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Improving the Quality of Sago Pith and Rumen Content Mixture as Poultry Feed Through Fermentation by *Bacillus amyloliquefaciens*

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Abstract: An experiment was conducted to improve the nutrient content of sago pith (*Metroxylon sago* Rottb) and rumen content mixture 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 sago pith and rumen content mixture 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 and C3 = 50°C). Results of study showed that optimum conditions of the fermentation of sago pith (*Metroxylon sago* Rottb) and rumen content mixture was at 2% dosage of inoculums, 9 days of fermentation length and 40°C temperature. This conditions can decrease 33% of crude fiber and increase 42% of crude protein which made the nutritional value of the product based on dry-substance was 15.79% crude protein, 2.75% crude fat, 18.54% crude fiber, 0.20% calcium, 0.16% phosphor, 2540 Kcal/kg metabolic energy, and 66.65% nitrogen retention.

Key words: Fermentation, sago pith (*Metroxylon sago* Rottb), rumen content and *Bacillus amyloliquefaciens*

Introduction

Plants are the main ingredients in poultry feed, particularly in the forms of grains and roots. In Indonesia, the use of these materials as ingredients for poultry feed is limited because it competes with human needs. The use of agricultural and agro-industrial wastes is one of the alternatives to overcome this problem. However, the use of these wastes in poultry feed is also limited because of their fibrous components. Poultry can not digest fiber because it does not have the cellulase to degrade it.

The mixture of sago pith and rumen content is one of the potential alternatives for poultry feed, but its utilization is restricted by its high fiber content. Rasyid *et al.* (1981) reported that an addition of 10% rumen content to broiler chicken feed did not affect its performance. Wizna (1997) reported that sago pith could substitute 75% corn in duck feed during egg-laying period without disturbing egg production, even though the egg yolk appeared to be pale yellow. Sago pith and rumen content was mixed together to improve the nutrients in sago pith needed by the microbe during fermentation.

Xanthophyll of rumen content (mainly consists of greens) is expected to fulfill the needs for egg yolk pigment or poultry skin pigment when the poultry consume the mixture of sago pith and rumen content as part of their feed. Anggorodi (1995) reported that xanthophyll content of yellow corn is 15 mg/kg, grass flour 200 mg/kg and alfalfa flour 150 mg/kg. Another advantage of using rumen content as feedstuff is its vitamin B_{12} as “animal protein factor” that elevated its biological value to the same level as fish flour and liver extract (Scott *et al.,* 1992).

Ozawa *et al.* (1996) stated that sago pith contained 58% starch, 23% cellulose, 9.2% hemicelluloses, 5.8% pectin and 3.9% lignin. Abbas (1987) reported the nutritional value of cow’s rumen content as follows: 9.29% water, 8.45% crude protein, 1.23% crude fat, 33.53% crude fiber; 0.20% Ca; 0.45% P; 16.19% ash and 31.60% NFE. The data showed that sago pith and rumen content mixture had sufficient nutritional value, only the fiber content was quite high.

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.

Sago pith is the inside part of sago palm trunk whose outer layer has been chopped away (Muller, 1976). In Indonesia, the utilization of sago for consumption, domestic industry and export was only 4-5% (210,000 ton/year) from the total production of 5-8.5 million ton dried sago flour/year with the total area around 740,000 ha (Haryanto and Pangloli, 1992).

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. Fermentation of cassava waste using *Bacillus amyloliquefaciens* as inoculum can increase the crude protein content by 360% and decrease the crude fiber content by 32% (Wizna *et al.*, 2005). *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 (Luzmeira.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 sago pith and rumen content mixture (70:30%) referred to the processing method of probiotics made from the yeast *Saccharomyces cerevisiae* according to Fardiaz (1987).

The mixture of sago pith and rumen content (70:30) 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.

Equipments and materials: Laminar air flow, test tubes with butyl rubber stoppers, tube rack. Magnetic mixer, Petri dishes, Bunsen lamp, vortex. Erlenmeyer 250 and 1000 ml, measuring glass 100 and 250 ml, incubator, oven, analytical balance, autoclave, fermentor (incubator), thermometer. *Bacillus amyloliquefaciens* inoculum, mixed medium of sago pith and rumen content (70:30) andaquadest.

Research methods: Completely randomized experimental design was chosen for this research with 3x3x3 factorial design and two replications. Treatment factor I had three levels of inoculum dose (2, 6 and 10%) factor II three levels of fermentation time (3, 6 and 9 days) and factor III three levels of fermentation temperature (30, 40 and 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 dry substance 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 sago pith and rumen content mixture. The data are shown in Table 1.

The effect of inoculum dose, fermentation time and temperature on crude protein 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 protein content of fermented sago pith and rumen content mixture is shown in Table 2.

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 sago pith and rumen content mixture is shown in Table 3.

Amino acid content, metabolic energy and nitrogen retention of fermented sago pith and rumen content mixture: Amino acid content of fermented sago pith and rumen content mixture before and after the fermentation can be seen in Table 4.

The amount of metabolic energy of fermented sago pith and rumen content mixture by *Bacillus amyloliquefaciens* can be seen in Table 5.

Discussion

Table 1 showed that longer fermentation time starting from 3 days, 6 days and 9 days, at 40°C and 2%

Table 1: The average value of dry matter content of fermented sago pith and rumen content mixture by Bacillus amyloliquefaciens at the interaction between inoculum dose, fermentation time and fermentation temperature(%)*.

Dose (%)	Time (day)	Temperature (°C) 30	40	50
2	3	32.10^{aA}	31.10^{abA}	31.60^{bA}
	6	32.10^{aA}	30.42^{abB}	31.33^{bA}
	9	30.83^{bB}	27.52^{cC}	32.10^{bA}
6	3	32.80^{aB}	30.16^{bC}	34.20^{aA}
	6	32.45^{aB}	30.41^{abC}	34.15^{aA}
	9	32.28^{aB}	30.33^{abC}	34.55^{aA}
10	3	32.66^{aB}	31.27^{abC}	34.20^{aA}
	6	31.10^{bB}	31.32^{aB}	34.15^{aA}
	9	31.05^{bB}	30.96^{abB}	34.55^{aA}
SE		0.34		

Different superscripted capital letter on the same row and different superscripted lower case letter on the same column indicated highly significant (P<0.01).

Table 2: The average value of crude protein content of fermented sago pith and rumen content mixture by Bacillus amyloliquefaciens at the interaction between inoculum dose, fermentation temperature and time (% DM)*.

Dose (%)	Time (day)	Temperature (°C) 30	40	50
2	3	12.13^{bcA}	12.66^{cA}	12.19^{bA}
	6	13.35^{bB}	14.76^{bA}	13.74^{aB}
	9	12.90^{bB}	16.37^{aA}	12.05^{bC}
6	3	11.01^{cdC}	13.01^{dA}	11.88^{bB}
	6	11.92^{bcA}	10.79^{dB}	11.16^{bcB}
	9	14.96^{aA}	14.10^{bB}	11.46^{bC}
10	3	13.28^{bA}	11.26^{dB}	10.88^{bcC}
	6	10.84^{cdA}	10.74^{dA}	10.49^{cA}
	9	10.93^{cdA}	10.67^{dA}	10.76^{bcA}
SE		0.45		

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 9,11%.

Inoculum dose produced lower dry-substance content. DMRT results indicated that the treatment of 2% inoculum dose and 9 day fermentation time resulted in the lowest dry-substance content, i.e. 27.52%. The decrease of dry substance content happened because with 2% dose there was enough available space for microbe development as inoculum.

Treveleyan (1974) reported that cassava fermentation by *Rhizopus oryzae* with inoculum concentration of 10^6 spores/gram substrate produced 20% more protein content compared to that of 10^7 spores/gram substrate. Longer time meant more chances for microbes to grow and produce useful enzymes to degrade crude fiber component found in the substrate. The end product of crude fiber degradation in the form of glucose was used by microbe as energy source which in turn reduced the dry-substance content of the substrate. This result was in line with Fardiaz (1992) who stated that microorganism utilized carbohydrate as energy source after degrading it into glucose. Glucose degradation continued until energy, water molecule and carbon dioxide were produced at the end.

When fermentation temperature 40°C interacted with 2% dose or 9 day fermentation time, it produced optimal condition for microbe growth; therefore it also produced maximum cellulase activity. The temperature 40°C was the same with optimal temperature for cellulase activity produced by *Sorangium* on cellulose medium (Hou *et al*., 2004). The temperatures 30°C and 50°C were not suitable for cellulase activity produced by the microbes. Winarno *et al*. (1982) reported that the higher temperature, the higher reaction rate with enzyme as catalyst. Almost all enzyme have optimal activity at 30 - 40°C. At higher temperature, protein structure which holds the key to cell metabolism and growth could be damaged. At very low temperature, cell metabolism activity would decrease rapidly resulted in lower metabolism product.

Table 1 showed that the treatment of inoculum dose at 6% (A2) and 10% (A3); and temperature at 30°C and 50°C did not influence the dry-substance content of sago pith and rumen content mixture. This happened because too high inoculum dose would decrease space available for the inoculum, so its growth and metabolism would be hampered. Raimbault and Alazard (1980) reported that optimal dose for the growth of *Aspergillus niger* on cassava flour as substrate was 10^6-10^7 spores/gram substrate, while at 10^8 spores/gram substrate inoculum growth was decreasing. Microscopic observation showed that some of the spores failed to grow.

Based on DMRT, the highest crude protein content after fermentation was 16.37%, obtained at the treatment combination of 2% dose, 9 day fermentation time and40°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 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.

Table 3: The average value of crude fiber content of fermented sago pith and rumen content mixture by Bacillus amyloliquefaciens at the interaction between inoculum dose, fermentation temperature and time (% DM)*

Dose (%)	Time (day)	Temperature (°C) 30	40	50
2	3	20.43^{bA}	19.87^{aA}	20.35^{cA}
	6	20.81^{abA}	19.63^{abB}	20.68^{bcA}
	9	18.54^{cB}	16.56^{dC}	20.54^{bcA}
6	3	18.66^{cB}	18.73^{abcB}	20.34^{cA}
	6	20.08^{bB}	18.61^{bcC}	20.86^{bA}
	9	19.71^{bA}	18.21^{cB}	20.24^{cA}
10	3	20.34^{bB}	19.55^{abB}	22.44^{aA}
	6	21.57^{aA}	19.58^{abB}	21.67^{bcA}
	9	20.08^{bB}	18.55^{bcC}	22.12^{abA}
SE		0.36		

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 23.73%.

Table 4: Amino acid content of fermented sago pith and rumen content mixture before and after fermentation by Bacillus amyloliquefaciens (%).

Amino acid component	Fermentation Before	Fermentation After
Non Essential		
Aspartic acid	0.19	0.36
Tyrosine	0.09	0.16
Serine	0.12	0.22
Glutamic	0.08	0.39
Proline	-	0.16
Glycine	0.17	0.21
Alanine	0.17	0.19
Cystine	-	-
Essential		
Valine	0.19	0.24
Methionine	0.09	0.11
Isoleucine	0.13	0.25
Leucine	0.16	0.29
Arginine	0.43	1.14
Phenilalanine	0.18	0.23
Histidine	0.08	0.09
Lysine	0.15	0.22
Threonine	0.11	0.17
Total	2.42	4.43

Table 2 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).

DMRT test showed that the treatment of 2% inoculum dose, 9 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 3 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 sago pith and rumen content mixture 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

Table 5: The amount of metabolic energy and nitrogen retention of fermented sago pith and rumen content mixture 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	59.85	66.88	2024	2634
2	56.78	66.99	1993	2543
3	60.47	67.81	2164	2654
4	61.12	67.41	2064	2544
5	59.52	67.71	2001	2461
6	58.98	66.29	2052	2622
7	60.37	66.32	2008	2598
8	59.85	67.78	2048	2478
Average	59.61	66.65	2008	2540

the increase of inoculum dose and fermentation time can decrease the crude fiber content of fermented sago pith and rumen content mixture. 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 amino acid content in fermented sago pith and rumen content mixture before and after fermentation on Table 4 indicated that there had been increases on most of the amino acid contents (83%) after fermentation by *Bacillus amyloliquefaciens*. For example, while there were no increase in histidine content, proline was synthesized during fermentation. This showed that *Bacillus amyloliquefaciens* reproduced during fermentation process and the cell as reproduction result was in the form of protein which would be counted later as amino acids. Ammonia content of fermented sago pith and rumen content mixture decreased after fermentation from 0.08% to 0.05%.

The average of nitrogen retention of fermented mixture of sago pith and rumen content mixture was 66.65%. This number was bigger than the average before fermentation i.e. 59.61%. 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. Suhartono (1989) 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 sago pith and rumen content mixture fermented by *Bacillus amyloliquefaciens* was 2540 kcal/kg. This number was bigger than metabolic energy before fermentation i.e. 2008 kcal/kg andt-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 sago pith and rumen content mixture 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.* (2005) added that fermentation of cassava waste by *Bacillus amyloliquefaciens* was able to reduce crude fiber content by 48% with the treatment of 2% inoculum dose, 6 day fermentation time and temperature 40°C.

Conclusion: Optimal condition for fermentation of sago pith and rumen content mixture 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 by 42%, which made the

nutritional value of the fermented product based on dry matter as follows: 15.79% crude protein, 2.75% crude fat, 18.54% crude fiber, 0.20% Ca, 0.16% P, ME 2540 kcal/kg and 66.65% nitrogen retention.

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