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The effect of treatment of shrimp waste with three microbial on nutrient content and digestibility of feed in native chicken

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Abstract

Shrimp shells and heads are waste materials that still contain protein but are constrained by the presence of chitin (15-20%) so they are difficult to digest by digestive enzymes. The research was conducted at the Faculty of Animal Husbandry, Padjadjaran University, Jatinangor, Sumedang. This study aims to obtain the optimum processing time at each stage of the bioprocess of shrimp waste with different microbes on the nutritional content and protein digestibility of the product as a concentrate of feed nutrients in native chickens. This research was conducted as an experiment in a laboratory, using a completely randomized design (CRD), 3 treatments, and 7 replications. Data were analyzed with variance and Duncan's Multiple Distance Test. Treatment of shrimp waste bioprocess is carried out in stages (W) W1 = *Bacillus licheniformis* (*Bl.*) 1 day + *Lactobacillus* sp. (*Ls.*) 1 day; + *Saccharomyces cerevisiae* (*Sc*) 1 day; W2 = *Bl.* 2 days + *Ls.* 2 days + *Sc* = 2 days; W3 = *Bl.* 3 days + *Ls*. 3 days + *Sc* = 3 days. Shrimp waste bioprocess products were used as a nutrient concentration in native chicken feed (CP 15%, ME 2750 kcal/kg). The best nutritional content showed a processing time of two days at each stage of the bioprocess with deproteination using *Bacillus licheniformis*, followed by demineralization by *Lactobacillus* sp. and finally fermented by *Saccharomyces cerevisiae* for 2 days each. The crude protein content of bioprocesses products is 48.5%, while crude fat, calcium, and phosphorus are 7.81%, 7.57%, and 3.14%, respectively. Protein digestibility is the best product, the concentration of nutrients in native chicken feed is 72.91%.

Keywords: Time at steps bioprocess; Shrimp waste; Nutrients concentrate; Protein digestibility; Native chicken

1. Introduction

Waste-product frozen shrimp processing industry (cold storage) form of the shell and head is a material with huge potential to be used as an alternative feed ingredient for poultry. It is based on nutritional content, namely: crude protein 43.41 percent, 18.25 percent crude fiber, crude fat 7.27 percent, 5.54 percent calcium, phosphorus 1.31 percent, 3.11 percent lysine, methionine 1.26 percent, cysteine 0.51%, and the gross energy 3892 kcal/kg [1]–[4]. Factors limiting the use of waste products as ingredients of poultry feed is the presence of chitin in the amount of about 15-20 percent. Chitin binds strongly with proteins, fats, and minerals covalent bond ß (1-4) making it difficult to digest by enzyme digestion of poultry [5]–[7].

Poultry does not have the enzymes that can break the glycosides bond β- (1-4) so before being used as feed material, waste-product are processed first. One effort to convert organic material into useful new products and has better nutritional value has been to use microbes through bioprocess. Bioprocess waste-product can be done through the steps deproteinated using *Bacillus licheniformis*, and demineralization by *Lactobacillus* sp. Bioprocess is terminated by

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Saccharomyces cerevisiae which according to [8], [9] is a yeast that can produce the enzymes amylase, lipase, protease, and other enzymes that can be petrified digestion of nutrients in the digestive organs.

Native chicken is one type of poultry that has been popular in the community and spread across the archipelago. Bioprocess products of shrimp waste used as a dietary ingredient of native chicken were expected to be better digestibility values because the nutrients have been relegated from the bonds of chitin. Compound at feed substance that effect digestibility is a crude fiber, one of these is chitin [6], [8], [10]. Apparent digestibility can be defined as part of feed substances are not secreted in the feces, so it can be interpreted that apparent digestibility is the number of nutrients were digested and absorbed in the digestive tract or not secreted in the feces compared to the nutrients consumed [11], [12]. The more nutrients were absorbed by the body, then the value of the higher digestibility. It is one indicator of the high quality of the feed.

2. Material and methods

2.1. Materials and Equipment

Isolates were *B. licheniformis, Lactobacillus* sp., and *S. 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 distilled water, glucose, yeast extract, technical glucose, tryptone, NaCl, NaOH, reagent azokasein, borate buffer, phosphate buffer, citrate buffer, bicarbonate buffer, TCA, oxygen gas, and Bovin Serum Albumin.

The tools used were jars steel (reactor), water bath, auto-shaker bath, autoclave, beaker, Bunsen burner, a petri dish, cup porcelain, centrifuge Nimac CR 21G, funnel, pH-meter Knick, spectrophotometer Novaspec II, test tubes, furnaces, HPLC, milling machines, and pellet machines. The nutrient concentrate was used for testing native chickens as many as 27 animals (average weight of 1139.86 grams - 111.86 grams) were placed in a cage measuring 20x40 x30 cm. The native chicken was obtained from the Development of Livestock Breeding Poultry, Jatiwangi, Majalengka, and West Java.

2.2. Scope of Research

- Bioprocess of waste shrimp using *B. licheniformis, Lactobacillus* sp., and *S. cerevisiae* then analyses the content of the nutrient product and used as nutrients concentrate.
- Determination of the quality of products (nutrients concentrate) biologically through measurement of the value of protein digestibility in native chicken.

2.3. Experimental Procedure

2.3.1. Bioprocess, with the following steps

Deproteinated

This step aims to protein degradation of chitin-binding. First, prepare a starter inoculum taking the bacterium *B. licheniformis* then cultivated in 125 ml Erlenmeyer flask containing 50 ml of sterile broth which is set at a pH of 7, which is set using 1N HCl. The solution which has been included bacterial broth was then incubated in an incubator for 2 days at a temperature of 50°C. Second, prepare a standard solution consisting of 0.5% (w/v) yeast extract; 0.5% (w/v) KH_2PO_4; 0.1% (w/v) $CaCl_2$; 0.5% (w/v) NaCl; and 0.05% (w/v) $MgSO_4$.

Third, do the auto-shaker bath fermentation. Shrimp waste is put into stainless jars and then inoculated with an inoculum of *B. 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 ºC with a rotation of 120 rpm [13].

Demineralized

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

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 45ºC.

Second, prepare a standard solution consisting of 0.5% (w/v) yeast extract, 0.5% NH_4NO_3; 0.05% KCl; 0.05% $MgSO_4$; 0.01% $FeSO_4$; and 0.001% $CuSO_4$.

Third, do fermentation in the auto-shaker bath. Deproteinated products were then added inoculum of *Lactobacillus* sp. 2% (v/w), then incubated for 1 day; 2 days; and 3 days at a temperature of 45ºC with a rotation of 120 rpm [13].

Fermented by *S. cerevisiae*

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

Second, prepare a standard solution consisting of 0.5% NH_4NO_3; 0.05% KCl; $MgSO_4.7H_2O$ 0.05%; $FeSO_4.7H_2O$ 0.01%; and $CuSO_4.5H_2O$ 0.001%.

Third, do fermentation in the auto-shaker bath. Product demineralization, then added inoculum of *S. cerevisiae* 3% (v/w), then incubated for 1 day; 2 days; and 3 days at a temperature of 35ºC [8].

Bioprocess products have then analyzed the content of protein, fat, calcium, and phosphorus, as well as the gross energy.

2.3.2. Measurement of protein digestibility

Native chickens 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 x 40 x 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 and 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 addlibitum. To obtain feces samples following the method [6] and modified by [6]. This experiment used internal indicators (lignin). After 14 hours, chickens were slaughtered and large intestines were removed to obtain feces samples. The feces sample was dried and then analyzed with dry ingredients, and proteins, whereas the indicator (lignin ration and feces), were analyzed by the method of [14]. Variables measured were the content of the ration, crude protein rations, lignin ration, feces dry matter, crude protein feces, feces, and lignin.

The protein digestibility calculation equation of [8], is as follows:

$$\text{Protein Digestibility} = 100\% - 100\ \text{X}\ \frac{\%\ \text{Dietary lignin}}{\%\ \text{Lignin in feces}}\text{X}\frac{\%\ \text{crude protein in feces}}{\%\ \text{dietary crude protein}}$$

3. Results and discussion

3.1. Effect of treatment of nutrient content

Nutrients Content (Crude protein, crude lipid, calcium, and phosphorus) of the time processing of shrimp waste at each step was presented in Table 1. The highest crude protein content (47.19%) in deproteinated by *B. licheniformis* was obtained from the time of processed two days treatment (W2), whereas W1 was the lowest (41.92%). Similarly, the highest content of phosphorus was obtained from treatment W2 (2.24%). The lowest crude lipid and highest calcium content were obtained in treatment W3, respectively 8.75% and 6.95%. *B. licheniformis* in this step acted to break chitin-binding for covalent bond ß (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 binds strongly with proteins, fats, and minerals covalent bond ß (1-4) making it difficult to digest by enzyme digestion of poultry [6], [15], [16]. The degradation product of protein-chitin binding by *B. licheniformis* must be followed by *Lactobacillus* sp. to release minerals from chitin. Table 1 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* release nutrient product with the lowest crude protein content of 43.5% and the highest of 48.5%. In the end step of bioprocess with *S. 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.

Treatment of time processing indicated that different significantly ($P < 0.01$) to crude protein, crude lipid, calcium, and phosphorus content. Results of the Duncan Test showed that bioprocess products (nutrient concentrate) by *B. licheniformis* followed by *Lactobacillus* sp. and *S. cerevisiae* at different times were presented in Table 2.

Table 1 Mean crude protein, crude fat, calcium, and phosphorus in nutrient concentrate products of time processing at each step of microbes on bioprocess shrimp waste

Treatments (Step of microbe over time)	Crude protein	Crude fat	calcium	phosphorus
	……%……			
*Bl.*W1	41.92	13.49	6.54	1.87
*Bl.*W2	47.19	9.37	6.79	2.24
*Bl.*W3	45.38	8.75	6.95	2.23
*+Ls.*W1	42.99	12.11	7.25	2.15
*+Ls.*W2	47.60	8.56	7.48	3.12
*+Ls.*W3	46.06	8.07	7.65	2.95
+Sc W1	43.50	11.44	7.35	2.31
*+Sc.*W2	48.50	7.81	7.57	3.14
*+Sc.*W3	47.69	7.42	7.72	2.96

Bl = *B. licheniformis* (first step)*; +Ls* = Product of first step *plus Lactobacillus* (second step); *+Sc* = Product of second step plus *S. cerevisiae* (third step)*;* W = time processing; W1 = 1 day; W2 = 2 days; W3 = 3 days.

Table 2 Effect of Time at Steps Bioprocess Shrimp Waste by *B. licheniformis, Lactobacillus* sp. and then by *S. cerevisiae* on nutrients Content of Products (P<0.01)

Treatments (Microbe/Time)	Crude protein	Crude fat	calcium	phosphorus
	…… % ……			
Bl+Ls +Sc. W1	43.50[a]	11.44[a]	7.35	2,31[a]
Bl+Ls +Sc.W2	48.50[b]	7.81[b]	7.57	3,14[b]
Bl+Ls +Sc.W3	47.69[b]	7.42[b]	7.72	2,96[b]

Bl = *B. licheniformis* (first step)*; +Ls* = Product of first step *plus Lactobacillus* (second step); *+Sc* = Product of second step plus *S. cerevisiae* (third step)*;* W = time processing; W1 = 1 day; W2 = 2 days; W3 = 3 days.

[a, b] means different superscripts within the same column significantly (P < 0.01)

The test results showed that time processing by *B. licheniformis, Lactobacillus* sp. followed by *S. cerevisiae* for two days (W2) was an effective time for the growth of microbes and activity of enzymes. A highly significant difference (P<0.01) against crude protein content, crude lipid, and phosphorus products. While the calcium content of W2 showed significant differences (P<0.01) and did not differ at the level of 1%. Differences in protein content on time processing due to the level of microbial growth. Based on the growth rate, the growth of microbes can be divided into three phases, namely the slow phase, or when cells do metabolic activity and physiological to prepare for division, the exponential phase or phase of accelerated growth, and the stationary phase or resting phase [2], [17]–[20].

Time processing each step of bioprocess with regarding several 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 fermentation caused the more the microbial population and the more substrate components were overhauled [13], [21], [22]. [23] suggest that microbes forming acidic conditions, such as *Lactobacillus* sp. resulted in the formation of the complex salt. Furthermore [18] reported that 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.

The release of phosphorus from chitin bind indicates that the inoculum used, namely *Lactobacillus* sp. in bioprocess occurred acidic precipitates 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 and affect the final product. The longer time processing caused more microbial population and more substrate components were overhauled. Microbes experiencing growth rates continue to rise until the stationary phase.

This is by the facts obtained from the research that a longer fermentation time, did not produce a higher content of phosphorus products.

B. licheniformis is a species of bacteria that is capable of producing protease and chitinase in relatively high amounts [1], [24]. Protease enzyme according to [15] can be obtained from proteolytic 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, supported by the data digestibility was for 2 days of time processing treatment (W2). These results were further tested to determine the quality of the product (concentrate nutrients) through ration digestibility value measurement on native chicken.

3.2. Effect of treatment of protein digestibility

Table 3 Effect bioprocess products (nutrient concentrate) by *B. licheniformis*, *Lactobacillus* sp., and *S. cerevisiae* on protein digestibility in dietary of native chicken

Repeated	Treatments		
	W1	**W2**	**W3**
	…… % ……		
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]

W = time processing; W1 = 1 day; W2 = 2 days; W3 = 3 days.
[a, b] means different superscripts within the same column significantly (P < 0.01)

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 product that has low digestibility was W1 (1 day) amounted to 62.90%. Statistical analysis showed that the protein digestibility value of bioprocess products (Nutrient Concentrate) on 3 days (W3) as well as time processing for 2 days, and both of them were significantly (P <0.01) higher than the digestibility of bioprocess products with time processing for a day (W1).

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 add 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.

Poultry has limitations in digesting food substances, especially those containing chitin and high crude fiber. This is because poultry cannot produce cellulase and chitinase enzymes, so chitin and crude fiber can bind nutrients that can be digested out with feces [25]. In line with the facts found from the research that the compound chitin shrimp waste without treatment was quite high, 20.11% [26].

Chitin is a chemical compound that cannot be digested by the digestive enzymes of poultry [27]; therefore, shrimp waste should be processed first. According to [28] suggested that the chitin polymer chains typically consisting of 2000 to 5000 monomer units of N-acetyl-Glucosamine (2-acetamino-2-deoxy-D-glucose) are adrift through bond β (1-4) glucoside.

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 *B. licheniformis* capable of producing protease and chitinase in relatively high amounts[29], [30], and acidic conditions created by *Lactobacillus* sp. dissolving mineral that is bound to a protein that has been unraveled. Further fermentation with *S. cerevisiae* helps improve digestion with carbohydrase and protease enzymes it produces.

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

4. Conclusion

Time processing at steps bioprocess shrimp waste with three microbes through a fermentation process using *B. licheniformis* and continued with *Lactobacillus* sp. and *S. 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 native chicken amounted to 72.91%.

Compliance with ethical standards

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Authors: Abun Abun, Tuti Widjastuti, and Kiki Haetami contributed to the paper's development. We now express our thoughts concerning our work.

Disclosure of conflict of interest

The authors declare no conflicts of interest.

Statement of informed consent

Everyone who participated in the study investigation gave his or her informed consent.

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