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EFFECT OF TIME PROCESSING AT STEPS OF BIOPROCESS SHRIMP WASTE BY THREE MICROBES ON PROTEIN DIGESTIBILITY AND METABOLIZABLE ENERGY PRODUCTS OF NATIVE CHICKEN

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Abstract

The shell and head of shrimp is a waste material whose protein content is constrained by its content of chitin (15-20%) making it difficult to digest. The objective of this research was to determine the optimum time required for different microbes bioprocess shrimp waste in order to improve nutrient availability, protein digestibility, and metabolizable energy of these materials in native chickens. Experiments were conducted using a completely randomized design with three microbial treatments and bioprocessing times. Data were statistically analyzed for variance using a Duncan's Multiple Range test. Shrimp waste materials were treated with each microbe sequentially over time (W) as follows: W1, Bacillus licheniformis (Bl) + Lactobacillus sp. (Ls.) + Saccharomyces cereviseae (Sc), each for 1 d; W2 = Bl+ Ls+ Sc, each for 2 d; W3 = Bl+ Ls+ Sc, each for 3 d. Bioprocessed products of shrimp waste materials were used as a nutrient concentrate in diets of native chickens (crude protein). The optimal crude protein content at W2 of the bioprocessed product was 48.5%, while extract ether, calcium, and phosphorous levels were 7.81%, 7.57%, and 3.14%, respectively. The highest protein digestibility obtained from the best nutrient (W2) with value 72.91%; the best of metabolizable energy of these materials was 2613.90 kcal/kg

Key words: *microbial bioprocessing time, shrimp waste, protein digestibility, metabolizable energy, native chickens.*

INTRODUCTION

Waste products of shrimp, especially the shell and head have great potential as alternative ingredients in poultry feed since their nutritional content includes 43.41% crude protein, 18.25% crude fiber, 7.27% crude lipid, 5.54% calcium, 1.31% phosphorus, 3.11% lysine, 1.26% methionine, 0.51% cysteine, and 3892 kcal/kg gross energy (Abun, 2008).

The main factor limiting the use of shrimp waste materials in poultry feed is the presence of chitin (15-20%).

Chitin binds strongly to proteins, lipids, and minerals with covalent β-(1-4)-glyosidic bonds making these materials difficult to digest by poultry (Leeson and Summers, 2001).

Because poultry do not possess enzymes that can break β-(1-4)-glyosidic bonds, shrimp waste materials must first be processed before being used as feed material.

One way to convert organic material into new and useful products with better nutritional value is by using microbial bioprocessing. Microbial bioprocessing of shrimp waste materials can be done sequentially by first deproteinating with *Bacillus licheniformis*, then demineralizing with *Lactobacillus* sp., and finally terminating the process by addition of *Saccharomyces cerevisiae* which produces amylase, lipase, protease, and other enzymes.

Native chicken is a type of poultry that is popular and across the archipelago. Bioprocessing of shrimp waste materials in order to use them as a dietary ingredient for native chicken is expected to improve digestibility because its nutrients will have been released from their bonds with chitin.

Apparent digestibility can be defined as the parts of feed substances that are not secreted in the feces. In other words, apparent digestibility can be interpreted as the amount of nutrients digested and absorbed in the digestive tract. Thus, the more nutrients that are absorbed by the body, the higher the digestibility of the feed substance, which is an indicator of high quality feed.

MATERIALS AND METHODS

Materials, Equipment, and Experiments

Microbial isolates used were *B. licheniformis, Lactobacillus* sp., and *S. cereviseae*. Fresh, raw shrimp waste materials (the head and shell) were obtained from a local company exporting frozen shrimp. Other ingredients included distilled water, glucose, yeast extract, tryptone, NaCl, NaOH, azokasein reagent, borate buffer, phosphate buffer, citrate buffer, bicarbonate buffer, oxygen gas, and bovine serum albumin. Equipment included (reaction vessel), a water bath, an auto-shaker bath, an autoclave, beakers, a Bunsen burner, petri dishes, a porcelain cup, a Nimac CR 21G centrifuge, funnels, a Knick pH meter, a Novaspec II spectrophotometer, test tubes, furnaces, a high-performance liquid chromatograph. The nutrient concentrate created after bioprocessing of shrimp waste materials was fed to 27 native chickens (average weight, 1139.86 ± 111.86 g) which were placed in a 20x40 x30 cm metal cage. Native chickens were obtained from the Jatiwangi, Majalengka, and West of Java, Indonesia.

Scope of Research

1) Bioprocessing of shrimp waste using *B. licheniformis*, *Lactobacillus* sp., and *S. cereviseae*, followed by analysis of the nutrient content in the processed product.
2) Determination of the quality of bioprocessed products (nutrient concentrate) by measuring their protein digestibility and metabolizable energy in native chickens.

Experimental Procedure

1) Microbial bioprocessing was completed with the following steps:

(a) Deproteination

First, a starter inoculum was prepared by cultivating *B. licheniformis* in a containing 50 ml of sterile broth. The bacterial broth was then incubated for 2 days at 50°C. Second, bacteria were allowed to ferment in an auto-shaker bath. Shrimp waste materials were put into stainless steel jars, inoculated with an inoculum of 2% (v/w), and then incubated inan auto-shakerbath for 1-3 d at 45°C.

(b) Demineralization

This step dissolves minerals from shrimp waste materials that were deproteinated as described above. First, a starter inoculum of *Lactobacillus* sp. was cultivated in a containing 50 ml sterile broth. The bacterial broth was then incubated for 2 d at 45°C. Second, bacteria were allowed to ferment in an auto-shakerbath. Then, a 2% (v/w) inoculum of *Lactobacillus* sp. was added to the deproteinated products (Saefulhadjaret.al, 2013), and then incubated for 1-3 d at 45°C with a rotation of 120 rpm.

(c) Fermentationby S. cerevisiae

First, an inoculum was made from a pure culture of *S. cerevisiae* grown on an agarose slant and incubated at 30°C for 3 d. An inoculum of 3% (v/w) *S. cerevisiae* was added to the demineralized products (Haetami, et al., 2010), and then incubated for 1-3 d at 35°C. Fully bioprocessed products were then analyzed for protein, lipid, calcium, and phosphorus content, as well as for gross energy.

2) Measurement of Protein Digestibility

Native chicken placed in individual cages. After 14 h, chickens were slaughtered and their large intestines removed to obtain feces samples. Feces samples were dried and analyzed for nutrient and protein content, whereas the indicator (lignin in rations and feces) was analyzed according to Van Soest (1979). Protein digestibility was calculated according to an equation presented by Ranjhan (1980):

$$\text{Protein Digestibility} = 100\% - 100\left[\left(\frac{\%\ dietary\ lignin}{\%\ lignin\ in\ feces}\right)\left(\frac{\%\ crude\ protein\ in\ feces}{\%\ dietary\ protein}\right)\right]$$

RESULTS AND DISCUSSIONS

The nutrient content (crude protein, crude lipids, calcium, and phosphorus) after each bioprocessing time point for each microbe is presented in Table1. The highest crude protein content (47.19%) was obtained after deproteination by *B.licheniformis* for 2 days (W2), and lowest after 1 day (W1,41.92%). Similarly the highest phosphorus content was

obtained at W2 was 2.24%. The lowest crude lipid and highest calcium content obtained at W3 were 8.75% and 6.95%, respectively. *B. licheniformis* served to break the covalent β-(1-4)-bonds between chitin and proteins within shrimp waste materials. A bioprocessing duration of 2 d was optimum for microbe growth, release of proteins from chitin, and increasing the protein content of the processed product.

Table 1.Mean crude protein, crude lipid, calcium, and phosphorus content in shrimp waste material over time after each microbial bioprocessing step

Treatments	Crude Protein	Extract ether	Ca	P
	……..…….....…….%.....			
*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, *Bacillus licheniformis*(step 1, deproteination);+*Ls*, product of step1 + *Lactobacillus sp.* (step 2, demineralization); +*Sc*, product of step 2 +*Saccharomyces cerevisiae*(step 3, fermentation);W, processing time; W1 = 1 d; W2 = 2 d; W3 = 3 d.

Chitin binds strongly to proteins, lipids, and mineralsvia covalent β-(1-4)-glyosidic bonds making them difficult forpoultry to digest (Leeson and Summers, 2001). Degradation of chitin-bound protein in shrimp waste materials by *B. licheniformis* must be followed by addition of *Lactobacillus* sp. to release minerals associated with denatured proteins and chitin. Table 1 indicates that a bioprocessing duration of 2 released the highest crude protein content at each step in the shrimp waste material degradation process. The bioprocessing times examined (W1-W3) produced significantly different ($P<0.05$) crude protein, crude lipid, calcium, and phosphorus content. Results of Duncan's Multiple Range analysis of bioprocessed products of shrimp waste materials (i.e., the nutrient concentrate) at W1-W3 are presented in Table 2.

Table2. Duncan's multiple range analysis of the effect of bioprocessing with steps of three microbe's duration on the nutrient content of shrimp waste materials

Treatments*	Crude Protein	Crude Lipid	Ca	P
	……..……..%.....			
W1	43.50 ^{A}	11.44 ^{A}	7.35 ^{A}	2.31 ^{A}
W2	48.50 ^{B}	7.81 ^{B}	7.57 ^{A}	3.14 ^{B}
W3	47.69 ^{B}	7.42 ^{B}	7.72 ^{A}	2.96 ^{B}

* Bl, *Bacillus licheniformis*(step 1, deproteination);+*Ls*, product of step1 + *Lactobacillus sp.* (step 2, demineralization); +*Sc*, product of step 2 + *Saccharomyces cerevisiae* (step 3, fermentation);
W, processing time; W1 = 1 d; W2 = 2 d; W3 = 3 d

The results showed that a bioprocessing duration of 2 d (W2) by *B. licheniformis*, *Lactobacillus* sp., and *S. cerevisiae* was optimal for microbial enzyme activity as shown by the highly significant differences ($P<0.01$) in crude protein, crude lipid, and phosphorus content of processed products (Table 2). Differences in protein content with bioprocessing duration were most likely due to the level of microbial growth obtained in that time frame. Microbial growth can be divided into three phases: the "slow phase," when cells undergo metabolic and physiological activities which prepare them for division; the "exponential phase, "a period of accelerated growth; and a stationary or resting phase (Battley and Edwin, 1987; Fardiaz, 1988; Balia, 1993). The ability of microbesto quickly

multiply their numbers and produce functional enzymes which break down substrates affects the quality and quantity of the final processed product, and is directly reflected in the bioprocessing time. Furthermore, a higher dose of inoculum and longer fermentation time has been shown to create larger microbial populations and increased degradation of substrates (Aisjah, 1995).Hall and De Silva (1992) suggested that microbes which create acidic conditions, such as *Lactobacillus* sp., result in the formation. For example, citric acid produced in the *Lactobacillus* sp. fermentation process reacts with calcium carbonate to form calcium citrate, carbon dioxide, and water.

Fermentation determines the amount of time needed to establish of microbial populations which is directly link to the development of enzymes which break down substrates and affect the final product. A longer bioprocessing time allows for an increase in the microbial population enabling more substrate components to be overhauled. Microbes experiencing high growth rates continue to rise until they reach the stationary phase. This is in accordance with current results which showed that a longer fermentation time (W3) did not produce a higher content of phosphorus in the processed product.

B. licheniformis is a species of bacteria that is capable of producing protease and chitinase in relatively high amounts (Alam et al., 1996). The highest content of crude protein, crude lipid, calcium, and phosphorus in bioprocessed products of shrimp waste materials was at W2. These results were further tested to determine quality of the processed product (nutrient concentrate) by examining their digestibility in native chicken rations.

Tabel 3. Effect bioprocessed products (concentrate nutrient) by *Bacillus licheniformis*, *Lactobacillus* sp. And *Saccharomyces cerevisiae* on protein digestibility and metabolizable energy in native chickens

Treatment	Protein Digestibility %	Metabolizable Energy kkal/kg
Bl+Ls+Sc. W1	62.90 B	2569.24 B
*Bl+Ls +Sc.*W2	72.91 A	2613.90 A
*Bl+Ls +Sc.*W3	71.73 A	2629.09 A

W, processing time; W1 = 1 d; W2 = 2 d; W3 = 3 d

Statistical analysis showed that the protein digestibility of the nutrient concentrate at W2 and W3 were both significantly ($P<0.05$) higher than its digestibility at W1. These results demonstrated that components of bioprocessed products have higher biological value than the original material. In addition, bioprocessing duration plays a key role in transforming organic material into other useful products, especially by utilizing biolysis and biosynthesis is events. Generation of additional microbial cells or biomass increases the concentration of enzymes, primary and secondary metabolites, and chemical compounds used by microbes to bioprocess materials (Laskin and Hubbert, 1973).

Poultry, like most animals, have digestive limitations, especially to foods containing chitin and a high amount of crude fiber. This is because poultry cannot produce cellulase or chitinase, leaving any chitin or crude fiber present in the feed to bind other key nutrients, causing them to be excreted in the feces (Tulung, 1987; Rev., 1997). Chitin is a compound that cannot be digested by poultry (Leeson and Summers, 2001); therefore shrimp waste materials should first be processed before being included in poultry feed.

Bioprocessed products created by *B. licheniformis*, *Lactobacillus* sp., and *S. cerevisiae* in the current study have better protein digestibility. This is likely because *B. licheniformis* produce proteases and chitinases in relatively high amounts (Alam et al., 1996; Rahayu et al., 2004), which degrade proteins and break protein-chitin bonds. Then, acidic conditions created by *Lactobacillus* sp. dissolve minerals that are bound to chitin and other proteins that have been unraveled by exposure to *B. licheniformis* enzymes. Finally, fermentation with *S. cerevisiae* helps improve the digestibility of deproteinated and demineralized

components with its carbohydrase and protease enzymes. Furthermore, the optimum bioprocessing time of 2 d provides these microbes with the opportunity to produce their respective enzymes and allow them to process the appropriate substrates.

The high value of metabolic energy as a result of the use of feed supplement of W3 in the ration of native chickens was 2629 kcal/kg, as reflection of the high quality of products. This is consistent with the opinion of Shurtleff is a and Aoyagi (1979), which states that the product bioprocess cause changes in complex molecules or organic compounds such as proteins, carbohydrates and fats into molecules simpler and easier to digest. Low metabolizable energy describes a lot of energy is lost through the stool, otherwise the value of a high metabolic energy describes the energy that is lost through the feces slightly.

The use of high chitinous feed ingredient in poultry rations, but can degrade components that are easy to digest also decreased enzyme activity solver food substances, such as enzymes that aid digestion of carbohydrates, protein, and fat (Parrakasi, 1983; Klasing, 2000). Chitin is a chemical compound that cannot be digested by the digestive enzymes of poultry (Leeson and Summers, 2001), therefore the shrimp waste should be processed first. Bastaman (1989) suggested that the chitin polymer chains each typically consisting of 2000 to 5000 monomer units of N-acetyl-D-glucosamine (2-acetamino-2-deoxy-D-Glucose) that through bond β (1-4) glucoside. Chitin is a macromolecule amorphous solid form, and can be biodegradable (biodegradable) mainly by bacteria producing enzymes protease and chitinase (Stephen, 1995).

Product of fermentation process with *Bacillus licheniformis* deproteinization and continued mineralization with *Lactobacillus* sp., and *Saccharomyces cerevisiae* has a better protein digestibility value. This is because the bacterial species *Bacillus licheniformis* capable of producing protease and chitinase in relatively high amounts (Alam et al., 1996; Rahayu et al., 2004), and acidic conditions created by *Lactobacillus sp*. mineral shed attached to the protein that has been unraveled. Furthermore, the fermentation process premises.

CONCLUSIONS

A bioprocessing time of 2 d was optimum for degradation of shrimp waste materials (the head and shell) using *B. licheniformis*, *Lactobacillus* sp., and *S. cerevisiae*, as it produced the highest nutrient concentrate and protein digestibility value (>70%). The highest crude protein, crude lipid, calcium, and phosphorus content were 48.50%, 7.81%, 7.57%, and 3.14%, respectively. Lastly, the best digestibility of bioprocessed products in the native chicken diet was 72.91%, and Metabolizable Energy value was 2629.09 kcal/kg.

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