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The Effect of Supplementation of Micro Nutrient on Nutrient Rice Bran Which Fermented by *Bacillus amyloliquefaciens*

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Abstract: An experiment was conducted to improve the nutrient content of rice bran which supplemented with Zn, Sulfur and urea through fermentation by using cellulolytic bacteria (*Bacillus amyloliquefaciens*) as inoculums. The objective of this experiment was to study the effect of supplementation of catalytic substrate consisting of Zn, sulfur and urea on the substrata characteristics and its microbial population.The experiment was determination of the optimum conditions (dosage of Zn, Sulfur and urea) for rice bran 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 treatments were : 1) A factor (Dose of urea: A1 = 1.0%, A2 = 1.5%, A3 = 2.0%), 2) B factor (Dose sulfur: B1 = 0.2%, B2 = 0.4%, B3 = 0.8%) and 3) C factor (Dose Zn: C1 = 0.0025%, C2 = 0.005%, C3 = 0.0075%). Results of study showed that optimum conditions of the fermentation of rice brand was at 2% urea, 0.0025% Zn and 0.2% sulfur. The protein level increased 100% and the mineral content 60%, accompanied by 8.2% of increase in the digestibility. The level of phytic acid decreased 97%. This conditions can increase 36% of metabolizable energy, 15% nitrogen retention, 98% crude fiber digestible.

Key words: Fermentation, rice bran, sulfur, Zn, urea, *Bacillus amyloliquefaciens*

INTRODUCTION

Rice bran was available paddy industry by product and has been used as poultry feed. In Indonesia rice bran produced about 50.7 million ton/year and consist of a 8-10% of total paddy weight. The amount of rice bran as poultry feed was limited due to high crude fiber, low protein content and containing anti nutrition phytate which affected to P and Ca availability (Luh, 1991). In term of increasing rice bran proportion on poultry feed, it was need increasing of nutrient content and availability through pre-treatment to decrease phytic acid and crude fiber and increase 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%.

Many kinds of processing method on animal feedstuff such as physical, chemical, biological and fermentation process, have been carried out to improve its efficiency. Fermentation process was treatment method beside as optimal food/feed storage also could increase nutrient content. Winarno *et al.* (1982) found that fermented food containing higher nutrient compared to a raw material. According to Pasaribu *et al.* (1998), substrate fermentation using *Aspergillus niger* could be use to increase agricultural by product, which a high crude fiber, low protein and digestibility. A *Aspergillus niger* produced protease, which increasing diluted protein by breaking disulfide bond of protein molecule chain. 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.*, 2009). *Bacillus amyloliquefaciens* has been known to produce many kinds of enzymes e.g. alpha-amylase, alpha-acetolactate, decarboxylase, beta-glucanase, hemicellulose, maltogenic amylase, protease and xylanase that have been produced commercially (Luizmera.com, 2005). *Bacillus amyloliquefaciens* can used produce phytase enzyme (Kim *et al.*, 1998). 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. This work aims to select biological treatments and conditions for the bioconversion of rice bran which supplemented with Zn, Sulfur and urea by *Bacillus amyloliquefaciens*, either individually or sequentially, into an enriched substrate with increased digestibility for use as nonruminant feed. The addition of nutrient into the fermentation media can be support and stimulate the growth of microorganisms. Urea or carbamide is an organic compound with the chemical formula $(NH_2)_2CO$. The molecule has two amine groups joined by a carbonyl functional group. Urea serves an important role in the metabolism of nitrogen-containing compounds by animals.

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MATERIALS AND METHODS

The method for inoculum preparation and fermentation of rice bran referred to the processing method of probiotics made from the yeast *Saccharomyces cerevisiae* according to Fardiaz (1987).

The mixture of rice bran which supplemented with Zn, Sulfur and urea was fermented using *Bacillus amyloliquefaciens* as inoculum to check optimal fermentation condition. Doses of Zn, Sulfur and urea were selected to obtain optimal condition of *Bacillus amyloliquefaciens* during fermentation so that maximum population of *Bacillus amyloliquefaciens* could be produced in order to higher the substrate's protein 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, medium of rice bran and micro nutrient of Zn, Sulfur and urea.

Research methods: Completely randomized experimental design was chosen for this research with 3 x 3 x 3 factorial design and three replications. Treatment factor I had three levels of urea dose (1.00, 1.50, 20%) factor II three levels of sulfur dose (0.20, 0.40, 0.80%) and factor III three levels of Zn (0.0025, 0.005, 0.0075%).

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, Calcium, Phosphor and crude fiber content (AOAC, 1984); determination of metabolic energy (Sibbald, 1976) protein quality, digestible of crude fiber, calcium and phosphor.

RESULTS

The effect of urea, sulfur and Zn dose supplementation on dry substance content of fermented rice bran: Statistical analysis showed very significant difference ($p<0.01$) in the effects of interactions between sulfur dose and Zn dose toward the dry-substance content of fermented rice bran. The data are shown in Table 1.

The effect of urea, sulfur and Zn dose supplementation on crude protein content of fermented rice bran: The average interaction between urea dose and Zn dose toward the average value of crude protein content of fermented rice bran is shown in Table 2.

The effect of urea, sulfur and Zn dose supplementation on crude fiber content of fermented rice bran: The average interaction between urea, sulfur and Zn dose toward the average value of crude fiber content of fermented rice bran is shown in Table 3.

The effect of urea, sulfur and Zn dose supplementation on calcium content of fermented rice bran: The average interaction between urea dose and Zn dose toward the average value of calcium content of fermented rice bran is shown in Table 4.

The effect of urea, sulfur and Zn dose supplementation on phosphor content of fermented rice bran: The average interaction between urea dose and Zn dose

Table 1: The average value of dry matter content of fermented rice bran by *Bacillus amyloliquefaciens* at the interaction between sulfur dose and Zn (%)*

	C (Zn)			
Factor B (Sulfur)	C1 (0.0025%)	C2 (0.0050%)	C3 (0.0075%)	Average
B1 (0.20%)	16.11^{a}	13.91^{b}	16.32^{a}	15.45
B2 (0.40%)	16.77^{a}	16.09^{a}	16.74^{a}	16.53
B3 (0.80%)	13.94^{b}	16.36^{a}	14.55^{a}	14.95
Average	01.06	15.45	15.87	SE = 0.73

Different superscript indicated very significant effect ($p<0.01$)

Table 2: The average value of protein content of fermented rice bran by *Bacillus amyloliquefaciens* at the interaction between urea dose and Zn (%)*

	C (Zn)				
Faktor A (Urea)	C1 (0.0025%)	C2 (0.0050%)	C3 (0.0075%)	Total	Average
A1 (1.00%)	18.46^{e}	15.59^{g}	19.98^{d}	54.03	18.01
A2 (1.50%)	20.75^{cd}	17.49^{f}	21.08^{bc}	59.32	19.77
A3 (2.00%)	22.62^{a}	21.41^{bc}	21.79^{ab}	65.82	21.94
Total	61.83	54.49	62.85	179.17	-
Average	20.61	18.16	20.95	-	-
SE	0.3				

Different superscript indicated very significant effect ($p<0.01$). *Initial crude protein content 12.11%

Table 3: The average value of crude fiber content of fermented rice bran by *Bacillus amyloliquefaciens* at the interaction between urea and Zn (%)*

Factor	A (Urea) A1 (1.00) Sulfur B1 (0.2)	A1 (1.00) Sulfur B2 (0.4)	A1 (1.00) Sulfur B3 (0.8)	A2 (1.50) Sulfur B1 (0.2)	A2 (1.50) Sulfur B2 (0.4)	A2 (1.50) Sulfur B3 (0.8)	A3 (2.00) Sulfur B1 (0.2)	A3 (2.00) Sulfur B2 (0.4)	A3 (2.00) Sulfur B3 (0.8)
C (Zn)									
C1 (0.0025)	7.68	7.74	8.32	7.20	8.84	8.26	8.14	7.91	7.62
C2 (0.0050)	6.56	7.76	7.97	8.14	7.80	8.67	7.34	7.64	7.97
C3 (0.0075)	7.22	7.81	7.69	8.31	7.78	7.94	7.73	7.88	7.89

*Initial crude fiber content 12.10%

Table 4: The average value of calcium content of fermented rice bran by *Bacillus amyloliquefaciens* at the interaction between sulfur dose and Zn (%)*

Factor B (Urea)	A (Zn) A1 (0.0025%)	A2 (0.0050%)	A3 (0.0075%)	Average
B1 (1.00%)	0.32	0.25	0.33	0.30[a]
B2 (1.50%)	0.26	0.24	0.27	0.26[b]
B3 (2.00%)	0.29	0.26	0.29	0.28[a]
Average	0.29[a]	0.25[b]	0.30[a]	
SE	0.016			

Different superscript indicated very significant effect ($p<0.01$). *Initial calcium content 0.12%

Table 5: The average value of phosphor content of fermented rice bran by *Bacillus amyloliquefaciens* at the interaction between sulfur dose and Zn (%)*

Factor B (Sulfur)	C (Zn) C1 (0.0025%)	C2 (0.0050%)	C3 (0.0075%)	Average
B1 (0.20%)	1.22[a]	1.19[ab]	1.14[ab]	1.12
B2 (0.40%)	1.12[ab]	1.01[b]	1.16[ab]	1.16
B3 (0.80%)	1.05[b]	0.99[b]	1.15[ab]	1.06
Average	1.13	1.06	1.15	
SE	0.066			

Different superscript indicated very significant effect ($p<0.01$). *Initial phosphor content 0.21%

toward the average value of p content phosphor of fermented rice bran is shown in Table 5.

Metabolic Energy (ME) and Nitrogen Retention (NR) and Crude Fiber (CF), Calcium (Ca) and Phosphor (P) digestible of before and after the fermentation rice bran: Metabolic energy and nitrogen retention and crude fiber, calcium and phosphor digestible of fermented rice bran before and after the fermentation can be seen in Table 6.

DISCUSSION

The research analysis showed that fermentation process caused a rice bran nutrient change, as presented at Table 1. The organic matter fermented rice bran decrease with a incubation. The dry matter of fermented rice bran was decrease ($p<0.01$) among Zn 0.0025% and 0.4% sulfur combination treatments which is 16.77%. The decreasing of dry matter caused by metabolic processes during fermentation process. Supriyati *et al.* (1998) stated that increasing of substrate temperature due to breakdown of carbohydrate releasing water from substrate. The organic matter decreasing due to the organic matter used as energy sources by *B. amyloliquefaciens* cells (Wizna *et al.*, 2007). The microbe need a media containing water and organic matter as carbon, nitrogen and ion organic sources, which taken from substrate organic matter, because of *B. amyloliquefaciens* was saprophyte microorganism does not produce a food for himself from CO_2 and water. According to Rahman (1992), a substrate organic matter used by microbe for cell biosynthesis and activation energy during molecule transport, maintaining cell structure and cell mobility.

The crude protein content of fermented rice bran was increase than a non treatment rice bran. The analysis of variance showed that different crude protein among combination treatments ($p<0.01$). The crude protein of fermented rice bran was increase ($p<0.01$) among Zn 0.0025% and 2.00% urea combination treatments which is 22.62%. According to Shin (1988), protein content of fermented substrate was affected by protein content of raw material. Beside that, nitrogen of urea was a crucial component needed by *B. amyloliquefaciens* after carbon and oxygen. A high crude protein content of substrata caused cell synthesis process running fast due to fast adaptation time, which need a much amino acid.

Table 3 showed that the combination treatments did not influence the crud fiber content of rice bran. This happened because too short fermentation time to hydrolyzed (24 h), so its metabolism not happening yet. Wizna *et al.* (2009) stated that optimal condition for the growth of *B. amyloliquefaciens* on sago pith and rumen content mixture as substrate was obtained at 2% inoculum dose, 9 day fermentation time and 40°C fermentation temperature.

The research analysis showed that fermentation process caused a rice bran calcium and phosphor change, as presented at Table 4 and 5. Calcium and phosphor content of fermented rice bran was increase than a non treatment rice bran. The analysis of variance

Table 6: The average value of metabolic energy, nitrogen retention, crude fiber, calcium and phosphor digestible of rice bran before and after the fermentation by *Bacillus amyloliquefaciens* at the interaction between sulfur dose and Zn (%)*

	Before fermentation					After fermentation				
No.	ME	NR	CF	Ca	P	ME	NR	CF	Ca	P
1	1724	50.86	28.85	74.97	64.84	2434	60.88	56.88	81.83	94.93
2	1893	54.08	32.78	82.81	65.95	2443	61.99	56.99	89.07	89.07
3	1764	53.84	30.47	80.57	64.24	2354	62.81	67.81	85.86	91.66
4	1664	51.12	27.12	89.33	64.04	2344	63.41	57.41	92.29	87.46
5	1801	54.52	29.52	88.40	64.11	2461	60.71	57.71	92.73	88.85
6	1752	58.98	28.98	85.47	60.18	2422	60.29	56.29	92.87	86.54
7	1808	50.37	30.37	89.73	59.01	2398	61.32	62.32	92.73	86.10
8	1748	51.85	29.85	76.27	67.51	2478	61.78	57.78	91.71	86.44
9	1769	53.20	29.74	78.67	82.02	2417	61.52	59.15	92.73	90.58
10	1848	56.68	29.68	90.04	64.30	2413	61.16	57.32	95.03	82.17
Average	1777	53.55	29.74	83.63	65.62	2417	61.59	58.97	90.69	88.38

showed that different calcium and phosphor among combination treatments ($p<0.01$). The calcium and phosphor of fermented rice bran was increase ($p<0.01$), calcium among Zn 0.0025% and 1.00% urea combination treatments which is 0.32% and phosphor among Zn 0.0025% and 0.20% sulfur combination treatments which is 1.22%. Calcium and phosphor content of fermented substrate was affected by phytic acid content of raw material. During fermentation process organic molecule complex was breaking into a simple molecule and phytic acid become diluted and this could be increase of calcium and phosphor of available.

The research analysis showed that energy metabolism, nitrogen retention, crude fiber, calcium and phosphor digestible of fermented rice bran was increase than a non treatment rice bran, can be seen in Table 6. Fermentation process, the activity of phytase enzyme breaking phytic acid into increase diluted phytic. The increasing of these metabolism caused a higher calcium and phosphor will breaks and produce a simply calcium and phosphor. Martin *et al.* (1998) stated that during fermentation process *B. amyloliquefaciens* produced phytase which hydrolyze phytic acid of rice bran, then protein, calcium and phosphor trapped by phytic acid release and become diluted and easy to digest.

Conclusion: Fermentation process of rice bran with *B. amyloliquefaciens* caused a change of nutrient content. The fermentation to increase digestibility of crude protein, calcium, phosphor, but does not decrease crude fiber.

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