The International Conference on Education, Social Sciences and Technology (ICESST)
Volume 3 Nomor 2 Tahun 2024
E-ISSN: 2964-2671; P-ISSN : 2964-2701, Hal 108-120
DOI: 10.55606/icesst.v3i2.425
Available online at: https://ijconf.org/index.php/icesst/

Rice Straw Processing Technology With Incubation Period On Quality Animal Feed Nutrition

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Abstract. *This study aims to identify the best cellulose, hemicellulose, and lignin content from rice straw processing using fermentation technology with bioactivators EM4, Win Prob, and Microbacter alfaafa 11 at various incubation durations (7, 14, and 21 days) to be used as animal feed. The study was conducted using the Complete Random Design (RAL) method of a 3 x 3 factorial pattern, involving 9 treatments with each treatment repeated three times. The factors tested include: factor A (Type of rice straw processing): 1. Fermentation with EM4; 2. Fermentation with Win Prob; 3. Fermentation with Microbacter alfaafa 11. As well as factor B: Incubation duration (7 days, 14 days, and 21 days). The parameters measured were cellulose, hemicellulose, and lignin content. The results showed that the lowest cellulose content was achieved in the A3B2 treatment (fermentation with Microbacter alfaafa 11 for 14 days) of 36.2591%. A1B1 treatment (fermentation with EM4 for 7 days) resulted in the highest hemicellulose content of 30.4997%. Meanwhile, the lowest lignin content was found in the A3B2 treatment with a value of 15.3618%. In conclusion, the fermentation treatment of rice straw with Microbacter alfaafa 11 for 14 days (A3B2) was the most optimal in reducing the content of cellulose (36.2591%), hemicellulose (25.8671%), and lignin (15.3618%).*

Keywords *Rice Straw, Fermentation, Cellulose, Hemicellulose, Lignin*

INTRODUCTION

The provision of forage in the maintenance of ruminant livestock is very important because forage functions as a source of fiber that will be converted into energy in the digestive tract (Haryanto, 2012). Forages include grass, leguminosa, and by-products of agricultural products. One alternative to overcome the limitation of forage is to utilize agricultural by-products, such as rice straw. Rice straw is agricultural waste in the form of leaves, stems, and stalks of rice plants that remain after the grain is separated. In addition to having various benefits, rice straw can be used as feed for ruminant livestock such as cows, goats, and buffaloes. Based on research, the production of rice straw from one hectare of rice fields reaches 12–15 tons per harvest or about 4–5 tons of dry matter, depending on the location and variety (Bata, 2008). The rice planting cycle that lasts throughout the year produces 11.89 tons/ha/harvest of fresh straw, 6.73 tons/ha/harvest of dry straw, and 5.94 tons/ha/harvest of dry matter (Syamsu, 2006).

The abundance of rice straw makes it a potential source of feed for ruminant livestock, due to its continuous availability. However, rice straw has a low crude protein

Received: September 29, 2024; Accepted: Oktober 22, 2024; Published: November 28, 2024
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content, high crude fiber, as well as lignin and silica which make the plant cell wall difficult for rumen microbes to digest. According to Hasan and Yanuarianto (2015), rice straw contains crude protein of 8.26%, crude fiber of 31.99%, NDF of 77.00%, ADF of 57.91%, cellulose of 23.05%, hemicellulose of 19.09%, and lignin of 22.93%. To increase its nutritional value, biotechnology such as fermentation is often used.

Fermentation is the process of preserving feed using starters (microorganisms) anaerobically. Microbes in fermentation are able to break down complex compounds into simpler ones so that they are easily digested by livestock. During fermentation, cellulose and hemicellulose are converted into simple sugars, while lignin is reduced thereby reducing its binding power to cellulose (Nisa et al., 2020). The addition of probiotics, such as starbio, has been proven to increase the crude protein content of rice straw (Syamsu, 2006). Fermentation with Microbacter alfaafa 11 (MA-11) is also able to reduce crude fiber levels (Syamsu, 2006).

Another study showed that rice husk fermentation using EM4 increased protein from 1.92% to 2.67% and decreased crude fiber levels, which signaled an increase in nutritional value (Telew et al., 2013). Fermentation of sugarcane sacks with Win Prob of 15% also succeeded in lowering the pH and accelerating the formation of lactic acid bacteria, which helped reduce coarse fiber (Sri Rahayu, 2021). This process is in line with the findings of Santoso et al. (2009), that lactic acid bacteria accelerate fermentation through the utilization of monosaccharides.

MA-11 itself is a decomposer that is able to break down organic chains quickly, improving the quality of animal feed materials, fertilizers, and bioethanol (Artarizqi, 2013). The bacteria in MA-11, including Rhizobium sp., have a major role in the process.

Based on this information, the use of various probiotics as fermentation starters is very helpful in improving the quality of rice straw. Further research comparing various starters and fermentation duration is needed to produce better quality rice straw, especially in terms of cellulose, hemicellulose, and lignin.

METHODS

Place And Time Study

This research was conducted at the Basic Sciences Laboratory of Panca Budi Development University. Time study from month Oktober until with November 2024.

Material And Tool Study

The materials used in the study were rice straw, sawdust, bran, lime, clean water, plastic bags, pipe rings and chemicals for lignin, cellulose and hemicellulose analysis.

The tools used are plastic basins, buckets, knives, scales, and stationery. The tools used in the nutritional chemistry test are : autoclave, cutting board, analytical balance and tools used for lignin analysis, cellulose, and hemicellulose.

Method Study

The research method used in this study is the Completely Randomized Design (CRD) method with a 3 x 3 factorial pattern, namely 9 treatments with each treatment repeated 3 times.

Factor A: Processing Straw Rice:
1. Fermentation (EM4).
2. Fermentation (Win Prob).
3. Fermentation (Micobacter alfaafa 11).

Factor B: Incubation period:
1. 7 days
2. 14 days
3. 21 days

The treatment combination is as follows:

A 1 B 1 = Straw fermented rice using EM4 5%, incubation period 7 days

A 1 B 2 = Straw fermented rice using EM4 5%, incubation period 14 days

A 1 B 3 = Straw fermented rice using EM4 5%, incubation period 21 days

A 2 B 1 = Straw fermented rice using Win Prob 5%, incubation period 7 day

A 2 B 1 = Straw fermented rice using Win Prob 5%, incubation period 14 day

A 2 B 1 = Straw fermented rice using Win Prob 5%, incubation period 21 day

A 3 B 1 = Straw fermented rice using Microbacter alfaafa 11 as much as 5% incubation period 7 days

A 3 B 2 = Straw fermented rice using Microbacter alfaafa 11 as much as 5%, incubation period 14 days

A 3 B 3 = Straw fermented rice using Microbacter alfaafa 11 as much as 5%, incubation period 21 days

Implementation Study

1. Procedure for Making Rice Straw Fermentation with EM4, Win Prob and Microbacter alfaafa 11:
   a) The first stage is to prepare all the ingredients (rice straw and the three bioactivators, then weigh them based on the percentage.
   b) The addition of this bioactivator will improve the quality of fermented processed feed.
   c) Prepare a bioactivator formula of 5% of the weight of the rice straw.
   d) Then it is dissolved in water that has been mixed with molasses (the amount of water and molasses mixture used is ± 5% of the total rice straw).
   e) Then the water that has been mixed with molasses and the three bioactivators is poured over the pile of ingredients, then stirred until homogeneous (even) according to the treatment.
   f) If the rice straw is wet, just sprinkle it.
   g) After that, put it in a tightly closed container, compact it by stepping on it, then close the closed plastic jar tightly so that there are no air gaps entering the rice straw and ferment for 7 days, 14 days and 21 days anaerobically.

2. Sampling Analysis

Samples for chemical analysis of nutrient content were taken randomly from all replications made based on the treatment. Sampling was taken from all research treatments (9 samples). The samples that had been taken were immediately dried (sun-dried/oven at 60 0 Celsius), then the samples were weighed and ground with a blender for further analysis in the laboratory.

Parameter Study

As for parameter Which observed in study This that is, analysis content cellulose, hemicellulose and lignin. To determine the levels of lignin, cellulose and hemicellulose, the ADF and NDF levels of the sample are first determined (Van Soest, 1985).

Determination Acid Detergent Fiber (ADF) content:

1. Weigh the sample approximately 0.4 grams then put it into a 50 ml test tube.
2. Add 40 ml of ADF solution then close the tube tightly.
3. Boil in boiling water for 1 hour while stirring occasionally.
4. Filter with sintered glass No. 1 of known weight (a gram) while being held by a vacuum pump.
5. Wash with approximately 100 ml of boiling water and 50 ml of alcohol.
6. Bake at 105 0 C for 8 hours or leave overnight.
7. Cool in a desiccator for approximately ½ hour then weigh (b grams) Calculation:

$$\text{Kadar ADF} = \frac{b-a}{\text{Berat contoh}} \times 100\%$$

Determination of Neutral Detergent Fiber (NDF):

1. Weigh a sample of approximately 0.2 grams.
2. Put it into a 50 ml test tube.
3. Add 30 ml of NDF solution, then close the tube tightly.
4. Boil in boiling water for 1 hour (shaking occasionally).
5. Filter into sintered glass No.1 of known weight (a gram) while being sucked with a vacuum pump.
6. Wash with hot water approximately 100 ml (as needed).
7. Wash with approximately 50 ml of alcohol.
8. Bake at 105 ^{0} C for 8 hours or leave overnight.
9. Cool in a desiccator for ½ hour then weigh (b grams) Calculation:

$$\text{Kadar NDF} = \frac{b - a}{\text{Berat contoh}} \times 100\%$$

% Lignin, Cellulose and Hemicellulose:

1. Sintered glass containing ADF is placed on a petri disk.
2. Add 20 ml of 72 % H_2SO_4.
3. Stir occasionally to ensure that the fibers are wetted with 72 % H_2SO_4.
4. Leave it for 2 hours.
5. Suck with a vacuum pump while rinsing with sufficient hot water.
6. Bake for 8 hours at 100 ^{0}C or leave overnight.
7. Put it in a desiccator then weigh it (c gran).
8. Put it in an electric furnace or heat it to 500 ^{0}C for 2 hours, let it cool slightly then put it in a desiccator for ½ hour.
9. Weighing (d grams) Calculation:

$$\text{Kadar Lignin} = \frac{c - d}{\text{Berat contoh}} \times 100\%$$

Kadar Selulosa = % ADF - % Lignin - % Abu yang tak larut

Kadar Hemiselulosa = %NDF-%ADF

RESULTS

Recapitulation Results Study

Recapitulation of the average results of cellulose, hemicellulose and lignin content with rice straw processing (fermentation with EM4; Win Prob and Microbacter alfaafa 11) with incubation duration (7, 14 and 21 days) in show on Table 1.

Table 1. Recapitulation of Cellulose, Hemicellulose and Lignin with Rice Straw Processing (Fermentation with EM4; Win Prob and Microbacter Alfaafa 11) with Incubation Duration (7,14 and 21 days)

Treatment		Average Parameter		
		Cellulose (%)	Hemicellulose (%)	Lignin (%)
A1	B1	39,4718	30,4997	19.6025
	B2	36,3501	26,4224	15,8437

	B3	37,0828	28,4679	16,5140
A2	B1	39,4543	30,2270	19,4206
	B2	36,2960	26,2697	15,6105
	B3	36,8885	28,4261	16,3655
A3	B1	39,4223	29,8005	19,3219
	B2	36,2591	25,8671	15,3618
	B3	36,7567	28,2292	16,0774

Cellulose

Average results study content cellulose with rice straw processing (fermentation with EM4; Win Prob and Microbacter alfaafa 11) with incubation duration (7,14 and 21 days) which different for each treatment can be seen on Table 2.

Table 2. Average k Content Cellulose with Rice Straw Processing (Fermentation with EM4; Win Prob and Microbacter Alfaafa 11) with Incubation Duration (7,14 and 21 days)

Bio- activator used (A)	Incubation Period (B) (Days)			Average A
	B_1 (7)	B_2 (14)	B_3 (21)	
	-----------------------%--------------------------			
A_1 (EM4)	39,4718	36,3501	37,0828	37.6349 ^{tn}
A_2 (Win Prob)	39,4543	36,2960	36,8885	37.5463 ^{tn}
A_3 (Microbacter alphaafa 11)	39,4223	36,2591	36,7567	37.4794 ^{tn}
Average B	39.4495 ^{B}	36,3017 ^{A}	36.9093 ^{A}	

Based on Table 2, on treatment fermentation to rice straw using EM4; Win Prob and Microbacter alfaafa 11 with a long fermentation period different (7.14 and 21 days), the highest average cellulose content was obtained, namely 39.4718 %. on treatment A_1B_1 (bio-activator EM4 And longtime incubation 7 day), and that lowest 36.2591% on treatment A_3B_2 (bio-activator Microbacter alfaafa 11 and long incubation time 14 days).

Hemicellulose

Average results study hemicellulose content with rice straw processing (fermentation with EM4; Win Prob and Microbacter alfaafa 11) with incubation duration (7,14 and 21 days) which different for each treatment can be seen on Table 3.

Table 3. Average k Content Hemicellulose with Rice Straw Processing (Fermentation with EM4; Win Prob and Microbacter Alfaafa 11) withIncubation period (7,14 and 21 days)

Bio- activator used (A)	Incubation Period (B) (Days)			Average A
	B_1 (7)	B_2 (14)	B_3 (21)	
	------------------------%---------------------------			
A_1 (EM4)	30,4997	26,4224	28,4679	28.4633 ^{c}
A_2 (Win Prob)	30,2270	26,2697	28,4261	28.3076 ^{b}
A_3 (Microbacter alphaafa 11)	29,8005	25,8671	28,2292	27.9656 ^{a}
Average B	30.1757 ^{B}	26.1864 ^{A}	28.3744 ^{A}	

Based on Table 3, on treatment fermentation to rice straw using EM4; Win Prob and Microbacter alfaafa 11 with a long fermentation period different (7,14 and 21 days), the highest average hemicellulose content was obtained, namely 30.4997 %. on treatment A 1 B 1 (bio-activator EM4 And longtime incubation 7 day), and that lowest 25.8671 % on treatment A 3 B 2 (bio-activator Microbacter alfaafa 11 and long incubation time 14 days).

Lignin

Average results study content lignin with rice straw processing (fermentation with EM4; Win Prob and Microbacter alfaafa 11) with incubation duration (7,14 and 21 days) which different for each treatment can be seen on Table 4.

Table 4. Average k Content Lignin with Rice Straw Processing (Fermentation with EM4; Win Prob and Microbacter Alfaafa 11) with Incubation Duration (7,14 and 21 days)

Bio- activator used (A)	Incubation Period (B) (Days)			Average A
	B_1 (7)	B_2 (14)	B_3 (21)	
	------------------------%---------------------------			
A_1 (EM4)	19.6025	15,8437	16,5140	17.3201 ^{b}
A_2 (Win Prob)	19,4206	15,6105	16,3655	17.1322 ^{a}
A_3 (Microbacter alphaafa 11)	19,3219	15,3618	16,0774	16.9203 ^{a}
Average B	19.4483 ^{B}	15.6053 ^{A}	16,3190 ^{A}	

Based on Table 4, on treatment fermentation to rice straw using EM4; Win Prob and Microbacter alfaafa 11 with a long fermentation period different (7,14 and 21 days), the highest average lignin content was obtained, namely 19.6025 %. on treatment A 1 B 1 (bio-activator EM4 And longtime incubation 7 day), and that lowest 15.3618 % on treatment A 3 B 2 (bio-activator Microbacter alfaafa 11 and long incubation time 14 days).

DISCUSSION

Cellulose

Recapitulation of the average results of cellulose, hemicellulose and lignin content with rice straw processing (fermentation with EM4; Win Prob and Microbacter alfaafa 11) with incubation duration (7, 14 and 21 days) in show on Table 1.

Based on results analysis statistics on Table 2, show that The lowest cellulose content in this study was found in the A 3 B 2 (bio-activator) treatment Microbacter alfaafa 11 and long incubation period 14 days), namely of 36.2591%. The decrease in cellulose content was caused by the cellulose degradation process by microorganisms in process fermentation, where according to (Artarizqi, 2012)bacteria there are on bio-activator Microbacter alfaafa 11 namely cellulose digesting bacteria, hemicellulose digesting bacteria, starch digesting bacteria, sugar digesting bacteria, and protein digesting bacteria. Cellulase digesting bacteria can produce cellulase enzymes that can break down cellulose into simpler molecules, such as glucose. Cellulase is an enzyme that can break down complex bonds in the cellulose structure (Baharuddin dkk., 2016).

The results of the study also showed that the average cellulose content decreased as the fermentation time increased. Fermentation occurs due to the activity of microorganisms that produce cellulase enzymes that function to decompose complex compounds from their substrates (Hasan & Yanuarianto, 2015). During fermentation, the process of decomposing the materials occurs. material organic Which functioning as source nutrition And energy for microorganisms.

To determine the effect of rice straw fermentation using bio-activators EM4, Win Prob and Microbacter alfaafa 11with long incubation 7,14 and 21 days against level content cellulose, so done analysis diversity. The results of the analysis of variance (Appendix 1) show that in factor A (bio- activator) provides a difference that is not significant (P>0.05) against content cellulose and factor B (length of fermentation time) gave a very significant difference (P<0.01) in content cellulose which was continued with a further honest significant difference (HSD) test. But there is no interaction between second factor the to content cellulose on rice straw fermentation. From Table 2 it can be seen that the cellulose content can be reduced at 14 days of incubation but there is an increase at 21 days although the results can reduce cellulose content more than 7 days of incubation. From Table 3 it can also be seen that the Microbacter alfaafa 11 bioactivator is the most able to reduce cellulose content in fermented rice straw compared to EM4 and Win Prob bioactivators although statistically tested with analysis of variance the results are not significantly different (P> 0.05).

Hemicellulose

Table 3 shows data on the treatment of rice straw fermentation using EM4 bioactivators; Win Prob and Microbacter alfaafa 11 differ in fermentation time (7.14 and 21 days). Treatment A 1 B 1 (EM4 bioactivator and 7-day incubation time) produced the highest hemicellulose content of 30.4997%, and treatment A 3 B 2 (Microbacter alfaafa 11 bioactivator and 14-day incubation time) could reduce the lowest cellulose content of 25.8671 %. The decrease in cellulose content is caused by the nutrients available in the material having been broken down and utilized by microorganisms, one of which is in the additional bioactivator (Irianto, 2015). The growth of microorganisms is closely related to the fermentation time, but is also related to the ideal fermentation time which differs between commercial bioactivators (Madusari, 2016).

To determine the interaction of rice straw fermentation treatment using bio-activators EM4, Win Prob and Microbacter alfaafa 11 with long incubation 7,14 and 21 days against level hemicellulose content , so done analysis diversity. The results of the analysis of variance (Appendix 2) show that in factor A (bio- activator) makes a difference significant (P<0.05) against hemicellulose content and factor B (length of fermentation time) gave a very significant difference (P<0.01) in content cellulose which was continued with a further honest significant difference (HSD) test. But the interaction between second factor This gave an insignificant difference (P>0.05) in hemicellulose content in rice straw fermentation. From Table 3 it can be seen that the hemicellulose content can be reduced at 14 days of incubation but there is an increase at 21 days although the results can reduce the hemicellulose content more than the 7-day incubation.

It can be concluded that the best ideal fermentation time that can reduce the hemicellulose content of the three bioactivators is around 14 days. From Table 3 it can also be seen that the Microbacter alfaafa 11 bioactivator is the lowest in reducing the hemicellulose content in fermented rice straw compared to the EM4 and Win Prob bioactivators with a fermentation incubation time of 14 days.

To see the impact of the interaction between rice straw fermentation using EM4, Win Prob, and Microbacter alfaafa 11 bioactivators , along with variations in incubation time for 7, 14, and 21 days, on the level of hemicellulose content , it is necessary to conduct an analysis of variance with the ANOVA table (Appendix 2). The results of the analysis, as listed in Appendix 2, show that factor A (type of bioactivator) has a significant/real effect ($P<0.05$) on the hemicellulose content , while factor B (fermentation time) shows a very significant difference ($P<0.01$) on the hemicellulose content , which is then strengthened by further honest significant difference (HSD) tests. However, the interaction between the two factors did not provide a significant difference ($P>0.05$) in the hemicellulose content in rice straw that had undergone the fermentation process.

Based on Table 3, it can be concluded that the lignin content can decrease at 14 days of incubation, although there is an increase at 21 days of incubation, but the effect is more effective in reducing lignin content when compared to 7 days of incubation. Therefore, it can be suggested that the optimal fermentation time that can reduce the hemicellulose content of the three types of bioactivators is around 14 days. This is in line with the data produced on the decrease in cellulose content. In addition, from Table 3 it can also be observed that the Microbacter alfaafa 11 bioactivator shows a better ability to reduce the hemicellulose content in rice straw that has undergone the fermentation process compared to the EM4 and Win Prob bioactivators, especially at a fermentation incubation time of 14 days.

Lignin

Lignin is a substance that together with cellulose and other fibrous materials forms the main part of plant cells. Lignin together with cellulose forms a component called lignocellulose, which has a very small digestibility coefficient. This causes lignin to be difficult to digest.

Based on the data in Table 4, research on rice straw fermentation using EM4 bioactivators, Win Prob, and Microbacter alfaafa 11 with variations in fermentation time (7, 14, and 21 days) showed that the highest lignin content was recorded in the A1B1 treatment, which was 19.6025% using EM4 bioactivator and a fermentation period of 7 days. Meanwhile, the lowest lignin content value was found in the A3B2 treatment, where the bioactivator used was Microbacter alfaafa 11 with a fermentation period of 14 days, reaching 15.3618%. The longer incubation time, which is fourteen days, gives microorganisms more time to break down organic matter, including lignin, naturally (Hadrawi, 2014). Because microorganisms use lignin as a source of energy or nutrients, biochemical processes can cause a decrease in lignin content. According to (Mursalim dkk., 2019)that the chemical bonds in lignin can be broken down by the enzymes produced, resulting in a decrease in lignin content. This is in line with the opinion (Suningsih dkk., 2019) that the complex processes that occur between microorganisms and substrate materials can contribute to the decrease in lignin content. The composition of the substrate material and the characteristics of the microorganisms involved are some of the factors that influence this. The lignin content in agricultural waste or feed raw materials can be broken by microorganisms by producing extracellular enzymes, microorganisms break the lignocellulose bonds found in crude fibers such as cellulose and hemicellulose into glucose so that it can be used as food by microorganisms.

CONCLUSION

Based on results research that Already done, can it was concluded that the use of Microbacter alfaafa 11 for the fermentation of rice straw with an incubation time of 14 days (A_3B_2) in study This is best treatment and can reducing cellulose content (36.2591%), hemicellulose (25.8671%) and lignin (15.3618%).

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