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RESEARCH ARTICLE

Improving the quality of Cassava Peel-Leaf Mixture (CPLM) through fermentation with *R. oligosphorus* as poultry ration

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ABSTRACT

Cassava (*Manihot utilissima*) is one of the potential food commodities in Indonesia. Cassava production in Indonesia is relatively high. Of course, it will produce much waste, such as Cassava Peel (CP) and Cassava Leaf (CL). Casava peel and casava leave has the potential to be used as feed ingredients but is constrained by low quality, and they can only be used 10% in poultry rations. For that, it is necessary to research to improve the quality of Cassava Peel-Leaf Mixture (CPLM) through fermentation with *R.oligosphorus* as a poultry ration. This experimental study uses a completely randomized design (CRD) with four treatments and five replications. The treatments were cassava peel-leaf mixture (CPLM) fermented with R. oligosphorus. The Treatments were A (9:1), B (8:2), C (7:3), and D (6:4). The variables observed were the digestibility of crude fiber (DCF), the activity of protease enzymes, crude protein (CP) nitrogen retention (RN), metabolic energy (ME), and crude fiber (CF). The diversity analysis showed a very significant effect ($P<0.01$) on (DCF), crude protein (CP), protease activity, crude fiber (CF), nitrogen retention (RN), and energy metabolism (ME) of CPLM fermentation. Based on the study's results, it can be concluded that CPLM (6:4) fermented with *R.oligosphorus* gave the best results.

Keywords: *R. oligosphorus*; Casava peel; Casava leave; Fermented; Poultry

INTRODUCTION

The cost of the poultry industry is high because many feedstuffs of poultry steel are imported, for example, corn, soybean meal and fish meal. Various efforts can be made to reduce feed costs in the poultry industry by looking for alternative feed ingredients that are cheap, continuously available, with complete nutritional content, and are not food for basic human needs. Alternative feed ingredients that can be used include agricultural waste. One of them is waste from the production of cassava plants in the form of cassava peel (CP) and cassava leaves (CL).

Cassava (*Manihot utilissima*) is one of the potential food commodities in Indonesia. Based on the central bureau of statistics of Indonesia (2018), cassava productivity in West Sumatra reached 201,833 tons per year. At the same time, its production in Indonesia is 19,341,233 tons per year. Cassava production in Indonesia is relatively high. Of course, it will produce a lot of waste, such as Cassava Peel (CP) and Cassava Leaf (CL). The potential of CP produced is approximately 16% of cassava production (Darmawan, 2006). So it is estimated that the number of cassava peel available in West Sumatra based on the 2018 BPS is around 32,293.28 tons/year. In addition to its abundant availability, CP also has a relatively good nutritional content. CP contains 89.17% dry matter, 4.01% crude protein, 4.70% crude fat, high crude fiber 26.27% and carotenoids 72.21μg/ml (Laboratoriun Nutrisi Non-Ruminansia, 2021). CP can only be used up to 7% in broiler rations (Suryana, 2016).

Another waste from cassava production potentially used as animal feed is cassava leaves (CL). Judging from the content of food substances, CL contains 88.40% dry matter, 14.84% crude protein, 2.63% crude fat and 15.33% crude fiber and carotenoids 386.11 g/ml (Laboratoriu Nutrisi Non-Ruminansia, 2021). According to Poodja and Padmaja (2014), cellulose content is 17.60%, hemicellulose is 27.65%, and lignin is 20.10%. Cassava leaves contain

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HCN ranging from 200-1300 ppm per kg fresh weight (Siritunga et al., 2003). cassava leaves are also rich in beta carotene ranging from 298.95-517.72 mg/kg (Priadi et al., 2009). The high nutritional content of cassava leaves has not been optimally utilized in broiler rations. So far, cassava leaves in broiler rations have only used a maximum of 5% (Iheukwumere et al., 2008).

The low utilization of CP and CL in the ratio was due to the low nutrient content and anti-nutrient HCN as a limiting factor. A feed processing technology by fermentation is essential to improve the quality and reduce the limiting factors of CP and CL and their utilization in rations. This fermentation uses the fungus *R.oligosphorus*. The fungus *R. oligosphorus* produces protease enzymes, lipases, alpha-amylase, glutaminase, and alpha-galactosidase (Han et al., 2003). CP fermentation with *R. oligosphorus* was carried out by Sabrina et al. (2001), and there was a decrease in HCN content from 228 ppm to 19.4 ppm, crude fat content from 3,61% to 2.99%, an increase in crude protein percentage from 7.24% to 18.78% and the protease activity was 24.58 U/ml. In addition, it can also be utilized up to 15% in broiler rations. Furthermore, Rizal et al. (2005) performed routine isolation of cassava leaves fermentation with *A. niger* where there was an increase in crude protein content, decreased crude fiber and HCN content from CL and its use in broiler rations to 9%. Anisa et al. (2020) also fermented a mixture of CL with tofu waste using *R. oligosphorus,* which also showed a rise in the crude protein percentage from 22.79% to 26.72% and a crude fiber reduction from 20.14% to 5.27%, crude fat from 8.46% to 6.74% and protease activity of 9.84 U/ml and can be used up to 15% in broiler rations.

Several factors need to be considered in fermentation. One of them is the composition of the substrate. The substrate is the place where microbes grow. Microbes work according to the inducers available on the substrate (Pratiwi et al., 2013; Mirnawati et al., 2019; Anisa et al., 2020). The balance between the composition of the substrate and nutrients needed by microbes to live so that the mass of microbes grows more and more (Muhiddin et al., 2000). In addition, the composition of the substrate will affect the enzymes produced. Microbes will produce enzymes according to the inducers available in the substrate (Pratiwi et al., 2013). The success of fermentation can be seen from the influence of the nutrient content in the substrate, primarily the carbon and nitrogen sources (Hidayat et al., 2006). CP in the fermentation medium also serves as a carbon source. However, it is necessary to add an N source to obtain a suitable C: N balance for mould growth. Another nitrogen source can be used in CL because it contains a relatively high crude protein value.

For this reason, in this study, the substrate used was a mixture of CP and CL (CPLM) so that the nutritional content in CPLM complemented each other. Based on the previous explanation, it is essential to determine the effect of the substrate composition of the fermented CPLM mixture with *R. oligosphorus* on the content and quality of the food substance of the fermented CPLM mixture.

MATERIALS AND METHODS

Research material

Some materials needed for this research are cassava peels (CP) and cassava leaves (CL), *Rhizopus oligosporus* inoculum, distillate water, buffer solution pH 7, NaOH, H2SO4, acetone and chemicals for proximate analysis and enzyme activity. Twenty-four broiler chickens aged six weeks. The tools used are an analytical balance, autoclave, plastic container, porcelain dish, metal cup, erlenmeyer, spray bottle, knife, oven, furnace, desiccator, incubator, beaker, grinder, filter paper, aluminium foil, tissue, syringe, ballistic bomb calorimeter, and metabolic cage (with drinking holder).

Experimental design

This study used the experimental method with a Completely Randomized Design (CRD) for four treatments and five replications. The treatment given was the ratio of substrate composition between CP and CL based on the results of Olowoyeye et al. (2019) research, namely A (9:1), B (8:2), C (7:3) and D (6:4). This study measured several parameters, specifically for nitrogen retention, crude protein, protease activity, digestibility of crude fiber, crude fiber and metabolic energy. The data analysis by statistically processed by analysis of diversity (Steel and Torrie,1991). The Duncan Multiple Range Test (DMRT) tested the differences between treatments.

The protease activity was measured by determining its proteolytic activity based on Cupp and Enyard (2008). Proximate analysis was used to measure the crude protein and crude fiber content, while crude fiber digestibility, nitrogen retention and metabolic energy were measured using the method of Sibbald (1976). Forty chickens aged four weeks were used in this experiment. Before the treatment was given, all experimental chickens were fasted for 36 hours to minimize the impact of the previously consumed feed. The treatment in this study was to provide as much as 20 grams of fermented feed per chicken, then the chickens were put in metabolic cages equipped with drinking containers and faeces separation areas. For 30 hours, faeces were collected every hour. They were then spraying 0.3 N H2SO4 to prevent nitrogen evaporation. The faeces are dried using an oven at a temperature of

50-60 Celsius. Furthermore, the faeces were ground into powder which would be analyzed for nitrogen retention, feces gross energy and crude fiber content.

RESULTS AND DISCUSSION

The effect of each treatment includes parameters of crude protein, nitrogen retention, protease activity, crude fiber, crude fiber digestibility and metabolic energy of mixed cassava peel leaves (CPLM) shown in Table 1.

The treatment effect for protease activity

Based on the data in Table 1, it can be seen that the more addition of CP in the substrate, the higher the protease activity. The increased protease activity was due to CL's high protein content, so *Rhizopus oligosporus* was encouraged to produce protease enzymes. Following Pratiwi's (2013) opinion, microbes work following the inducers available on the substrate. The food source or inducer influences the work of moulds in releasing enzymes. The higher the inducer content, the higher the effort of the mould in secreting protease enzymes into the environment to decompose proteins into amino acids. Then, the decomposition products are transported into cells using a transport system for growth so that the microbes grow more and more (Oetari, 2006).

More protease enzymes will be produced if more and more microbes grow. Therefore, an increase in protease activity will be produced at the end of the fermentation process. This result follows Mirnawati et al. (2019a) research that the high protease activity is due to many growing microbes, so many enzymes are produced during fermentation, especially protease enzymes. The effect of treatment on protease activity can be seen in Fig. 1.

This study found the best protease activity in treatment D with protease activity of 7.25 U/ml. This result is lower than the result obtained by Annisa et al. (2020), namely cassava leaves and tofu dregs fermented with *R. oligosporus* produced a protease activity of 9.84U/ml. Differences in the substrate composition used in this study will cause differences from the results of Annisa et al. (2020) used more cassava leaf substrates.

The treatment effect for crude protein

Table 1 shows that the crude protein content in the CPLM mixed substrate was higher due to the more CL added. The high content of crude protein is due to the growth of more and more moulds. The more the growth of the fungus, the more protein donations from the body of the fungus. The results of the research by Krishna et al. (2005) state that the supply of microbial protein caused by its growth which produces single cell protein or cell biomass, will increase protein by 40-65%. The treatment affects crude protein, as shown in Fig. 2.

In addition, treatment D showed higher crude protein content by the accumulation of enzymes produced by microbes. It is known that enzymes also belong to proteins. More enzymes will be produced during fermentation if more microbes grow. Mirnawati et al. (2010) explained that enzymes produced by microbes would increase crude protein. Thus, one of the determining factors for protein content in the feed is the enzyme content contained therein. In further research, Mirnawati et al. (2012) described that microbes as single-cell proteins greatly determine the protein content of the feed during the fermentation process.

The treatment effect for crude fiber

Table 1 shows that treatments B, C and D resulted in a significant reduction in crude fiber content caused by high fungal growth. The adequacy of elements C and N in the substrate for the growth of *R. oligosphorus* influences optimal mould growth. In treatment B, the balance of C: N is 30:1, C C: N is 28:1, and D C: N is 28:1. This follows the opinion of Deublein and Stainhauser (2008) that the optimum C/N value for microbes in the substrate is between 20-30. The results of Musnandar's research (2003) explained that mould needs carbon (C) to form its body framework during its growth. Nitrogen (N) for mould is needed to form amino acids, pyrimidines, purines, lipids and carbohydrates. Fertility of the mould caused the contribution of body protein to the substrate to be more

Table 1: The treatment effect for protease activity (U/ml), crude protein (%), crude fiber (%), nitrogen retention (%), crude fiber digestibility (%) and metabolic energy (Kcal/kg) of CPLM

Substrate Composition	Parameters Measured					
	Protease Activity	Crude protein	Crude Fiber	Nitrogen Retention	Crude Fiber Digestibility	Energy Metabolism
A (9:1)	5.70^{a}	17.54^{a}	14.62^{b}	55.44^{a}	46,29^{b}	1869^{b}
B (8:2)	6.40^{b}	18.34^{b}	12,24^{ab}	57.26^{b}	56,76^{ab}	2171^{ab}
C (7:3)	6.82^{c}	20.85^{c}	11,53^{a}	58.46^{c}	61,42^{a}	2458^{ab}
D (6:4)	7.25^{d}	21.23^{d}	19.80^{a}	69.59^{d}	62,99^{a}	2671^{a}
SEM	0.09	0.06	1.78	0.22	0,50	182
p	0.01	0.01	0.01	0.01	0.01	0.01

Different superscripts ($^{a\ b,c,d}$) shows a significant effect (P<0.01)

than treatment A. The crude fiber content of the results of the study is presented in Fig. 3.

The number of growing microbes will determine the number of enzymes produced. One is the cellulase enzyme which can break crude fiber into glucose. Following Wattiheluw (2012) opinion, *R. oligosporus* with a substrate consisting of a CPLM mixture as an inducer can utilize cellulose and hemicellulose as an energy source resulting in a decrease in crude fiber content. Mirnawati et al. (2019b) also expressed the same thing to reduce the amount of crude fiber and produce energy. The cellulase enzyme will break down cellulose into glucose on the substrate.

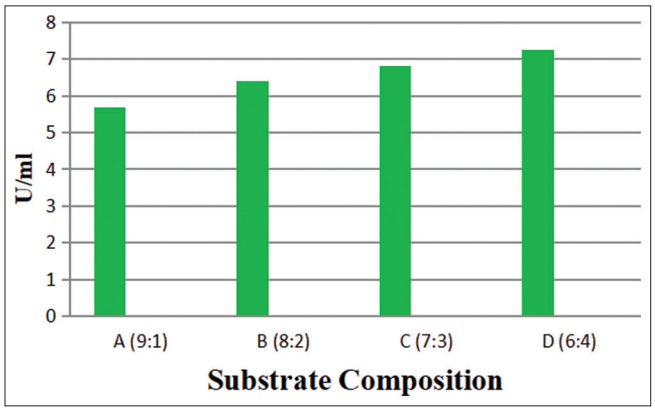

Fig 1. The treatment effect for protease activity.

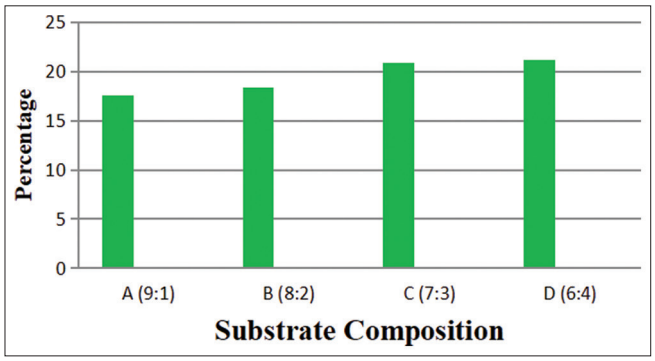

Fig 2. The treatment effect for crude protein.

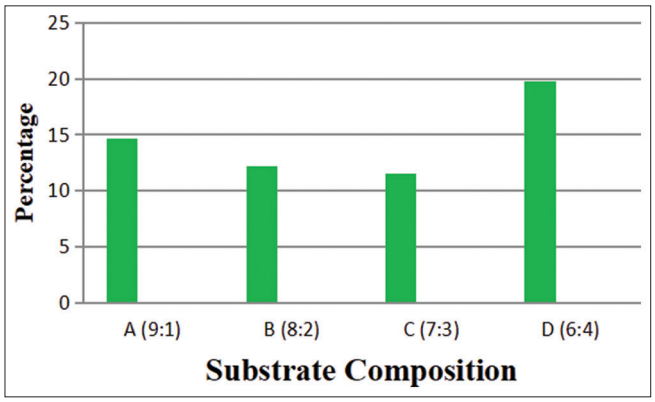

Fig 3. The treatment effect for crude fiber.

The treatment effect for nitrogen retention

Based on the data (Table 1), it can be seen that the more addition of CP, the higher the nitrogen retention value produced. The high protein content in treatment D will result in high nitrogen retention. McDonald et al. (2002) decided which states that the protein content in animal feed is determined based on the amount of nitrogen retention. The higher the protein feed given to the treatment, the more nitrogen retention also increased. The level of nitrogen in excreta affects the amount of nitrogen retention. Following the research of Mirnawati et al. (2017) that the nitrogen retention value will be positive if the nitrogen consumed by livestock is more than the nitrogen excreted through faeces. The nitrogen retention values obtained in this study are presented in Fig .4.

In addition, the nutritional quality becomes better after fermentation due to increased nitrogen retention and is supported by complete amino acids. Along with the opinion of Mirnawati et al. (2019a), who described that better nutritional quality would be obtained from increased retention of nitrogen and more amino acids formed after fermentation.

The treatment effect for digestibility of crude fiber

Based on the data (Table 1), it can be seen that treatments A and B have low crude fiber digestibility because treatments A and B have high crude fiber content. In line with the research of Mirnawati et al. (2017), the amount of crude fiber in the feed will impact the level of digestibility of the crude fiber. The digestibility of crude fiber will be higher if the content is low in the feed, due to the limited ability of poultry to digest crude fiber.

Conversely, the digestibility of crude fiber will be lower if the amount is high in the feed. Tillman et al. (2005) stated that the amount of crude fiber in the ration and the amount of crude fiber consumed determined the digestibility level of crude fiber in livestock. The Maynard et al. (2005) opinion explained that several factors influence the level of fiber digestibility, namely the fiber content in the feed and the activity of microorganisms. The digestibility level of fiber in this study is presented in Fig. 5.

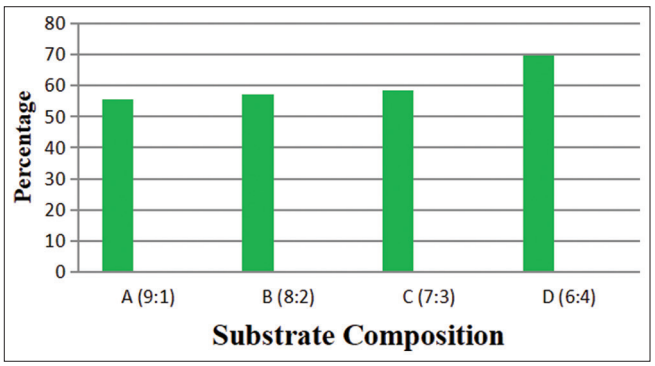

Fig 4. The treatment effect for nitrogen retention.

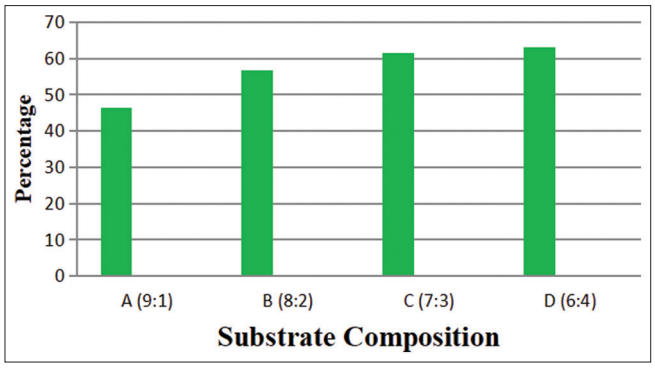

Fig 5. The treatment effect for crude fiber digestibility.

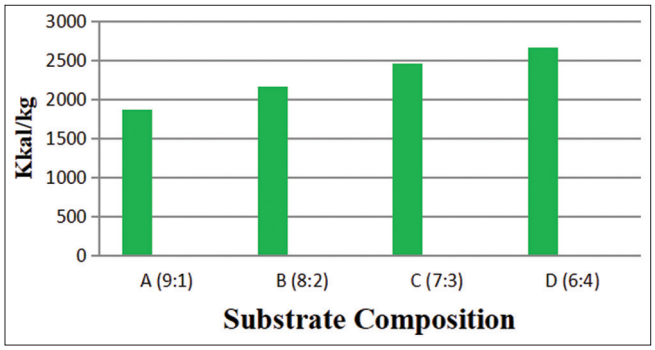

Fig 6. The treatment effect for metabolic energy.

Treatments C and D showed high levels of crude fiber digestibility due to the higher cellulase enzyme activity produced, proving that cellulase enzyme activity can increase the digestibility of crude fiber in the ratio. Our results are in line with the statement of Mirnawati et al. (2019b) that there is a directly proportional relationship between the production of cellulase enzymes produced by mould and its ability to break down cellulose into glucose thereby increasing the digestibility of crude fiber at the end of fermentation.

The treatment effect for energy metabolism

Based on the data (Table 1), it can be seen that the more addition of CL in the substrate there is a tendency to increase the metabolic energy of CPLM products. The high energy metabolism is due to the degradation of complex food substances into simple ones, which are ultimately easier to digest. The high digestibility of crude fiber is also associated with high metabolic energy due to increased energy required during the digestive process, supported by the opinion of Prabowo et al. (2002), which stated that differences in crude fiber content in each treatment would result in differences in metabolic energy. The higher the crude fiber, the lower the metabolic energy. Conversely, the lower the crude fiber, the higher the metabolic energy. McDonald et al. (2002) stated that crude fiber is a food substance that affects digestibility, and digestibility is a factor that affects the energy metabolism of feed ingredients. Rations containing high crude fiber are amba and produce low energy (Amrullah, 2004). The effect of treatment on metabolic energy can be seen in Fig. 6.

This study's results follow Sukaryana (2007) opinion that fermentation can increase the metabolic energy of fermented products. After the fermentation process, there will be an increase in metabolic energy content. The overhaul of hard-to-digest crude fiber causes this to make it easier to digest due to the cellulase enzyme's role, which can degrade cellulose into glucose.

CONCLUSIONS

The results of the study concluded that the composition of the substrate in the mixture of CP and CL (6:4) fermented with *R. oligosporus* gave better results in terms of protease activity 7.25 U/ml, crude protein 21.23%, crude fiber 19, 80%, nitrogen retention 59.65%, digestibility of crude fiber 62.99% and metabolic energy 2671.44 kcal/kg.

Authors' contributions

Mirnawati designed the concept, searched for funding, and drafted and reviewed the paper. Gita Ciptaan supervised the field and laboratory work. Ferawati conducted field and laboratory work, data tabulation, and data analysis.

CONFLICT OF INTEREST

All of the authors declare that they have no conflict of interest.

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