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Improving the Quality of Tapioca By-Products (Onggok) as Poultry Feed Through Fermentation by *Bacillus amyloliquefaciens*

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Abstract: An experiment was conducted to improve the nutrient content of tapioca by-products (onggok) through fermentation by using cellulolytic bacteria (*Bacillus amyloliquefaciens*) as inoculums. The experiment was determination of the optimum conditions (dosage of inoculums, fermentation length and temperature) for tapioca by-products (onggok) fermentation based on nutrient quality and quantity of these fermented products. The study was conducted in experimental methods, using the completely randomize design in factorial with 3 treatment were: 1) A factor (Dosage of inoculums: A1 = 2%, A2 = 6%, A3 = !0%), 2) B factor (Fermentation length: B1 = 3 days, B2 = 6 days, B3 = 9 days) and 3) C factor (Temperature: C1 = 30°C, C2 = 40°C, C3 = 50°C). Results of study showed that optimum conditions of the fermentation of tapioca by-products (onggok) was at 2% dosage of inoculums, 6 days of fermentation length and 40°C temperature. This conditions can decrease 32% of crude fiber and increase 360% of crude protein which made the nutritional value of the product based on dry-substance was 7.9% crude protein, 2.75% crude fat, 11.55% crude fiber, 0.26% calcium, 0.17% phosphor, 2190 Kcal/kg metabolic energy and 65.95% nitrogen retention.

Key words: *Bacillus amyloliquefaciens*, fermentation, tapioca by-products (onggok)

INTRODUCTION

Tapioca by-products is the inside part of cassava whose starch has been separated away. It is one of the potential alternatives for poultry feed, but its utilization is restricted by its low protein and high fiber content. In Indonesia, the total production is 1.2 million ton dried/year (Tabrani *et al*., 2002). The tapioca by-products contained 14.27% crude fiber cellulose 2.57 crude protein. Wiharto (1986) stated that chicken's digestive tolerance toward crude fiber was very low, while the limit of crude fiber content in broiler chicken feed was 2-5%. To overcome this problem, it is important to reduce crude fiber content and increase other nutritional values. Many kinds of processing method on high-fiber animal feed, such as physical, chemical, biological and fermentation process, have been carried out to improve its efficiency. Wizna *et al.* (2007) stated that the cellulase activity of *Bacillus amyloliquefaciens* was 1.200 unit ml^{-1} to C1(b-exoglucanase) and 0.488 unit ml-1 to Cx (b-endoglucanase). The bacteria were isolated from the litter of swampy forest in Pesisir Selatan Indonesian. Optimal condition for fermentation of sago pith and rumen content mixture with *Bacillus amyloliquefaciens* was obtained at 2% inoculum dose, 9 day fermentation time and 40°C fermentation temperature. This fermentation process was able to reduce crude fiber content by 33% and increase crude protein content by 42% (Wizna *et al*., 2008). *Bacillus amyloliquefaciens* has been known to produce many kinds of enzymes e.g. alpha-amylase, alpha-acetolactate, decarboxylase, beta-glucanase, hemicellulase, maltogenic amylase, protease and xylanase that have been produced commercially (Luizmera.com, 2005). These enzymes are expected to be able to transform complex molecules particularly lignocelluloses, which become the limiting factor in animal feed, into simpler molecule components.

MATERIALS AND METHODS

The method for inoculum preparation and fermentation of tapioca by-products (onggok) referred to the processing method of probiotics made from the yeast *Saccharomyces cerevisiae* according to Fardiaz (1987). The tapioca by-products was fermented using *Bacillus amyloliquefaciens* as inoculum to check optimal fermentation condition. Inoculum dose, fermentation time and temperature were selected to obtain optimal condition of *Bacillus amyloliquefaciens* during fermentation so that maximum cellulase could be produced in order to lower the substrate's fiber content maximally.

Research methods: Completely randomized experimental design was chosen for this research with 3 x 3 x 3 factorial design and two replications. Treatment factor I had three levels of inoculum dose (2, 6, 10%), factor II three levels of fermentation time (3, 6, 9 days) and factor III three levels of fermentation temperature (30, 40, 50°C).

The data were subjected to the analysis of variance of factorial experiment under completely randomized design (Steel and Torrie, 1989). The differences of treatments were tested by Duncan's Multiple Range Test (DMRT).

The fermentation product were measured for dry-substance content, crude protein content and crude fiber content (AOAC, 1984); amino acid content (Nur *et al*., 1992) and determination of metabolic energy and protein quality (Sibbald, 1975).

RESULTS

The effect of inoculum dose, fermentation time and temperature on crude protein content of fermented sago pith and rumen content mixture: Statistical analysis showed very significant difference ($p<0.01$) in the effects of interactions between inoculum dose, fermentation time and fermentation temperature toward the dry-substance content of fermented tapioca by-products (onggok). The data are shown in Table The average interaction between inoculum dose, fermentation time and temperature toward the average value of crude protein content of fermented tapioca by-products (onggok) is shown in Table 1.

Table 1: The average value of crude protein content of fermented tapioca by-products (onggok) by *Bacillus amyloliquefaciens* at the interaction between inoculum dose, fermentation temperature and time (% DM)*

Dose (%)	Time (day)	Temperature (°C) 30	40	50
2	3	4.11^{aB}	4.44^{aC}	4.10^{aA}
	6	5.57^{bcB}	5.54^{bB}	5.27^{aA}
	9	5.07^{cdB}	6.53^{bC}	5.84^{cA}
6	3	5.86^{bB}	5.50^{cB}	5.65^{abA}
	6	6.07^{cdB}	7.14^{cdC}	5.96^{bcA}
	9	7.25^{eB}	7.69^{dC}	6.48^{fA}
10	3	6.72^{dB}	7.64^{cB}	7.07^{cA}
	6	8.31^{eB}	8.49^{cC}	7.31^{deA}
	9	8.70^{fB}	9.62^{dC}	7.68^{fA}
SE		0.52		

Different superscripted capital letter on the same row and different superscripted lower case letter on the same column indicated highly significant ($p<0.01$). *Initial crude protein content 2.57%

The effect of inoculum dose, fermentation time and temperature on crude fiber content of fermented sago pith and rumen content mixture: The average interaction between inoculum dose, fermentation time and temperature toward the average value of crude fiber content of fermented tapioca by-products (onggok) is shown in Table 2.

Table 2: The average value of crude fiber content of fermented tapioca by-products (onggok) by *Bacillus amyloliquefaciens* at the interaction between inoculum dose, fermentation temperature and time (% DM)*

Dose (%)	Time (day)	Temperature (°C) 30	40	50
2	3	13.87^{aA}	13.71^{aA}	13.96^{aA}
	6	13.58^{aA}	11.40^{bB}	13.61^{bcA}
	9	13.52^{aA}	11.25^{bB}	13.70^{abcA}
6	3	13.75^{aB}	13.49^{aC}	14.02^{aA}
	6	11.44^{bB}	10.34^{cC}	12.47^{dA}
	9	11.33^{bB}	10.15^{cC}	11.76^{eA}
10	3	13.66^{aA}	13.23^{abB}	13.41^{cAB}
	6	11.24^{bB}	10.10^{cC}	12.16^{dA}
	9	11.20^{bA}	10.01^{cB}	11.57^{eA}
SE		0.39		

Different superscripted capital letter on the same row and different superscripted lower case letter on the same column indicated highly significant ($p<0.01$). *Initial crude fiber content 14.22%

Metabolic energy and nitrogen retention of fermented sago pith and rumen content mixture: The amount of metabolic energy and nitrogen retention of fermented tapioca by-products (onggok) by *Bacillus amyloliquefaciens* can be seen in Table 3.

DISCUSSION

Table 1 indicated that at 2% dose, increased crude protein content was in line with increased fermentation time at 40°C. This happened because during that time the microbes were in rapid growth phase. After that microbes entered stationary phase in which growth rate was decreasing because less nutrients were available and also there was accumulation of metabolic substances that slowed down the growth. Besides, there was enzyme as secondary product formed after stationary phase that explained enzymatic activity after this phase. Afterward, the growth rate would decrease again until its value equaled with zero (the number of new cells produced equaled the number of dead cells) and eventually the number of living cells would decrease because of lysis and cell mass would continue decreasing (Wang *et al*., 1979). In stationary phase, some of the microbes were already found dead (Hou *et al*., 2004). Based on DMRT, the highest crude protein content after fermentation was 7.14%, obtained at the treatment combination of 2% dose, 6 day fermentation time and 40°C temperature. Maximum microbe growth at that interaction was caused by suitable condition especially the density of substrate and nutrient (Standbury and Whitaker, 1984). High microbe population resulted in high crude protein content because microbes mostly consist of protein. Crueger

Table 3: The amount of metabolic energy and nitrogen retention of fermented tapioca by-products (onggok) before and after fermentation by *Bacillus amyloliquefaciens* (%)

Replications	Nitrogen retention before fermentation (%)	Nitrogen retention after fermentation (%)	Metabolic energy before fermentation (kcal/kg)	Metabolic energy after fermentation (kcal/kg)
1	55.83	66.82	1765	2178
2	54.78	65.44	1693	2182
3	54.95	65.32	1864	2154
4	56.07	66.45	1762	2144
5	54.58	65.79	1801	2164
6	55.98	66.97	1752	2222
7	55.37	64.98	1707	2098
8	56.52	65.78	1744	2078
Average	55.51	65.95	1798	2190

and Crueger (1984) reported that protein content of different kinds of microbes varied, bacteria contained 70-78% protein. Moreover, fermentation process can be seen as protein enrichment process using certain kind of microorganism. Protein enrichment process was identical with the making of Single Cell Protein, but in protein enrichment microbe cells were not separated from the remaining substrate.

DMRT test showed that the treatment of 2% inoculum dose, 6 day fermentation time and 40°C temperature produced the lowest crude fiber content. The decrease of crude fiber content could happen because the longer available time, the more chances for the inoculum to work in fermentation process and the more substrate being degraded. Inoculum dose had not influenced crude fiber content, probably because high dose did not equal with maximum microbe growth. It could be caused by nutrient imbalance in the substrate. High inoculum dose logically should accelerate fermentation process because a large amount of inoculum would produce a large number of microbes, which consequently would produce more enzymes to degrade the substrate faster. This was in accordance with Sulaiman (1988) who stated that the higher inoculum dose used and the longer fermentation time, the faster fermentation process would be and therefore more substrate being degraded. However, high inoculum dose or high inoculum density made the inoculum difficult to germinate perfectly which in turn caused the death of the microbes. In line with Raimbault and Alazard (1980) who reported that the optimal dose for *Aspergillus niger* to grow on cassava flour as substrate was 10^6-10^7 spores/gram substrate, while 10^8 spores/gram substrate would result in decreased inoculum growth and after microscopic observation, it was shown that some of the spores had failed to grow. Table 2 showed that at the treatment of 2% dose, fermentation time and 40°C temperature, there was significant decrease of crude fiber content, while at other treatments the interaction between inoculum dose and fermentation temperature did not show significant decrease of crude fiber content. This could happen because the longer fermentation time at ideal temperature, the more chances for the inoculum to work in fermentation process and the more substrate being degraded; consequently the crude fiber content of fermented tapioca by-products (onggok) was reduced. Optimal temperature for cellulase activity of *Sorangium* on cellulose medium was 40°C (Hou *et al*., 2004). The treatments of 6 and 9 day fermentation time at each dose treatment (2, 6 and 10%) did not produce significant decrease in crude fiber content, because at that time *Bacillus* sp. had just entered stationary phase. The decline of crude fiber content was influenced more by fermentation time, while the effect of inoculum dose did not show significant difference. However, in general the increase of inoculum dose and fermentation time can decrease the crude fiber content of fermented tapioca by-products (onggok). Furthermore, it was assumed that the substrate contained more amorphous cellulose than crystalline cellulose, so there were time difference in producing Cx and C_1 cellulase. Chahal *et al*. (1992) reported that cellulase production profile was determined by the components of substrate cellulose (amorphous and crystalline). Amorphous part was consumed during the first exponential phase, while crystalline part was consumed during the second exponential phase. Wizna and Rizal (2003) observed that there were two exponential phases in cellulase production by *Bacillus amyloliquefaciens*, the first exponential phase occurred on the third day, with Cx specific activity 15.79 U/mg and the second exponential phase on the eighth day with highest C_1 specific activity 20.58U/mg. Damude *et al*. (1996) reported that cellulose-degrading microbes generally secreted several different cellulase enzymes that reacted in synergy when hydrolyzing substrate.

The average of nitrogen retention of fermented tapioca by-products (onggok) was 65.95%. This number was bigger than the average before fermentation i.e. 55.51%. Nitrogen retention average after fermentation was a little lower than nitrogen retention of adult broiler i.e. 67% as reported by Scott *et al*. (1992). This might be caused by the presence of nucleic acid which was part of microbe's protein in the substrate that could not be utilized by poultry. Young and Scrimshaw (1975) stated that in maintaining microorganism's nutritional value, besides protein content it was also important to pay attention to

essential amino acid content, digestibility and other factors like antinutritional factor. Hariyum (1986) reported that fungi had lower protein content, but its nucleic acid content was only 5% compared to that of bacteria and yeasts. Nucleic acid content of bacteria and yeasts were 8-16% and 6-12% respectively. Nucleic acid could be poisonous if consumed continuously.

The average of metabolic energy of tapioca by-products (onggok) fermented by *Bacillus amyloliquefaciens* was 2190 kcal/kg. This number was bigger than metabolic energy before fermentation i.e. 1798 kcal/kg and t-test determined that the difference of energy before and after fermentation was significantly different. This happened because of increased glucose content which was the hydrolysis product of cellulose from tapioca by-products (onggok) by the cellulase of *Bacillus amyloliquefaciens* during fermentation and later the glucose was counted as metabolic energy. In accordance with Geharzt (1990) who stated that cellulase was actually an enzyme complex that worked gradually or simultaneously breaking down cellulose into glucose unit. Wizna *et al.* (2008) added that fermentation of sago pith and rumen content mixture by *Bacillus amyloliquefaciens* was able to reduce crude fiber content by 33% with the treatment of 2% inoculum dose, 9 day fermentation time and temperature 40°C.

Conclusion: Optimal condition for fermentation of tapioca by-products (onggok) was obtained at 2% inoculum dose, 6 day fermentation time and 40°C fermentation temperature. This fermentation process was able to reduce crude fiber content by 32% and increase crude protein by 360%, which made the nutritional value of the product based on dry-substance was 7.9% crude protein, 2.75% crude fat, 11.55% crude fiber, 0.26% calcium, 0.17% phosphor, 2190 Kcal/kg metabolic energy and 65.95% nitrogen retention.

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