

Production of Probiotic Supplement Based on Agriculture Industrial Waste

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Production of Probiotic Supplement Based on Agriculture Industrial Waste

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Abstract: A study to produce good quality of probiotic supplement based on agriculture industrial waste was done. The study was conducted in three laboratories, namely Feed Technology Laboratory, Animal Feed Science Laboratory and Microbiology Laboratory, Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang. The processing of probiotic manufacture was done using Utama's *et al.* (2009) method. Fermentation of agriculture industrial waste process was become the next step after then. The evaluated parameters were the proximate components, total lactic acid bacteria, total *Saccaromyces cerevise*, gram positive/negative; *Salmonella* and *Coliform* according to the Atlas method (2005). The results showed that the treatment of the type of feed ingredients and the incubation period showed the significantly effect ($p>0.05$) and the components have interaction with proximate parameters such as moisture content, ash content, crude fat, crude protein, crude fiber and nitrogen free extract material. Furthermore, microbiological treatment suggests that career probiotics containing *Lactobacillus* sp., *Saccaromyces cereviseae* and *Rhizopus* sp. On the other hand, determination of *E. coli* and *Salmonella* have negative result. Based on the results of this study concluded that the best treatment on a polard carrier with 4 days incubation period has crude protein content of 16.09% and total lactic acid bacteria 3×10^4 .

Key words: Probiotics, fermentation, microorganism, *Lactobacillus*, *Saccaromyces*

INTRODUCTION

Agriculture industrial waste such as pollard, cassava and rice bran are now widely used by the farmers as a source of energy whether for animal feed ingredients. Pollard, cassava and rice bran are widely available because they are not for human consuming. Pollard is the rest milling of wheat that can be used as an animal feed which rich of protein, fat, mineral substances and vitamins compared to whole grains. Pollard also has polysaccharides structural in a significant amount. The structure of polysaccharides are composed by cellulose, hemicellulose, cellobiose, lignin and silica materials which suitable to be used as ruminant feed (Maynard and Loosli, 1973). Church and Pond (1980) stated that pollard has a bulky, laxative and palatable characteristics for the cows, but if it given in a large amounts (more than 40-50%) in the diet it can reduce the feed intake.

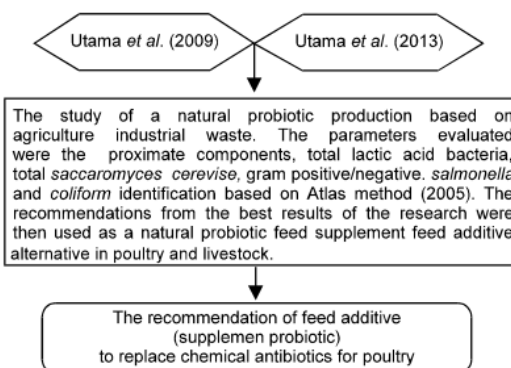
Rice bran has varies nutritional value depends on the origin of rice seeds, planting rice and ways of processing/machine used. *Onggok* or cassava starch processing waste has highly significant of nutritional content as a source of energy. Agriculture industrial waste recently used only for animal feed especially for ruminants. Therefore, the price is still relatively cheap. Agriculture industrial waste could be used as a potential career probiotic feed supplement for livestock especially for feed poultry to increase economic value and the necessary diversification agriculture industrial waste.

Otherwise, the utilization of agriculture industrial waste has not been highly used which is make the economic value still low. Furthermore, there is still required more effort to find out the natural antibiotics as a natural feed additive for organic livestock productivity.

This study is expected to produce a natural probiotic feed supplement which save for poultry production made from agriculture industrial wastes thus could reduce or even eliminate the use of chemical antibiotics in poultry or livestock productivity. The environment pollution also could be overcome by producing a natural probiotic feed supplements made from agriculture industrial waste.

MATERIALS AND METHODS

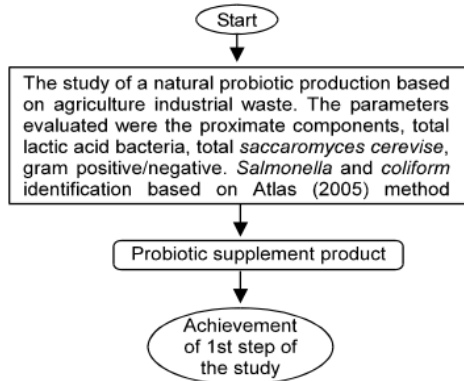
Research framework:



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Place of study: Study was conducted in the Feed Technology Laboratory, Animal Food Science Laboratory and Microbiology Laboratory Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang. Goal achievement of the study can be seen in the Table 3.

Design of study:



A study to produce good quality of probiotic supplement based on agriculture industrial waste use a completely randomized design (CRD) 3x3 factorials with 3 replications. The first factor is the type of agriculture industrial waste (S2, S4, S6). The second factor is the period of time for incubation (L2, L4, L6). The treatments combination as follows:

- S2L2: Rice bran with 40% ELKAF addition and 2 days incubation period
- S2L4: Rice bran with 40% ELKAF addition and 4 days incubation period
- S2L6: Rice bran with 40% ELKAF addition and 6 days incubation period
- S4L2: Wheat bran with 40% ELKAF addition and 2 days incubation period
- S4L4: Wheat bran with 40% ELKAF addition and 4 days incubation period
- S4L6: Wheat bran with 40% ELKAF addition and 6 days incubation period
- S6L2: Onggok with 40% ELKAF addition and 2 days incubation period
- S6L4: Onggok with 40% ELKAF addition and 4 days incubation period
- S6L6: Onggok with 40% ELKAF addition and 6 days incubation period

The parameters evaluated were the proximate components, total lactic acid bacteria, total *Saccaromyces cerevise*, gram positive/negative. *Salmonella* and *coliform* identification based on Atlas method (2005).

Procedures of the study: The study was begin by making ELKAF (*Ekstrak Limbah Kubis dan Sawi fermentasi/Cabbage and Mustard Fermented Waste Extracts*). The fermented solution was then dismantled and taken for appropriate treatment and being incubated for 2, 4 and 6 days incubation period. The proximate components, total lactic acid bacteria, total *Saccaromyces cerevise*, gram positive/negative. *Salmonella* and *coliform* identification based on Atlas method (2005) were then evaluated.

RESULTS AND DISCUSSION

The result on the Table 2. Show that all the treatment result in significantly and between the 1st and 2nd factor has been interacted. Otherwise, there was not interaction treatment between ash and crude fat content. The factors that affect the fermentation process were temperature, moisture content of the materials, environmental humidity and the media for fermentation. For this study, the water content for fermentation undergo was ranged from 60-70% whereas 70% was the best water content condition for conducted fermentation. The 70% of water content was the best condition for fermentation due to starter bacteria from cabbage and mustard green dominantly were lactic acid bacteria from the genus *Lactobacilli* and *Saccharomyces*. Those lactic acid bacteria is optimum growth at 70% water content and for 4 days incubation period. Both are expected potentially as probiotics and have capability to survive in the gastrointestinal tract of poultry in general. *Saccharomyces cerevisiae* is commonly used for industrial fermentation which containing immunostimulant such as beta-glucan, mannan oligosaccharides and anti-cancer. *Saccharomyces cerevisiae* and *Aspergillus oryzae* are known as a type of fungi that widely used for animal feed. *Saccharomyces cerevisiae* has a special characteristic for animal feed because of their ability to produce glutamic acid which can increase the palatability of feed. In contrast with bacteria, fungi is a microorganism that have a high level of resistant and can survive as well being reproductive in the acidic conditions of pH 1.5. Given *Saccharomyces* in feeding animal can improve the digestibility of protein and fiber such as cellulose and hemicellulose (Tawwab *et al.*, 2008). The purposes of fermented a product are for improving utility value of the product, eliminate the anti nutrition and for create a biomass. Fermented of rice bran, pollard and onggok as agriculture industrial waste also increasing biomass content for 4 day incubation period which could be seen from increasing total lactic acid bacteria content and *Saccharomyces* even though it does not imply for increasing crude protein content. According to Fardiaz (1989) the process and fermentation product are affected by the amount and the

Table 1: Average proximate components feed treatments

Parameter	Incubation period/feedstuff								
	2 days			4 days			6 days		
	RB	Poll	Ongg	RB	Poll	Ongg	RB	Poll	Ongg
C. Water	9.77	8.68	6.81	9.07	7.04	7.24	8.59	9.17	7.57
C. Ash	12.07	10.81	7.36	12.28	11.63	7.56	12.05	11.32	7.35
Crude fat	13.64	3.60	1.75	13.69	1.78	0.97	13.40	2.91	1.37
Crude fiber	14.03	6.71	13.85	15.12	14.64	14.10	13.05	14.03	14.04
Crude protein	12.75	15.47	1.41	14.31	16.09	1.79	13.69	16.72	1.65
NFE	47.51	63.23	75.10	43.92	55.86	75.61	46.8	55.03	73.92
RB: Rice Bran	Ongg: Onggok		Poll: Pollard (wheat bran)						

Table 2: Proximate analysis of variance parameters (moisture content, ash content, crude fat, crude fiber, crude protein extracts and NFE)

Analysis of variance	Parameters					
	Water content	Ash content	Crude fat	Crude fiber	Crude protein	NFE
Feedstuff	***	*	***	***	***	***
Incubation time	***	Ns	*	***	***	***
Feedstuff x Incubation time	***	Ns	Ns	***	*	***

Significancy level: *p<0.05; **p<0.01 dan ***p<0.001

Table 3: Microbiological content in feedstuff treatments

Parameter	Incubation time/feedstuff								
	2 Days			4 Days			6 Days		
	Rice bran	Poll	Ongg	Rice bran	Poll	Ongg	Rice bran	Poll	Ongg
Total Bacteria	2.10 ³	20.10 ³	50.10 ³	38.10 ³	>	>	15.10 ³	>	160.10 ³
Total Yeast	18.10 ³	-	>	-	13.10 ³	>	2.10 ³	>	7.10 ³
Total LAB	1.10 ⁴	2.10 ⁴	-	2.10 ²	3.10 ⁴	-	-	-	-
Gram+/-	+	+	+	+	+	+	+	+	+
Salmonella	-	-	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-	-
Bacteria type	Lactobacillus sp.								
Yeast Type	Sacaromyces cereviseae dan Rhizopus sp.								

Note: >TNTC (Too Numerous Too Count)

genus of starter bacteria, substrate media, pH and temperature also the period of time incubation. Mulyono *et al.* (1989) said that biomass as a form of microorganism biological process. The material is converted into protein by the microorganisms. However, the protein created by the bacteria would not be read as N of NPN. Therefore, the crude protein would not be increase even though they has the greater number of total acid bacteria and *Saccharomyces* content.

The fermentation process has some advantages among others process such as it does not give any negative effects, easy to do, relatively no need specific equipment and low cost production. The fermentation process is done by giving specific fungi or bacteria as starter microorganisms which is appropriate to the media and the purpose of fermentation. The chosen of starter for fermentation process based on their ability to give optimum bioconversion for the fermentation specific purposes, easily survive, readily available and low cost production. The purpose of fermentation is to produce a better product (material feed) that have a nutrient content, texture and biological availability, besides that it also can reduce the anti-nutritional (Winarno, 1984).

Only the Gram+bacteria was found out in feedstuffs treatment. Their group as *Lactobacillus* sp., while yeast species contained in the feed material is *Saccharomyces cerevise* and *Rhizopus* sp. For kapang's species. Pollard as a feedstuff with 4 days incubation time is the best feed ingredients of the treatments. Lactic acid bacteria will decreasing the pH value and produce acid flavor during fermentation process. The acid condition inhibits the growth of pathogenic bacteria. These phenomenon shown that negative result for Gram-present such us *Salmonella* and *E. Coli*. According to Supardi and Sukamto (1999), the acidification process result in Gram-bacteria were going to collapse thus the Gram+bacteria that grows dominantly.

Lactobacillus sp. as a lactic acid bacteria Gram+ were found in the feedstuffs will begin to grow and produce the acid from carbohydrate compounds which is available in the feed material. Lactic acid bacteria Gram+are follows: (1) *Streptococcus thermophilus*, *Streptococcus cremoris*; which all are Gram+spherical bacteria (cocci shape); (2) *Pediococcus cereviseae* is a Gram+bacteria with round shape and plays an important role in the fermentation process of meat and vegetables;

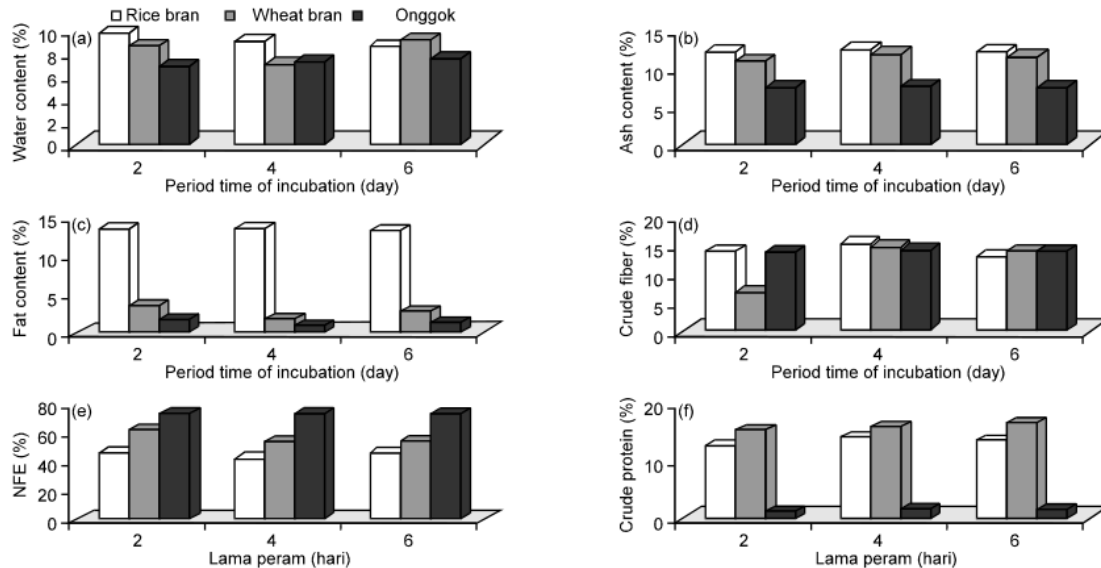


Fig. 1(a-f): Effect of Treatments for Moisture content, (a) Water content (b) Ash Content, (c) Crude Fat Content, (d) Crude Fiber, (e) Nitrogen Free Extract (NFE) and (f) Crude Protein Content and

(3). *Leuconostoc mesenteroides*, *Leucostoc dextranicum*; a spherical Gram+ bacteria that play an important role in the initiation of vegetable and fruit juices fermentation; (4) *Lactobacillus lactis*, *L. Acidophilus*, *L. Bulgaricus*, *L. Plantarum*, *L. Delbrueckii*; are a rod shape Gram+ bacteria which are more resistant to acids condition in the vegetables and milk fermentation (Buckle *et al.*, 1987). Those bacteria which have capability producing lactic acid could be used for a fermentation bacteria. The requirements as a starter bacteria are follows: the pH in acidic condition, safe to use and have ability to inhibit pathogenic bacteria. Based on this research it is feasible to develop a career as a ground breaking development of probiotics which are easy, inexpensive and applicable.

Conclusion and recommendation: The conclusion based on the study is pollard which had fermented for 4 days incubation time appropriate as a career probiotic in term of the protein content and bacteria availability. For the further research on probiotics, pollard career can be studied in vivo in order to determine their ability as a career probiotics and the achievement to replace antibiotics application.

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