogen is a component of protein and carbon is a component of carbohydrate and lipid are also required for the mycelial growth of *G. lucidum*. The addition of supplement in this research such as urea was found to accelerate mycelial growth. The mycelium can be grown well by using dry matter of sugarcane bagasse, so that dry matter content decreases until day 60 of fermentation. Dry matter content of the substrate decreased as much as 27.45% in urea treatment, which was higher than non-urea supplementation (15.45%). The mycelial growth and the production of bioactive components were accelerated by the addition of another supplement such as fatty acids [37]. The increasing of mycelium number can be seen on the growth of *G. lucidum*. The main mechanism through which fungi and other microorganisms degrade plant biomass consists of production and secretion of enzymes acting synergistically in the plant cell wall, releasing monomers that can be used by the microorganism as chemical energy [33].

Ganoderma lucidum is one of the saprophytic. Saprophytism, one of the most common lifestyles of microorganisms, involves living in dead or decaying organic matter, mainly composed of plant biomass. In this context, microorganisms developed cellular mechanisms in order to take energy from plant biomass, and one of these mechanisms involves the production and secretion of carbohydrate-active enzymes. These enzymes degrade the plant cell wall, releasing sugar monomers that can be used as substrates for the metabolism of the microorganism.

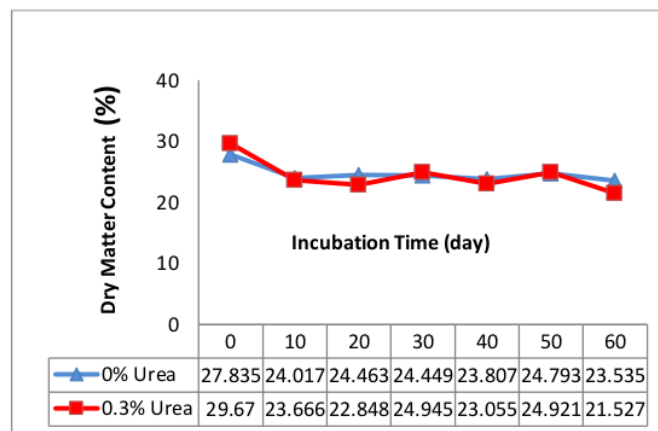


Figure 3. Dry matter content of fermented sugarcane bagasse.

3.1.4. Organic matter content of fermentation product of *G. lucidum*

Changes in organic matter content followed the pattern of dry matter content, which is influenced by the fermentation time ($P < 0.01$); and also there is an interaction between the factors adding urea with the fermentation time ($P < 0.01$). In Figure 4 it can be seen that the point of intersection of the fermentation time occurs in 50 days, where the value of organic matter content in sugarcane bagasse plus urea was continued to decline compared with bagasse without the addition of urea. The decline in organic matter content is a result of the *G. lucidum* in the fermentation process. At that time, nutrient of substrate is reduced. At the urea treatment, organic matter content of the substrate decreased as much as 31.64%, which was higher than non-urea supplementation (21.86%). *G. lucidum* can utilize the fraction of

fibre that are difficult to degrade by producing laccase enzyme that can degraded lignin. Laccase produced by *G. lucidum* will reduce the content of organic material. Laccase enzyme activity increases with increasing duration of fermentation as described below.

Plant cell wall [38] polysaccharides are the most abundant organic compounds found in nature. These compounds consist mainly of polysaccharides such as cellulose, hemicelluloses and pectin, as well as the phenolic polymer lignin. Together, the polysaccharides and lignin provide high complexity and rigidity to the plant cell wall. Fungi play a central role in the degradation of plant biomass, producing an extensive array of carbohydrate-active enzymes responsible for polysaccharide degradation [wagner shouza].

Concerning to lignin degradation, many white-rot basidiomycetes and some actinomycetes are able to produce lignin-degrading enzymes, especially peroxidases. For instance, *Phanerochaete chrysosporium* and *Phlebia radiata* are well known producers of extracellular peroxidases [39] as well as *Coriolus versicolor*, which was shown to produce the intracellular haem peroxidase upon the induction by phenolic compounds [40]. A white-rot basidiomycete, *Rigidoporous lignosus*, is known to secrete two oxidative enzymes, laccase and Mn peroxidase, responsible for solubilizing the lignin in a synergistic way [41].

By using white rot fungi, lignin component can be degraded and among white rot fungi, *Ganoderma lucidum* is efficiency producer of ligninolytic enzyme such as laccase.

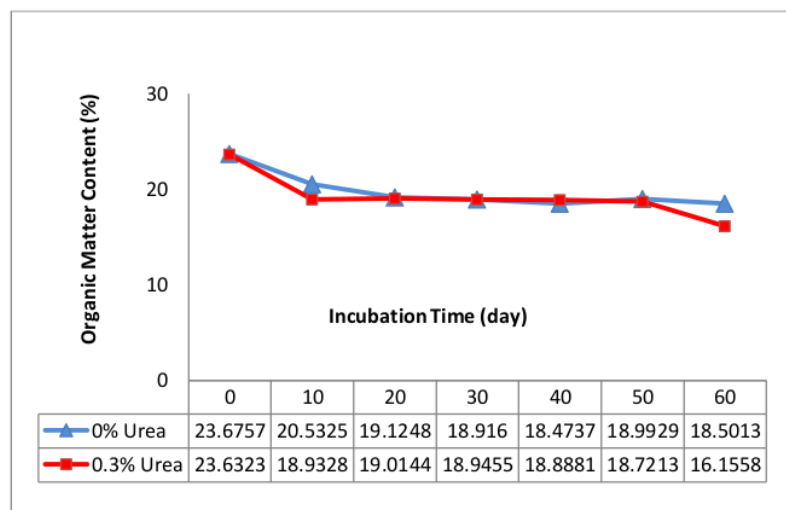


Figure 4. Organic matter content of Fermentation Product of *G. Lucidum*.

3.1.5. Laccase activity

Laccase activity showed that the addition of urea and long fermentation gave a different effect on the enzyme activity of laccase produced by the *G. lucidum* (Figure 5). Based on Figure 5 appears that the addition of urea to produce laccase was very significantly higher than the fermented sugarcane bagasse without the addition of urea, at every length of fermentation 10 days to 60 days. The addition of 0.3% urea will increase laccase activity until fermentation 60 days, with the highest value of enzyme activity is 6.54 U mL⁻¹. Laccase activity decreased after fermentation 50 days, but the fermentation of 30 days, the activity of the enzyme laccase almost identical, both plus urea and non plus urea with a value of 6.13 U mL⁻¹ and 5.91 U mL⁻¹ respectively.

Ganoderma lucidum (Curt, Fr) P. Karst is a white-rot fungi, a species of the class Basidiomycetes, which can degrade substances which contain lignin by producing ligninolytic enzyme, such as laccase [9, 27]. Laccases produced by some wood-rotting fungi from the genus *Basidiomycete* play a major

role in the biodegradation of lignin [42] Laccases can catalyze the oxidation of non-phenolic benzyl alcohols in the presence of a redox mediator, such as 2,2'-azinobis-[3-ethylthiazoline-6-sulfonate] (ABTS) [43] [44]. Approximately 70% of lignocellulosic biomass is made of cellulose and hemicellulose. They are tightly linked to the lignin through hydrogenic and covalent bonds, making the structure resistant for any treatment.

In particular, the ability to decompose the aromatic lignin polymers in wood is mostly restricted to the white rot basidiomycetes. The white-rot decay of wood is possible due to secretion of organic acids, secondary metabolites, and oxidoreductive metalloenzymes, heme peroxidases and laccases, encoded by divergent gene families in these fungi [45].

Laccase is an enzyme involved in lignin degradation, beside lignin peroxidase a manganese peroxidase. Laccases were found in eukaryotes (fungi, higher plants, insects), [46]. The first gene and/or cDNA sequences were recorded for laccase from the *Ganoderma lucidum*, *Phlebia brevispora*, *Lentinula edodes* and *Lentinus tigrinus* [47].

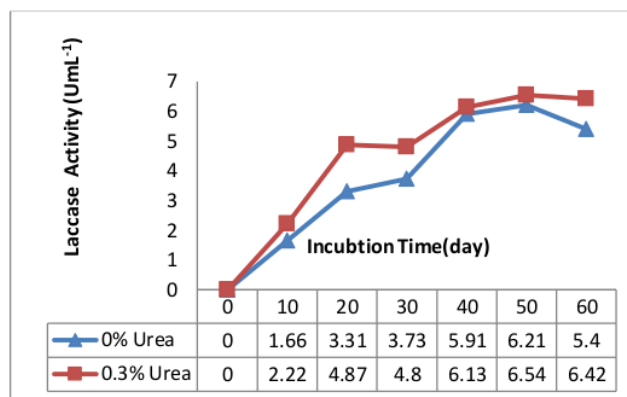


Figure 5. Laccase activity of fermented bagasse using *G.lucidum*.

3.2. The nutritive value of bagasse

3.2.1. Rumen pH

The growth and the activity of rumen microbial are influenced by many factors, one of them is rumen pH. The results showed that the pH value of rumen fluid in the in-vitro experiment of fermented bagasse with the addition of urea was highly significant different ($P < 0.01$) and the fermentation time was not significant ($P < 0.05$), although at 60 days fermentation period there was a decrease in pH to 6.8. The pH value of 6.8 is an ideal pH for the growth and microbial activity in the rumen. The resulting pH value is still in the normal range for rumen microbial growth with a pH value of 6.8-7.06 (Figure 6). Decreasing pH in 60 days fermentation is closely related to cellulose content and cellulose digestibility of fermented bagasse because cellulose is fermented into VFA and an increase in the amount of fermented cellulose also increases the VFA produced. Most VFAs are fermented products from carbohydrates, especially cellulose. VFA is a fermentation product in the rumen which is the main energy source for ruminants. If the pH value drops below 6 (< 6) then the microbial activity to digest the fiber fraction will be disrupted and consequently can reduce the digestibility value of the fiber fraction, but in this study the pH value did not drop below 6 (< 6), even the pH value was very support so that microbes are able to produce cellulolytic enzymes.

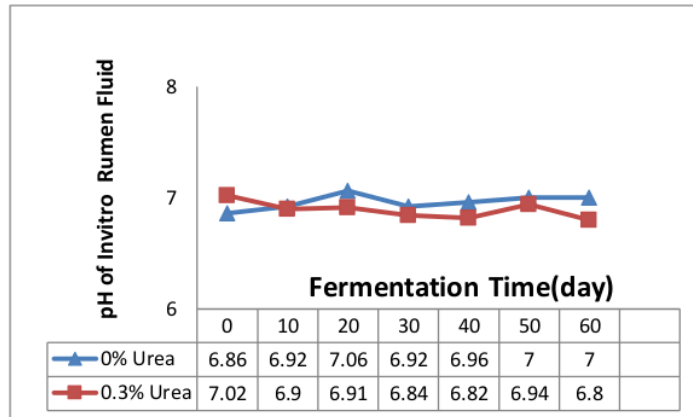


Figure 6. Rumen pH of fermented bagasse using *G. lucidum* by adding urea at fermentation times of 0, 10, 20, 30, 40, 50 and 60 days.

3.2.2. Levels of VFA of rumen liquid from fementation products of sugar cane using *Ganoderma lucidum*

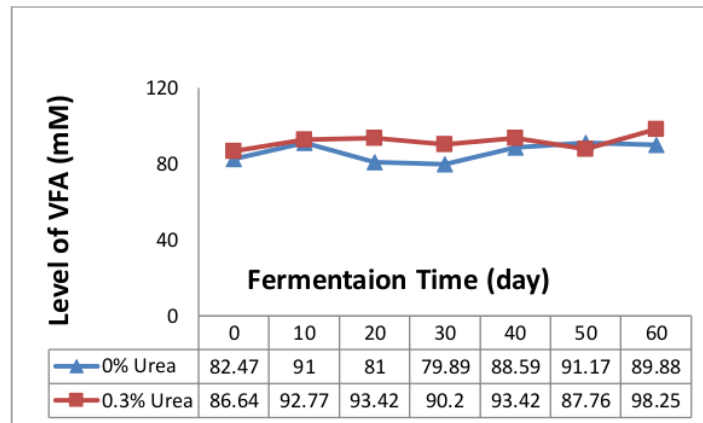


Figure 7. Level of VFA in vitro from fermented bagasse using *G. lucidum* with the addition of urea at 0, 10, 20, 30, 40, 50 and 60 days.

Based on the results of the study it was found that the VFA levels in vitro from fermented bagasse using *G. lucidum* added with urea showed significantly different ($P < 0.05$). VFA levels from fermented bagasse added with urea were significantly higher than those without urea added during the fermentation process to 60 days. After 50 days of fermentation, it was seen that the VFA levels of fermented bagasse without added urea began to decline, while the bagasse added with urea, the VFA levels increased and even reached the highest value of 98.25 mM. This shows that the fiber fraction contained in bagasse pulp fermentation products is easily degraded in the rumen resulting in the highest fiber fraction fermentation product, VFA. The ease of degradation of this fiber fraction is the result of the work of an enzyme that begins with the enzyme laccase produced by *G. lucidum* in the process of bagasse fermentation outside the rumen, then continued by enzymes produced by rumen microbes.

3.2.3. Levels of NH₃ in vitro rumen liquid

NH₃ levels of rumen fluid showed significantly different values ($P < 0.01$), higher in sugarcane ampas fermented by the addition of urea. The addition of urea in the bagasse fermentation process using *Ganoderma lucidum* will be utilized by *G. lucidum* as a source of nitrogen to increase the growth of *G. lucidum* mycelium, which in turn will increase the protein content of bagasse pulp fermentation products. This protein from bagasse is degraded in the rumen with the help of enzymes produced by rumen microbes. Fermentation product of protein in the rumen is NH₃. NH₃ produced in this rumen will be utilized by rumen microbes as a source of nitrogen for microbial protein synthesis. Increased microbial population will increase the amount of enzymes produced by microbes that will be used to digest feed. This increase in rumen microbial population is not only determined by the availability of a source of Nitrogen in the form of NH₃, but is also determined by the availability of VFA which has a dual role, namely as an energy source and carbon framework for rumen microbial protein synthesis. So there must be synchronization between NH₃ produced with VFA which is formed from the degradation of carbohydrates in the rumen.

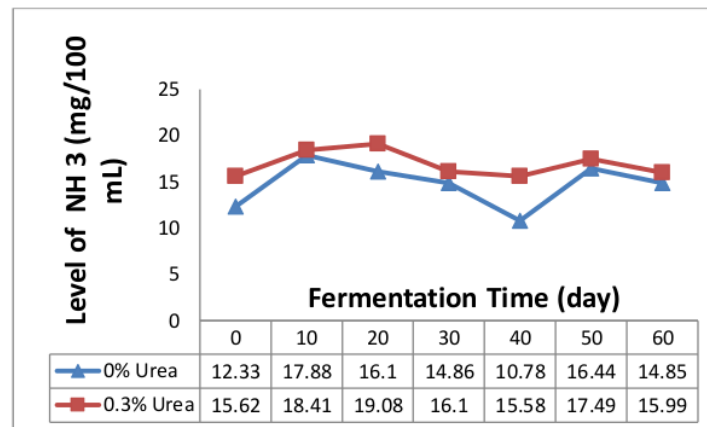


Figure 8. Level of NH₃ in vitro from fermented bagasse using *G. lucidum* with the addition of urea at 0, 10, 20, 30, 40, 50 and 60 days.

3.2.4. In vitro digestibility of dry matter of fermented bagasse with *G. lucidum*

The results showed that there was a very significant difference ($P < 0.01$) between the addition of urea to bagasse and without urea in fermentation to the digestibility of dry matter. Digestibility of dry matter up to 10 days fermentation did not show any difference, but after 10 days of fermentation, the addition of urea gave a different response with no addition of urea. The highest value of dry matter digestibility occurred at 40 days fermentation period, namely in fermented bagasse added with urea with digestibility value of 43.39% (Figure 9); after that there was a decrease in digestibility of dry matter until fermentation of 60 days; while the fermented bagasse without urea showed results that decreased the dry matter digestibility until the fermentation time was 50 days, then the dry matter in fermentation was 60 days.

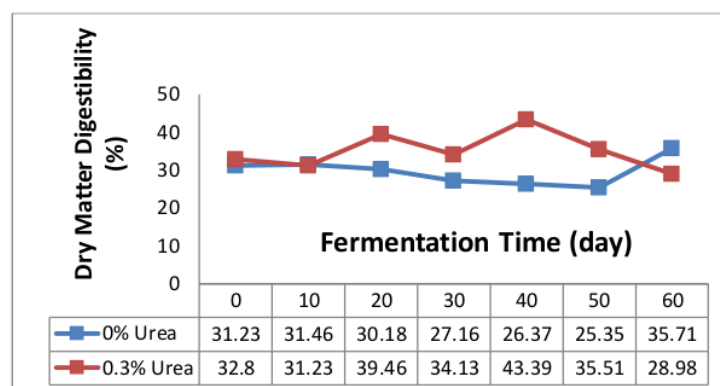


Figure 9. In vitro digestibility of dry matter from fermented bagasse using *G. lucidum* with the addition of urea at 0, 10, 20, 30, 40, 50 and 60 days.

3.2.5. In vitro digestibility of organic matter of fermented bagasse with *G. lucidum*.

The digestibility value of organic matter has a pattern similar to the digestibility value of dry matter, because indeed most of the dry matter itself is organic material. The results of the addition of urea in the cementation of bagasse with *G. lucidum* showed that the digestibility of organic matter increased with the addition of urea in the bagasse fermentation process. The highest organic matter digestion occurs at 40 days of fermentation, after which there is a decrease in digestibility of organic matter.

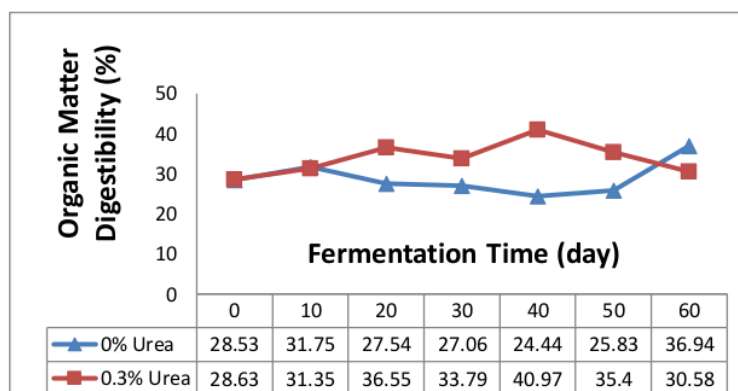


Figure 10. In vitro digestibility of organic matter from fermented bagasse using *G. lucidum* with the addition of urea at 0, 10, 20, 30, 40, 50 and 60 days.

4. Conclusion

- The percentage of growth of *Ganoderma lucidum* on the basic substrate of sugarcane with the addition of different energy sources is 100% because in all treatments, *G. lucidum* can grow well fully with different growth speeds, ranging from 10 days to 60 days.
- Urea can be used 0.3% as nitrogen source in fermentation process using *G. lucidum*.
- The highest VFA content of fermented bagasse was 98.25 mM in the length of fermentation up to 60 days with 0.3% urea with the NH₃ value was 15.99 mg%.

- d. The highest dry matter and organic digestibility occurs in bagasse which is fermented with the addition of using *Ganoderma lucidum* which is at 40 days fermentation time with dry matter digestibility value 43.39% and organic matter digestibility value 40.97%.

5. References

- [1] F. Agustin, T. Toharmat, D. Evvyernie, D. Taniwiryono, S. Tarigan. 2012. Effect of Supplemental Organic Chromium and Fungi *Ganoderma lucidum* on Milk Production and Immune Response in Lactating Cows, in *Proc. The 2nd International Seminar. Feed Safety for Healthy Food*. paper 22. p 156-163.
- [2] F. Agustin, T. Toharmat, D. Evvyernie, D. Taniwiryono, S. Tarigan. 2012. Blood Profile of Lactating Cows Supplemented with Organic Chromium and *Ganoderma lucidum*. *Pakistan J. of Nutrition*. Vol. 11 (10). Pp 991-997.
- [3] Xu Z., X. Chen, Z. Zhong, L. Chen, Y. Wang. 2011. *Ganoderma lucidum* Polysaccharides: Immunomodulation and Potential Anti-Tumor Activities. *Am. J. Chin. Med.*, 39 (1): 15-27.
- [4] G. C. Yen, J Y. Wu. 1999. Antioxidant and radical scavenging proepcties of extract from *Ganoderma lucidum*. *Food chem*. 65: 375-379.
- [5] Y. H. You, Z B. Lin³. 2003. Antioxidant effect of *Ganoderma* polysaccharide. *Acta Pharm. Sinica*. 38: 85-88.
- [6] J. Sun., H. He, B. J. Xie. 2004. Novel antioxidant peptides from fermented mushroom *Ganoderma lucidum*. *J Agric Food Chem*. 52. 6645 – 6652.
- [7] Z. X. Yang., Y. Y. Sou, & W. An. 2006. Studies on the capability of *Ganoderma lucidum* rich in Chromium. *J. CEPS*.
- [8] F. Agustin, T. Toharmat, D. Evvyernie, D. Taniwiryono, S. Tarigan. 2013. The Incorporation of Chromium in Rice Straw Fermented with *Ganoderma lucidum*. *Journal of Animal Science and Technology*. Vol. 36. No.1. pp 64-69.
- [9] S. T. Chang, & P. G. Miles. 2004. *Mushrooms: Cultivation, Nutritional Value, Medical Effect, and Environmental Impact*. 2nd Ed. London: CRC Press. Boca Raton.
- [10] A. K. Misra, AK, A. S. Mishra, M. K. Tripathi, R. Prasad, S. Vaithiyanaathan, & R. C. Yalchmola. 2007. Optimization of solid state fermentation of mustard (*Brassica campestris*) straw for production of animal feed by white rot fungi (*Ganoderma lucidum*). *Asian-Aust J Anim Sci* 20 (2): 208-213.
- [11] F. Agustin, T. Toharmat, D. Evvyernie, D. Taniwiryono, S. Tarigan. 2010. The Incorporation of Chromium by *Ganoderma lucidum* in Oil Palm by-Product as Substrate. *Journal of Animal Science and Technology*. Vol. 33. No. 1.
- [12] De Vries R. P., Visser J. 2001. *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides *Microbiol. Mol. Biol. Rev.* 65, 497–522.
- [13] Couto SR, Sanromán MA. 2005. Application of solid-state fermentation to ligninolytic enzyme production. *Biochem. Eng. J.*, 22 (3): 211-219.
- [14] Couto SR, López E, Sanromán MA. 2006. Utilization of grape seeds for laccase production in solid-state fermentors. *J. Food Eng.*, 74 (2): 263-267.
- [15] V. Madhavi and S. S. Lele. 2009. Laccase: Properties and Applications. *BioResource* 4 (4) pp 1694-1717.
- [16] C. F. Turston. 1994. The Structure and Function of Fungal Laccases. *Review Article. Microbiology*, 140 pp 19-25.
- [17] Placido, J and Capareda, S. 2015. Ligninolytic Enzymes: A Biotechnological Alternative for Bioethanol Production. *Bioresources and Bioprocessing*. 2: 23.
- [18] Baldrian P. 2006. Fungal laccases-occurrence and properties. *FEMS Microbiology Reviews*. 30 (2): 215–242.
- [19] Sanches C. Lignocellulosic Residue; biodegradation and Bioconversion by Fungi. *Biotechnol. Adv.* 27 (2): 185-194.

- [20] Hildén K., Hakala T. K., Maijala P., Lundell T. K., Hatakka A. 2007. Novel thermotolerant laccases produced by the white-rot fungus *Physisporinus rivulosus*. *Applied Microbiology and Biotechnology*. 77 (2): 301–309.
- [21] Sharma P., Goel R., Capalash N. 2007. Bacterial laccases. *World Journal of Microbiology and Biotechnology*. 23(6):823–832.
- [22] Diamantidis G., Effosse A., Potier P., Bally R. 2000. Purification and characterization of the first bacterial laccase in the rhizospheric bacterium *Azospirillum lipoferum*. *Soil Biology and Biochemistry*. 32(7):919–927.
- [23] Alberts J. F., Gelderblom W. C. A., Botha A., van Zyl W. H. 2009. Degradation of aflatoxin B1 by fungal laccase enzymes. *International Journal of Food Microbiology*. 135(1):47–52.
- [24] Isroi, Millati R, Syamsiah S, Niklasson C, Cahyanto MN, Lundquist K. 2011. Biological Pretreatment of Lignocelluloses with White-Rot Fungi and Its Applications: A Review. *Bioresources*. 6 (4): 5224-59.
- [25] P. J. Van Soest PJ. 2006. Rice straw, the role of treatments to improve quality. *Anim Feed Sci Tech* 130 (3-4): 137-171.
- [26] F. Agustin, T. Toharmat, D, Evvyernie, D, Taniwiryo, S, Tarigan. 2013. The Incorporation of Chromium in Rice Straw Fermented with *Ganoderma lucidum*. *Journal of Animal Science and Technology*. Vol. 36. no. 1. pp. 64-69.
- [27] F. Agustin, Y. Marlida, Erpomen. 2015. The Fermentation of Bagasse with Fungi *Ganoderma lucidum* and The Evaluation of the In vitro Digestibility. Proc. Nasional Seminar, Paper 18, p124-132.
- [28] K. A. Gomez, & A. A. Gomez. 1995. *Statistical Procedure for Agriculture Research*. Indonesia University Press.
- [29] J. Buswell, J. A. Y. Cai and S. Chang. 1995. Effect of nutrient and manganese on manganese peroxidase and laccase production by *Lentinula* (*Lentinus edodes*). *FEMS microbial Lett.* 128: 81-88.
- [30] Chang S. T, Buswell J. A. *Ganoderma lucidum* (Curt.: Fr.) P. Karst. 1999. (Aphyllorphomycetidae): A mushrooming medicinal mushroom. *Int J Med Mushrooms*. 1: 139-46.
- [31] Wasser S. P, Coates P, Blackman M, Cragg G, Levine M, Moss J, White J. 2005. Encyclopedia of Dietary Supplements. Reishi or Lingzhi (*Ganoderma lucidum*). New York: Marcel Dekker, pp. 680–90.
- [32] Boh B, Berovic M, Zhang J, Zhi-Bin L. 2007. *Ganoderma lucidum* and its pharmaceutically active compounds. *Biotechnol Annu Rev*. 13:265–301.
- [33] De Souza W. R. 2003. Submerged Cultivation of *Ganoderma lucidum*, *Food Technol. Biotechnol*. 41 (4): 371–382.
- [34] Habijan J, Berovic M. 2000. The relevance of solid-state substrate moisturing on *Ganoderma lucidum* biomass cultivation. *Food Technol Biotechnol*. 38: 225–8.
- [35] Kim H. M, Park M. K, Yun J. W. 2006. Culture pH affects exopolysaccharide production in submerged mycelial culture of *Ganoderma lucidum*. *Appl Biochem Biotechnol*. 134: 249–62.
- [36] P. Shrestha, B. Joshi, J. Joshi, R. Malla, and L. Sreerama. 2016. Isolation and Physicochemical Characterization of Laccase from *Ganoderma lucidum*-CDBT1 Isolated from Its Native Habitat in Nepal. *Biomed Res Int*. 3238909.
- [37] Yang F. C, Liau C. B. 1998. The influence of environmental conditions on polysaccharide formation by *Ganoderma lucidum* in submerged cultures. *Process Biochem*. 33: 547–53.
- [38] Aro N, Pakula T, Penttila M. 2005. Transcriptional regulation of plant cell wall degradation by filamentous fungi. *FEMS microbiology reviews*. 29 (4): 719-39.
- [39] Lee S, Ha, JK, Kang, HS, McAllister T, Cheng K-J. 1997. Overview of energy metabolism, substrate utilization and fermentation characteristics of ruminal anaerobic fungi. *Korean Journal of Animal and Nutritional Feedstuffs*. 21: 295–314.

- [40] Lobarzewski J. 1990. The Characteristics and Functions of the Peroxidases from *Trametes Versicolor* in Lignin Biotransformation. *J Biotechnol.* 13 (2-3): 111-7.
- [41] Galliano H, G. Gas, J. L. Sevis and A. M. 1991. Boudet: Lignin degradation by *Rigidoporus lignosus* involves synergistic action of two oxidizing enzymes: Mn peroxidase and laccase. *Enzyme Microb Technol.* 13: 478-82.
- [42] Coll PM, Fernandez-Abalos JM, Villanueva JR, Santamaria R, Perez P. 1993. Purification and characterization of a phenoxidase (laccase) from the lignin-degrading basidiomycete PM1 (CECT 2971). *Appl Environ Microbiol.* 59: 2607-2613.
- [43] Bourbonnais R, Paice MG. 1990. Oxidation of non-phenolic substrates: An expanded role for laccase in lignin biodegradation. *FEBS Lett* 1267: 99-102.
- [44] Wong, D. W. S. 2009. Structure and Action Mechanism of Ligninolytic Enzyme. *Appl. Biochem. Biotechnol.* 157 (2): 174-209.
- [45] Lundel T. K, Makela M. R, Hilden K. 2010. Lignin-modifying enzyme in filamentous basidiomycetes-ecological, functional and phylogenetic review. *J. Basic Microbiol.* 50 (1): 5-20.
- [46] Martínez AT, Speranza M, Ruiz-Dueñas FJ, Ferreira P, Camarero S, Guillén F, Martínez MJ, Gutiérrez A, del Río JC. 2005. Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *Int Microbiol.* 8 (3): 195-204.
- [47] D'Souza TM, Boominathan K, Reddy CA. 1996. Isolation of laccase gene specific sequences from white rot and brown rot fungi by PCR. *Appl Environ Microbiol.* 62: 3739-3744.

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