Bioconversion of Shrimp Waste with Fermentation Stage Process on Proximate Analysis and Digestibility Values of Feed

Abun Hasbuna, Tuti Widjastuti, and Kiki Haetami

ABSTRACT

Frozen shrimp processing waste has the potential to be used as feed, but the characteristics of the shrimp shells need to be improved so that they can be digested. Fermentation using three types of microbes in stages has been studied, to determine the optimal processing time that yields proximate values and protein digestibility of shrimp waste concentrate. Completely randomized design (CRD), 3 treatments and 7 replications, conducted with treatments of shrimp waste bioconversion in stage over time, T1 = *Bacillus licheniformis* (Bl.) 1 day + *Lactobacillus* sp. (Ls.) 1 day; + *Saccharomyces cerevisiae* (Sc) 1 day; T2 = Bl. 2 days + Ls. 2 days + Sc = 2 days; T3 = Bl. 3 days + Ls. 3 days + Sc = 3 days. Product of shrimp waste bioconversion was used as a nutrient concentrate in dietary of local poultry (CP 15%, ME 2750 kcal/kg). The best proximate analysis value showed that each stage overtime two days of bioconversion with *Bacillus licheniformis*, followed by *Lactobacillus* sp. and finally fermented by *Saccharomyces cerevisiae*. The proximate analysis resulted that the Crude Protein of product bioconversion was 48.5%. Extract ether, Calcium and Phosphorous contents respectively were 7.81%, 7.57% and 3.14%. The best value of digestibility of protein feed containing bioconversion product of concentrations of local poultry was 72.91%.

Keywords: digestibility value, local poultry, proximate analysis, stage of bioconversion, the waste of shrimp processing.

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Abun Hasbuna*

Department of Animal Nutrition and Feed Technology, Padjadjaran University, Sumedang-West Java, Indonesia.
(e-mail: abunhasbunap@gmail.com)

Tuti Widjastuti

Department of Animal Production, Padjadjaran University, Sumedang-West Java, Indonesia

Kiki Haetami

Department of Fisheries, Padjadjaran University, Sumedang-West Java, Indonesia.

**Corresponding Author*

I. INTRODUCTION

Industry the processing of the export product of shrimp (cold storage) has leaving wasted in the shell of crustacean material with huge, but potentially used as feed formulation component for poultry. The Chemical content of proximate analysis result of shrimp waste includes crude protein 43.41 per cent, 18.25% crude fibre, and extract ether 7.27%. 5.54% calcium, phosphorus 1.31%, 3.11% lysine, methionine 1.26%, cysteine 0.51%, and the calory 3892 kcal/kg [1]. Factors are limiting shrimp waste as a formulation component of poultry feed. This is a binding structure of covalent strongly in nutrients with chitin bond β (1-4), so it is problematic in gut digestive because enzyme digestion of poultry cannot destruct [2].

Bioconversion of shrimp waste through fermentation with microbe activity that can break the glycosides bond β - (1-4) was an effort to convert shrimp waste into organic material products with better nutrient content. The fermentation technique in this research used microbes through a three-stage bioconversion. Waste-product through the steps protein break by *B. licheniformis*, and demineralization by *Lactobacillus* sp. [3]–[5]. Bioconversion is terminated by *S. cerevisiae* which according to [6] is a species of yeast with metabolites that includes amylase, lipase, and protease, that can be petrified nutrients digestion in gut organs.

Local chicken is one type of poultry that has been popular in the community and spread across the archipelago.

Fermented products of shrimp waste are used as a dietary component of local poultry. Compound at feed substance that effect digestibility is a crude fibre, one of these is chitin [7], [8]. Apparent digestibility can be defined as part of feed substances that are not secreted in the faeces, so it can be interpreted that the apparent digestibility is the number of nutrients that were digested and absorbed in the tract digestive or not secreted in the faeces compared to the nutrients consumed [9], [10]. The value of good digestibility indicates the better quality of the diet [11].

II. MATERIALS AND METHODS

A. Materials and Equipment Experiment

Isolates were *Bacillus licheniformis*, *Lactobacillus* sp. and *Saccharomyces cerevisiae*. The main raw materials waste in the form of the head and shell shrimp fresh shrimp obtained from the company exporting frozen shrimp. Other ingredients were aquadest, D-glucose, the extract of fungi, technical glucose, Sodium chloride, sodium hydroxide, azo-casein, Buffers (Sodium **Borate**, Sodium Phosphate pH 6.0, citrate, and bicarbonate), TCA, oxygen gas and BSA.

Equipment used includes jars, water, and auto-shaker bath. Steamer, beaker, burner, dish, cup ceramic, centrifuge, funnel, pH-measure (Knick Merck), spectrometer Novaspec II, reaction tubes, furnaces, HPLC, milling machines, and pellet machines. Nutrient concentrate used for testing local

chicken as much as 27 animals (average weight of 1139.86 grams 111.86 grams) were placed in a cage measuring 20×40×30 cm. The local chicken was obtained from the Development of Livestock Breeding Poultry, Jatiwangi, Majalengka, West Java.

B. Scope of Research

1) Bioprocess of waste shrimp using *B. licheniformis*, *Lactobacillus* sp., and *S. cerevisiae*, then the proximate analyses of the ingredient product used as nutrients concentrate.

2) Determination of the quality of products (nutrients concentrate) biologically through measurement of the value of protein digestibility in local chicken.

C. Experimental Procedure

1) Bioprocess, with the following steps

1.1) Deproteinized

This step aims to protein degradation of chitin-binding. First, prepare a starter inoculum was taking bacterium *Bacillus licheniformis* then cultivated in Erlenmeyer flask with 50 ml of sterilized broth which is set at a pH of 7, which is set using 1N HCl. The solution which has been included in bacterial broth was then incubated in an incubator for 48 hours at 50 °C.

Second, prepare a standard solution consisting of 0.5% (w/v) yeast extract; 0.5% (w/v) KH₂PO₄; 0.1% (w/v) CaCl₂; 0.5% (w/v) NaCl; and 0.05% (w/v) MgSO₄.

Third, do the auto-shaker bath fermentation. Shrimp waste is put into stainless jars, then inoculated with an inoculum of *Bacillus licheniformis* with a dose of 2% (v/w). Subsequently incorporated into the auto-shaker bath for 1 day; 2 days; and 3 days at a temperature of 45 °C with a rotation of 120 rpm [12].

1.2) Demineralized

This step aims to dissolve the minerals from shrimp waste that had previously been in-deproteinized,

First, prepare a starter inoculum, namely by taking *Lactobacillus* sp., then cultivated in 125 ml Erlenmeyer flask containing 50 ml of sterile broth which is set at pH of 7, which is set using 1N HCl. Broth solution that has been put *Lactobacillus* sp. then incubated in an incubator for 2 days at a temperature of 45 °C.

Second, prepare a standard solution consisting of 0.5% (w/v) yeast extract, 0.5% NH₄NO₃; 0.05% KCl; 0.05% MgSO₄; 0.01% FeSO₄; and 0.001% CuSO₄.

Third, do fermentation in the auto-shaker bath. Deproteinized products then added inoculum of *Lactobacillus* sp. 2% (v/w), then incubated for 1 day; 2 days; and 3 days at a temperature of 45 °C with a rotation of 120 rpm [12].

1.3) Fermented by *Saccharomyces cerevisiae*

First, the manufacture of a pure culture of *Saccharomyces cerevisiae*, grown on agarose slant, then put into an incubator and set the temperature 30 °C, for incubated for 3 days, then made inoculum.

Second, prepare a standard solution consisting of 0.5% NH₄NO₃; 0.05% KCl; MgSO₄·7H₂O 0.05%; FeSO₄·7H₂O 0.01%; and CuSO₄·5H₂O 0.001%.

Third, do fermentation in the auto-shaker bath. Product demineralization, then added inoculum of *Saccharomyces*

cerevisiae 3% (v/w), then incubated for 1 day; 2 days; and 3 days at a temperature of 35 °C [13].

Bioprocess products have then analysed the content of protein, fat, calcium and phosphorus, as well as the gross energy [12].

2) 2) Measurement of protein digestibility

Local chicken as many as 27 animals (average weight of 1139.86 grams 111.86 grams) were placed in individual cages of 27 units at random. The cages used were metabolic cages measuring 20×40×30 cm and each unit enclosure was fitted with a feed and drinking water. At the base enclosure coated plastic tray can be installed and removed for easy storage of excreta. Chickens were placed into individual cages, then fasted for 18 hours to eliminate the ration before the rest of the digestive tract. A ration of 100 grams per head. Drinking water supplied ad-libitum. This experiment used the lignin component as a marker in the hind intestine, and at 14 hours after dietary treatments were detached to obtain faeces. The faeces sample was dried and then analysed dry ingredients, and proteins were, whereas the indicator (lignin ration and faeces) were analysed by the method of ±[14]. Variables measured were the content of the ration, crude protein rations, lignin ration, faeces dry matter, crude protein faeces, faeces, and lignin.

The protein digestibility calculation equation of [15], as follows:

$$\text{Digestibility} = 100\% - 100 \times$$

$$\times \left\{ \frac{\% \text{ Dietary lignin}}{\% \text{ Lignin in faeces}} \times \frac{\% \text{ crudeprotein in faeces}}{\% \text{ dietary of crude protein}} \right\}$$

III. RESULTS AND DISCUSSIONS

A. The Proximate Analysis Content

Nutrients Content (Crude protein, crude lipid, calcium, and phosphorus) of the time processing of shrimp waste at each step were presented in Table I. The highest crude protein content (47.19%) in deproteinized by *Bacillus licheniformis* was obtained from the time of processed two days treatment (T2), and also the phosphorus (2.24%), whereas T1 was the lowest (CP 41.92%). The lowest crude lipid and highest calcium content were obtained in treatment T3, respectively 8.75% and 6.95%. *Bacillus licheniformis* in this step acted to break chitin-binding for covalent bond β (1-4) with protein. Time processing for 2 days was optimum for growth microbe, release protein for chitin binding, and then increasing protein content.

Chitin material which is a covalent bond with proteins, fats and minerals in bind β (1-4) glycosidic was difficult to digest with enzyme endogenous of monogastric [7], [15], [16]. The degradation product of protein-chitin binding by *B. licheniformis*, must be followed by *Lactobacillus* sp. to release minerals from chitin. Table I indicated that the time of processing for two days was higher crude protein content in the amount of 47.60%. Finally, fermentation of shrimp waste product by *S. cerevisiae* resulted in the nutrient product according to the crude protein content 43.5% until 48.5%. The end step of bioprocess with *Saccharomyces cerevisiae* added protein which converted from sugar and

chitin in the product of degradation by *Bacillus* sp., and then lactic acid fermented with *Lactobacillus* sp.

TABLE I: MEAN PROTEIN CONTENT, CRUDE FAT, CA, AND P IN NUTRIENT CONCENTRATE PRODUCTS OF A STAGE OF MICROBES SHRIMP WASTE

Treatments (Step of microbe over time)	BIOCONVERSION			
	Crude Protein	Crude Fat	Ca	P
			%	
Bl. T1	41.92	13.49	6.54	1.87
Bl. T2	47.19	9.37	6.79	2.24
Bl. T3	45.38	8.75	6.95	2.23
+Ls.T1	42.99	12.11	7.25	2.15
+Ls.T2	47.60	8.56	7.48	3.12
+Ls.T3	46.06	8.07	7.65	2.95
+Sc T1	43.50	11.44	7.35	2.31
+Sc.T2	48.50	7.81	7.57	3.14
+Sc.T3	47.69	7.42	7.72	2.96

Notes: Bl = *Bacillus licheniformis* (first step); +Ls = Product of first stage plus *Lactobacillus* (second stage); +Sc = Product of second stage plus *Saccharomyces cerevisiae* (third step); T = time processing; T1 = 1 day; T2 = 2 days; T3 = 3 days.

Treatment of time processing indicated that different significantly ($P < 0.05$) to crude protein, crude lipid, Ca, and P content. The result of the Duncan Test showed that bioprocess products (nutrient concentrate) by *Bacillus licheniformis* furthermore with *Lactobacillus* sp. and *Saccharomyces cerevisiae* at different times were presented in Table II.

TABLE II: THE NUTRIENTS CONTENT OF STAGE OF SHRIMP WASTE BIOCONVERSION PRODUCTS BY *BACILLUS LICHENIFORMIS*, *LACTOBACILLUS* SP. AND THEN BY *SACCHAROMYCES CEREVISIAE*

Treatments	Crude Protein	Crude Lipid	Ca	P
Microbe/Time		%		
Bl+Ls +Sc. T1	43.50 ^A	11.44 ^A	7.35	2.31 ^A
Bl+Ls +Sc.T2	48.50 ^B	7.81 ^B	7.57	3.14 ^B
Bl+Ls +Sc.T3	47.69 ^B	7.42 ^B	7.72	2.96 ^B

Note Significance ($P < 0.01$).

The test results showed that time processing by *Bacillus licheniformis*, *Lactobacillus* sp. followed by *Saccharomyces cerevisiae* for two days (T₂) was an optimum time for the growth of microbes and activity of the enzyme.

A highly significant difference ($P < 0.01$) against crude protein content, crude lipid, and phosphorus products. While the calcium content T₂ showed significant differences ($P < 0.05$) and did not differ in the level of 1%. The proximate analysis resulted from the Crude Protein (CP) content on-time processing due to the level of microbial growth. Based on the curve the growth of microbes with three phases includes the adaption phase namely the slow phase or when cells do metabolic activity and physiological to prepare for division, the exponential phase or phase of fast growth and stagnant or stationary phase [4], [17]–[19].

Time processing each step of bioprocess with regarding many microbial populations to quickly the development of microbes, and then produce enzymes to break down the substrate, which in turn affect the final product. The higher dose of inoculum and longer time fermentation caused a large population and substrate components were overhauled [20], [21].

According to [22] suggest that microbes forming acidic conditions, such as *Lactobacillus* sp. resulted in the formation of the complex salt. The mineralization process

can be done by dissolving the mineral found in shrimp waste acid through a fermentation process. Citric acid produced in the fermentation process with *Lactobacillus* sp. reacts with the calcium carbonate to form calcium citrate, carbon dioxide and water [22], [23].

The release of phosphorus from chitin bind indicates that the inoculum used, namely *Lactobacillus* sp. in bioprocess occurred acidic precipitates so that formed the mineral phosphorus. Fermentation determines the amount of time to achieve microbial populations on the next link in the development of microbes that produce enzymes to break down the substrate affect the final product. The longer time processing caused the more microbial population and the more substrate nutrients were repaired. Microbes experiencing growth rates continue to rise until the stationary phase. This is under the facts obtained from the research that a longer fermentation time, did not produce a higher content of phosphorus products.

B. licheniformis is a bacteria that is capable of producing protease and chitinase [24], [25]. Protease enzyme according to [26]–[28] can be examined from activity proteolytic with microbial metabolites among which is *B.licheniformis*.

The best nutrient content in the form of crude protein, crude fat, calcium, and phosphorus product of shrimp waste bioprocess, that supported by the data digestibility was for 2 days of time processing treatment (W2). These results were further biological tested to examine the product quality (nutrients) through ration digestibility value measurement on local chicken.

B. Effect of Treatment of Protein Digestibility

TABLE III: EFFECT BIOPROCESS PRODUCTS (NUTRIENT CONCENTRATE) BY *B. LICHENIFORMIS*, *LACTOBACILLUS* SP., AND *S. CEREVISIAE* ON PROTEIN DIGESTIBILITY IN DIETARY OF LOCAL CHICKEN.

Repeated	Treatments		
	T1	T2	T3
	%		
1	63.18	73.37	70.65
2	63.38	73.95	72.39
3	62.32	72.32	71.67
4	62.53	70.24	71.57
5	63.30	73.99	73.02
6	61.93	73.46	72.61
7	63.68	73.07	70.21
Average	62.90 ^A	72.91 ^B	71.73 ^B

The highest average value of protein digestibility Bioprocess Products (Nutrient Concentrate) by *B. licheniformis*, *Lactobacillus* sp., and *S. cerevisiae* obtained at the length of time processing for 2 days (48 hours) amounted to 72.91%. While the bioprocess products that have low digestibility was T1 (1 day) amounted to 62.90%. Statistical analysis showed that the protein digestibility value of bioconversion products (Nutrient Concentrate) on 3 days (T3) as well as time processing for 2 days, and both of them were significantly ($P < 0.05$) higher than the digestibility of bioprocess products with time processing for a day (T1).

Feed ingredients processing products have better biological value than the original material. In line with the opinion of time processing could transform organic material into other useful products and added value better, especially by utilizing biolysis and biosynthesis events. Products that can be generated are microbial cells or biomass, enzymes,

primary and secondary metabolites, as well as chemical compounds by microbe results bioprocess [26].

Poultry has limitations in digesting food substances, especially those containing chitin and high crude fibre. This is because poultry cannot produce cellulase and chitinase enzyme, so that chitin and crude fibre can bind nutrients that can be digested out with faeces [29], [30]. In line with the facts found from the research that the compound chitin shrimp waste without treatment was quite high, 20.11% [31]–[33].

Chitin is a chemical compound that cannot be digested by the digestive enzymes of poultry [34], [35]; therefore, the shrimp waste should be processed first. The chitin polymer chains typically consisting of 2000 to 5000 monomer units of N-acetyl-D-glucosamine (2-acetamino-2-deoxy-D-glucose) are adrift through bond β (1-4) glucoside [36]–[38].

Products of Steps Bioprocess by *B. licheniformis* continued by *Lactobacillus* sp., and then by *S. cerevisiae* have a better protein digestibility value. This is because the bacterial species *Bacillus licheniformis* capable of producing protease and chitinase in relatively high amounts [18], [39], [40], and acidic conditions created by *Lactobacillus* sp. dissolving mineral that is bound to a protein that has been unravelled. Further fermentation with *S. cerevisiae* helps improve digestion with carbohydrase and protease enzymes it produces [6], [41].

Time processing for 2 days was the optimum time which provides an opportunity for microbes producing enzymes to conduct their activities.

IV. CONCLUSION

Time processing at steps bioprocess shrimp waste with three microbes through a fermentation process using *Bacillus licheniformis* and continued with *Lactobacillus* sp. and *Saccharomyces cerevisiae*, respectively for two days produced the best products (Nutrient-concentrate) and had a protein digestibility of is high (above 70%). The best nutrient content (crude protein and crude lipid) was 48.50% and 7.81%, Ca and P respectively 7.57% and 3.14%. The best value products on the digestibility of the dietary at local chicken amounted to 72.91%.

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