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Digestibility of Coconut Coir Fiber Fermented by Buffalo Rumen Fluid Microbes in Vitro

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ABSTRACT. This study aimed to examine the digestibility of fiber, including crude fiber, neutral detergent fiber (NDF), and acid detergent fiber (ADF) digestibility of coconut husk fermented using selected buffalo rumen microbes with varying percentages of inoculum and fermentation periods. The study utilized a completely randomized design (CRD) with a factorial pattern (3×3) and 3 replications. The first factor was the percentage of inoculum (0%, 2.5%, and 5% mL of inoculum per gram of dry matter of coconut husk), and the second factor was the fermentation period (0, 7, and 14 days). The collected data were analyzed using analysis of variance (ANOVA) and, where applicable, Duncan's multiple range test. The results indicated that there was no significant interaction (P>0.05) between the different percentages of buffalo rumen fluid as inoculum and the varying fermentation periods on crude fiber and ADF digestibility of coconut husk. However, the percentage of buffalo rumen fluid as inoculum had a significant effect (P<0.05) on crude fiber and ADF digestibility, and the fermentation period also significantly influenced (P<0.05) ADF digestibility. An interaction between the percentage of buffalo rumen fluid and fermentation period had a significant effect (P<0.05) on NDF digestibility. The findings concluded that the percentage of buffalo rumen fluid as inoculum and the fermentation period did not increase crude fiber and ADF digestibility; however, the interaction between 2.5% buffalo rumen fluid inoculum and a 14-day fermentation period resulted in improved NDF digestibility of coconut husk.

Keywords: ADF, buffalo rumen microbes, coconut husk, crude fiber, NDF

INTRODUCTION

Feed is one of the most important factors in determining the success of livestock farming. The largest proportion of expenditure in a livestock enterprise, whether large or small, is allocated for feed (59.98%), followed by workers' wages (8.86%), and other costs such as medicines (1.96%), fuel and lubricants (1.36%), and electricity and water (0.60%) (Badan Pusat Statistik, 2020).

As the livestock population continues to grow, the demand for feed supply will inevitably increase. However, the availability of feed is becoming increasingly limited due to shrinking land areas used for agricultural development (Samadi et al., 2010). The challenge of limited feed availability is also exacerbated by seasonal factors; for instance, feed may be abundant during the rainy season, but scarce during the dry season due to limited water resources. The quality of feed also tends to decline during the dry season, and the quantity of feed from the plantation sector is often insufficient to meet livestock needs (Agustono et al., 2018).

Plantation waste, such as coconut fiber, is abundantly available. National coconut production in 2019 reached 2.92 million tons (Honorisal et al., 2020). However, agricultural waste like coconut fiber has limitations, including low protein content, a high crude fiber component, and low digestibility (Wati et al., 2012). These limitations can be improved through processing methods, such as fermentation.

Fermentation is one method of enhancing the quality of feed ingredients using microorganisms (Hastuti et al., 2011). In the coconut coir fermentation process, buffalo rumen fluid microbes are employed, as these microbes have been selected through specific activity tests of cellulase enzymes, enabling them to degrade fiber more effectively and improve feed digestibility (Aminah et al., 2020). This study aims to evaluate the digestibility of crude fiber, NDF, and ADF in coconut coir fermented using buffalo rumen microbial inoculum with varying steeping times. The benefit of this research lies in providing valuable insights and information on the optimal provision of selected buffalo rumen microbes and fermentation times for coconut fiber, as measured by the digestibility of crude fiber, NDF, and ADF.

MATERIALS AND METHODS

Research Time and Location

The research was conducted from December 2019 to February 2020. The research and in vitro digestibility measurements were carried out at the Nutrition and Feed Science Laboratory, Faculty of Animal and Agriculture Sciences, Diponegoro University. The measurement of NDF and ADF

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levels was conducted at the Ciawi Livestock Research Center, Bogor.

Research Material

The material used in the study was coconut coir fermented with buffalo rumen microbial inoculum. Buffalo rumen microbial inoculum was obtained from buffalo rumen fluid taken from the slaughterhouse, then incubated at 39ºC for 16 hours by adding 1% avicel PH 101 and 2% glucose. Cultures of fiber-digesting microbes were obtained by isolating fiber-digesting microbes from buffalo rumen fluid using a liquid selective medium and a substrate in the form of peanut shells. The isolation medium was a liquid medium composed of mineral solution I, mineral solution II, yeast extract (Merck, Germany), tryptone (Sigma-Aldrich, USA), centrifuged buffalo rumen fluid, H_2O, 12% Na_2CO_3, cysteine HCl, and 0.1% resazurin. Mineral solution I was composed of 0.3% K_2HPO_4, while mineral solution II was composed of 0.3% K_2HPO_4; 0.6% $(NH_4)_2SO_4$; 0.6% NaCl; 0.06% $MgSO_4$, and 0.06% $CaCl_2.H_2O$.

Isolation was carried out using 50 ml of liquid medium. The materials used included beef cattle rumen fluid, McDougall solution, 0.2% HCl pepsin solution, aquadest, 0.3% NH_2SO_4 solution, NaOH, 70% alcohol, neutral detergent solution (NDS), acid detergent solution (ADS), and Na_2SO_3. The tools used for isolation and experimentation were test tubes (fermenters), water bath, centrifuge, thermometer, 250 ml beaker glass, 50 ml measuring cup, Whatman 41 filter paper, Buchner funnel, analytical balance, desiccator, fume hood, vacuum pump, oven, furnace, electric stove, porcelain cup, and porcelain crucible. The parameters studied included digestibility of crude fiber (CF), neutral detergent fiber (NDF), and acid detergent fiber (ADF) of fermented coconut fiber.

Research Design

The study was conducted using a completely randomized design (CRD) with a 2×3 factorial pattern and 3 replications. The first factor was the difference in the percentage of inoculum use (0%, 2.5%, and 5% ml inoculum/g dry matter of coconut fiber), and the second factor was the length of curing (0, 7, and 14 days). The treatment combinations were as follows:

P_0H_0 = coconut fiber + 0% inoculum matured for 0 days
P_0H_1 = coconut fiber + 0% inoculum matured for 7 days
P_0H_2 = coconut fiber + 0% inoculum matured for 14 days
P_1H_0 = coconut fiber + 2.5% inoculum matured for 0 days
P_1H_1 = coconut fiber + 2.5% inoculum matured for 7 days
P_1H_2 = coconut fiber + 2.5% inoculum matured for 14 days
P_2H_0 = coconut fiber + 5% inoculum matured for 0 days
P_2H_1 = coconut fiber + 5% inoculum matured for 7 days
P_2H_2 = coconut fiber + 5% inoculum matured for 14 days

Research Procedure

The stages of the research consist of two main phases: the preparation stage and the analysis of digestibility (crude fiber, NDF, and ADF). Preparation Stage: Tool and Material Preparation: Preparation of all tools and research materials, including the coconut fiber, is done at this stage.

Coconut Fiber Fermentation: Coconut fiber is ground into smaller pieces of approximately 1-2 cm. Around 1 kg of dry matter (DM) of coconut fiber is used in each treatment. The microbial culture, as studied by Aminah et al. (2020), is re-inoculated in a liquid medium and incubated for 16 hours. This culture is then used as the inoculum for the fermentation process. The coconut fiber is then mixed with 0.1% urea, 3% molasses, and distilled water (adjusted to 60% moisture content). The inoculum is added at different percentages: 0%, 2.5%, and 5%. The fiber mixture is placed in airtight jars and fermented for varying durations of 0, 7, and 14 days.

Analysis Stage: In Vitro Digestibility: This method simulates the conditions of the rumen. For each sample of fermented coconut fiber, 0.55-0.56 g is placed in a fermenter tube. The McDougall solution (40 ml) and rumen liquid (10 ml) are added to the sample, followed by CO_2 gas. The tube is sealed with a rubber cap and incubated in a waterbath at 38-40ºC for 48 hours. Every 6 hours, the mixture is shaken. After incubation, centrifugation is done for 15 minutes at 3000 rpm. The residue is then incubated in a waterbath with a pepsin HCl solution for another 48 hours, with shaking every 6 hours. The sample is filtered using a vacuum pump and filter paper to determine digestibility (Hambakodu et al., 2020).

1. Calculation of crude fiber digestibility (Lestari et al., 2020):

$$\text{CF digestibility} = \frac{\text{feed CF weight} - (\text{residual CF weight} - \text{weight of CF blank})}{\text{feed CF weight}} \times 100\%$$

2. Calculation of NDF digestibility according to Tilley dan Terry (1963) which is particularized by Hambakodu et al. (2020):

$$\text{NDF digestibility} = \frac{\text{feed NDF weight} - (\text{residual NDF weight} - \text{weight of NDF blank})}{\text{feed NDF weight}} \times 100\%$$

3. ADF digestibility calculation according to Tilley dan Terry (1963) which is particularized by Hambakodu et al. (2020):

$$\text{ADF digestibility} = \frac{\text{feed ADF weight} - (\text{residual ADF weight} - \text{weight of ADF blank})}{\text{feed ADF weight}} \times 100\%$$

Data Analysis

Data analysis in this study was conducted using Analysis of Variance (ANOVA) at the 5% significance level to assess the effects of different treatments. If significant differences were found, the analysis was followed by a Duncan's Multiple Range Test (DMRT) to determine which specific groups differed from each other. The linear model for the observation values, which follows a Completely Randomized Design (CRD) with a 2×3 factorial pattern, is outlined as follows (Angriany et al., 2019):

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

RESULTS AND DISCUSSION

Crude Fiber Digestibility

The results of the analysis of variance indicated that the treatments involving different percentages of inoculum and varying soaking durations did not show any significant interaction (P>0.05) on the digestibility of crude fiber in coconut coir. Specifically, the combination of a 5% inoculum percentage and a 14-day soaking period did not result in any enhanced crude fiber digestibility. This lack of interaction is likely due to the concentration of microbes in the inoculum not being sufficient to compensate for the combined effect of the fermentation time. As Suningsih et al. (2019) noted, effective microbial concentrations during fermentation play a critical role in breaking down chemical bonds in feed ingredients, leading to improved digestibility. However, in this case, the microbial concentration may not have been high enough to achieve optimal feed ingredient breakdown and digestion. The results showed that as the percentage of inoculum increased, the average crude fiber digestibility of coconut coir also improved. The crude fiber digestibility for each treatment was 48.74% (P_0), 63.13% (P_1), and 63.25% (P_2). The analysis of variance revealed that the inoculum percentage factor significantly affected (P<0.05) the digestibility of coconut coir crude fiber. This indicates that the use of fiber-digesting microbes from buffalo rumen, with inoculum levels ranging from 0 to 5%, can enhance the digestibility of coconut coir crude fiber.

Table 1. Crude fiber digestibility of fermented coconut fiber

Inoculum (%)	Fermentation time (days)			Average
	H_0	H_1	H_2	
	---------- (%) ----------			
P_0	42.22	50.32	53.67	48.74[b]
P_1	60.33	65.03	64.04	63.13[a]
P_2	59.73	64.13	65.88	63.25[a]
Average	54.09	59.83	61.20	

Note: [a,b] different superscript letters in the same column indicate significant differences (P<0.05), P_0= Use of 0% inoculum; P_1= Use of 2.5% inoculum; P_2= Use of 5% inoculum; H_0= 0 days maturation; H_1= 7 days maturation; H_2= 14 days aging.

Duncan's test showed that the crude fiber digestibility for the P_2 treatment (63.25%) was not significantly different (P>0.05) from the P_1 treatment (63.13%), but both P_2 and P_1 were significantly higher (P<0.05) than P_0 treatment (48.74%). Crude fiber digestibility increased up to a 5% inoculum percentage. The increase in digestibility can be attributed to the differences in inoculum levels (0%, 2.5%, and 5%), where the inoculum-buffalo rumen fluid containing cellulolytic bacteria-played a crucial role in enhancing the breakdown of fiber. As Trihatma et al. (2018) pointed out, a higher number of microorganisms in the starter culture leads to increased cellulase enzyme production, which helps degrade the crude fiber fraction more effectively.

NDF Digestibility

The results of the analysis of variance showed a significant interaction (P<0.05) between the treatment of different percentages of inoculum

and the length of steeping on the NDF digestibility of coconut fiber. This indicates that both treatment factors-percentage of inoculum and fermentation time-contributed to the improvement of NDF digestibility. The combination of higher inoculum percentages and longer fermentation periods led to increased NDF digestibility. As Septianto et al. (2019) observed, extending the length of the maceration time allows microbes more time to grow during fermentation, thereby enhancing their ability to degrade feed. Thus, the longer the maceration time, the greater the opportunity for microbial activity to break down fiber, ultimately improving feed digestibility.

Table 2. NDF digestibility of fermented coconut fiber

Inoculum (%)	Fermentation time (days)			Average
	H_0	H_1	H_2	
	---------- (%) ----------			
P_0	30.60^{c}	30.65^{c}	30.93^{c}	30.73
P_1	31.02^{c}	36.59^{b}	43.06^{a}	36.89
P_2	30.85^{c}	38.33^{b}	37.43^{b}	35.54
Average	30.82	35.19	37.14	

Note: ^{a,b} different superscript letters in the same column and row indicate significant differences ($P<0.05$), P_0= Use of 0% inoculum; P_1= Use of 2.5% inoculum; P_2= 5% inoculum; H_0= 0 days maturation; H_1= 7 days maturation; H_2= 14 days aging.

The results of the Duncan multiple range test (Duncan multiple range test) on the interaction between the percentage of inoculum and the length of fermentation on NDF digestibility of fermented coconut coir showed that the P_1H_2 treatment (43.06%) was significantly different ($P<0.05$) from the other treatments. NDF digestibility increased as the combination of the percentage of inoculum and the length of fermentation increased. However, NDF digestibility in the P_1H_2 treatment (43.06%) was relatively higher than in the P_2H_1 treatment (38.33%). This indicates that the combination of 2.5% inoculum and 14 days of fermentation was more effective in enhancing NDF digestibility of coconut fiber, providing more opportunity for fiber-digesting microbes to grow during fermentation. Ahmad et al. (2020) stated that higher levels of inoculum, up to a certain point, along with a sufficient fermentation period, can enhance the digestibility of fermented feed ingredients. This is because bacteria require time to grow during the fermentation process. The influence of the interaction between inoculum percentage and fermentation time on NDF digestibility can be observed in Figure 1.

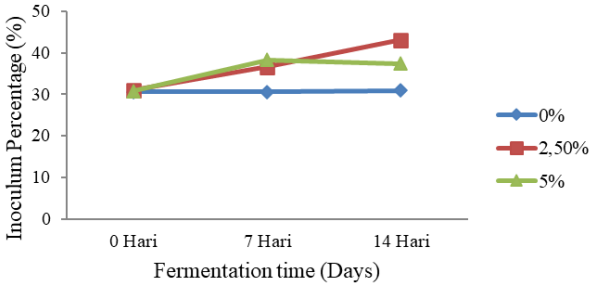

Figure 1. Interaction of Factor 1 (percentage of inoculum) and Factor 2 (length of aging) on coconut coir NDF digestibility

NDF digestibility in the P_2H_1 treatment (38.33%) was lower than in the P_1H_2 treatment (43.06%). This is likely due to the higher crude fat content in P_2H_1 (13.53%) compared to P_1H_2 (10.10%). High fat content (ranging from 7.04% to 16.03%) in coconut coir can affect microbial development and activity during in vitro digestion, thereby influencing fiber digestibility. According to Wina and Susana (2013), an increase in fat content in feed can negatively impact microbial function because fat can coat the feed fiber, preventing rumen microbes from degrading the fiber optimally.

ADF Digestibility

The results of the analysis of variance showed that there was no interaction effect ($P>0.05$) between the combination of inoculum percentage and different soaking durations on ADF digestibility. This indicates that these two factors do not together influence ADF digestibility of coconut fiber. The lack of interaction between the microbial inoculum percentage and soaking time is likely due to insufficient protein availability to meet microbial needs during fermentation. When the protein content in the substrate is low, it can result in decreased ammonia concentration and pH levels, which can hinder microbial growth and activity in the rumen. This, in turn, leads to reduced ADF digestibility. According to Nurkhasanah et al. (2020), low protein content can decrease ADF digestibility because it inhibits the growth and activity of rumen microbes.

The results of the Duncan multiple range test on the inoculum percentage factor indicated that treatment P_0 was significantly different ($P<0.05$) from P_1, but not significantly different ($P>0.05$) from P_2. Treatment P_1 was not significantly different ($P>0.05$) from P_2, but was different from P_0. The effect of inoculum percentage on ADF digestibility was greatest in treatment P_1 (33.05%) and lowest in treatment P_0 (29.75%). This implies

that a 2.5% inoculum percentage containing buffalo rumen microbial isolates is effective in improving ADF digestibility. Buffalo rumen microbes are capable of degrading ADF fractions, leading to increased ADF digestibility. Anam et al. (2012) suggested that ADF digestibility can improve due to the microbial breakdown of cell walls into simpler components like hemicellulose and glucose during fermentation.

Table 3. ADF digestibility of fermented coconut fiber

Inoculum (%)	Fermentation time (days)			Average
	H_0	H_1	H_2	
	---------- (%) ----------			
P_0	29.47	30.07	29.70	29.75^{b}
P_1	30.08	32.47	36.60	33.05^{a}
P_2	29.12	32.73	31.44	31.10^{ab}
Average	29.56^{b}	31.76^{a}	32.58^{a}	

Note: ^{a,b} different superscript letters in the same column and row indicate significant differences ($P<0.05$), P_0= Use of 0% inoculum; P_1= Use of 2.5% inoculum; P_2= 5% inoculum; H_0= 0 days maturation; H_1= 7 days maturation; H_2= 14 days aging.

The results of the analysis of variance showed that different lengths of curing significantly affected ($P<0.05$) ADF digestibility. This suggests that the duration of fermentation, ranging from 0 to 14 days, significantly enhances ADF digestibility. Duncan's test on the aging factor showed that treatment H_2 (14 days) was not significantly different ($P>0.05$) from H_1 (7 days), but was significantly different ($P<0.05$) from H_0 (0 days). The highest ADF digestibility was found in the H_2 (14 days) treatment at 32.58%, followed by H1 (7 days) at 31.76%, and the lowest in the H_0 (0 days) treatment at 29.56%. The longer steeping time provided microbes more opportunity to degrade the substrates, improving ADF digestibility. Prastyawan et al. (2012) noted that extending the soaking time increases microbial growth and fermentation opportunities, thereby enhancing the degradation of coconut fiber.

CONCLUSION

The interaction between the percentage of buffalo rumen inoculum and the duration of soaking time did not improve the digestibility of crude fiber and ADF in coconut fiber. However, the combination of a 2.5% buffalo rumen fluid inoculum and 14 days of soaking significantly increased the NDF digestibility of coconut fiber. This suggests that while the inoculum percentage and soaking time did not interact to enhance crude fiber or ADF digestibility, the specific combination of the 2.5% inoculum and 14-day soaking period was effective in promoting the breakdown of NDF, improving its digestibility.

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