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Research Article
Value of Metabolizable Energy and Digestibility of Nutrient Concentrate from Fermented Shrimp Waste for Domestic Chickens

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Abstract

Background and Objective: Improving the quality of waste containing high levels of chitin through bioprocesses that utilize the services of the microbes *Bacillus licheniformis*, *Lactobacillus* sp. and *Saccharomyces cereviseae* can generate a high-quality product that can meet the requirements of domestic chickens. The objective of this study was to determine the optimal bioprocessing conditions to make a nutrient concentrate, as well as to describe its biological quality for the domestic chicken. **Materials and Methods:** This study utilized experimental methods in the laboratory that consisted of a completely randomized design with six treatment rations (R_0, R_1, R_2, R_3, R_4 and R_S) that were replicated five times. The data were subjected to analysis of variance and the differences between treatments were tested by Duncan's multiple range test. **Results:** Bioprocessing shrimp waste with *Bacillus licheniformis* for two days, followed by *Lactobacillus* sp. for two days and finally, *Saccharomyces cereviseae* for two days resulted in the best nutrient content (48.50% crude protein, 7.81% crude fat, 7.57% calcium and 3.14% phosphorus), The metabolizable energy value and protein digestibility of the nutrient concentrate for the domestic chicken were 2613.90 kcal kg^{-1} and 72.91%, respectively. **Conclusion:** Processing shrimp waste for poultry feed, especially for domestic poultry, can be achieved through multilevel fermentation technology that uses microbial services to produce a nutrient concentrate with good chemical and biological qualities.

Key words: Bioprocess, digestibility, metabolizable energy, nutrient concentrate, shrimp waste

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The optimal performance of domestic chickens can only be realized by providing quality, balanced rations that meet the dietary requirements in sufficient quantities. The food substances in the ration can be supplemented by adding feed additives (bioprocessing products), so it is necessary to find alternative, non-food feed additives that are inexpensive, easy to obtain and of high quality. One approach is the utilization of shrimp waste that is processed by fermentation technology[1,2].

Due to its high protein and mineral contents, shrimp waste can potentially be used as an alternative source of nutrients for chicken feed. However, the constraint on the use of shrimp waste is that the nutrients are bound with chitin, which is resistant to the digestive enzymes of the chicken[2-4].

Bioprocessing can be performed in two stages, namely, deproteination using *Bacillus licheniformis* and demineralization with *Lactobacillus* sp. and *Saccharomyces cereviseae*. The bacterium *Bacillus licheniformis* is capable of producing relatively high amounts of proteases and chitinases[5-7]. *Lactobacillus* sp. is a microbe that decomposes glucose, sucrose, maltose and lactose into lactic acid, which results in mineral deposits[8]. S*accharomyces cereviseae* is a yeast that can produce amylase enzymes, lipases, proteases and other enzymes that can facilitate the breakdown of food substances in the digestive organs[9,4].

The nutritional potency of feed can be determined through chemical analysis; the true value is shown by what remains after the ingredients are ingested, absorbed and metabolized[6,10]. The metabolizable energy and digestibility values of feed increase with the amount of food substances that can be absorbed by the chicken and are indicators of the quality of feed processing products[11]. The use of the nutrient concentrate that is a product of bioprocessing in chicken rations is expected to improve the digestibility value of the feed because the nutrients are free from the chitin bond; the crude fibre/chitin ratio of food substances affects their digestibility[10].

The objectives of this study were (1) To describe the effects of bioprocessing conditions (fermentation time by *Bacillus licheniformis*, *Lactobacillus* sp. and *Saccharomyces cereviseae*) on the content of the nutrient concentrate and (2) To determine the biological quality of the nutrient concentrate product for the domestic chicken by measuring the metabolizable energy and protein digestibility values.

MATERIALS AND METHODS

Producing concentrated nutrients: The experimental materials included shrimp waste; *Bacillus licheniformis*, *Lactobacillus* sp. and *Saccharomyces cereviseae* isolates; distilled water, glucose, yeast extract, tryptone, NaCl, NaOH, azocasein reagent, borate buffer, phosphate buffer, citrate buffer, bicarbonate buffer and bovine serum albumin. The tools were steel jars (reactor), a water bath, an auto-shaker bath, an autoclave, beakers, Bunsen burners, Petri dishes, porcelain dishes, a Nimac CR 21G centrifuge, funnels, a pH meter, a spectrophotometer, test tubes, a furnace and a machine grinder.

Stages of making the nutrient concentrate

Deproteination: A starter *Bacillus licheniformis* inoculum was cultivated in 50 mL of broth and incubated for two days at a temperature of 50°C and a dose of 2% inoculum (v/w)[2] and fermentation liquid substrates with standard solution were placed in an auto-shaker bath for one, two and three days at 45°C and 120 rpm.

Demineralization: A starter *Lactobacillus* sp. inoculum was cultivated in a mixed standard solution (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% $CuSO_4$) and fermented in an auto-shaker bath. *Lactobacillus* sp. inoculum was added to the deproteination products according to treatment (one, two and three days at a temperature of 45°C at 120 rpm).

Fermentation with *Saccharomyces cerevisiae*: Pure *Saccharomyces cerevisiae* cultures were incubated for three days and an inoculum was prepared in a standard solution (0.5% NH_4NO_3, 0.05% KCl, 0.05% $MgSO_4.7H_2O$, 0.01% $FeSO_4.7H_2O$ and 0.001% CuSO4.5H2O) and fermented in an auto-shaker bath. After obtaining the demineralization product, 3% *Saccharomyces cereviseae* inoculum (v/b)[12] was added and then incubated for one, two and three days at 35°C.

Biologically testing the nutrient concentrate: Up to 42 domestic chickens (Sentul strain) were placed in individual cages (20×40×30 cm). The chickens were fasted for 24 h before being given the feed treatment.

Measurement of metabolizable energy: After fasting for 24 h, each chicken was treated; 100 g $bird^{-1}$ of nutrient concentrate was given by force feeding. Drinking water was provided *ad libitum*. After feeding, the excreta were collected

and sprayed with 5% boric acid. The excreta were collected from the shelter after 24 h and then cleaned of impurities, weighed, dried and analysed in the laboratory. The measurement of metabolizable energy followed previous methods[13].

Measurement of digestibility values: The nutrient concentrate (100 g bird^{-1}) was provided by force feeding. A stool sample was obtained following the methods of Sklan and Hurwitz[3] as cited by Wiradisastra[14] with modifications[15], using internal indicators (lignin). After 14 hours of feeding, the chickens were slaughtered and the large intestines were removed. The stool samples were then dried and analysed in the laboratory.

Observed variables

- **Chemical analysis of the products:** The protein, fat, calcium and phosphorus contents of the nutrient concentrate were tested using proximate analysis methods[16]

- **Metabolizable energy of the nutrient concentrate test:**

$$MEn\left(\frac{kcal}{kg}\right)=\frac{(GEr\times C)-(Ne\times GEe)-\left(\frac{C\times Nr}{100}\right)-\left(\frac{Ne\times En}{100}\right)}{C}\times 8.22$$

Where

MEn = Metabolizable energy of a bioprocess product (nutrient concentrate) corrected for a fixed nitrogen content (kcal kg^{-1})
GEr = Gross energy of bioprocess products (kcal kg^{-1})
GEe = Gross excreta energy (kcal kg^{-1})
C = The number of bioconversion products consumed (kg)
Ne = Amount of excreta (kg)
Nr = Nitrogen bioconversion products (%)
En = Excretory nitrogen (%)
8.22 = Constant energy value (kcal g^{-1})

- **Digestibility of the nutrient concentrate test:**

$$Digestibility(\%)=100\%-100\left(\frac{\%lignin\ ration}{!\%lignin\ faeces}+\frac{\%protein\ in\ faeces}{\%protein\ in\ ration}\right)$$

Experimental design: The experiments were performed in the laboratory using a complete randomized design consisting of six treatment rations (R_0, R_1, R_2, R_3, R_4 and R_S) that were replicated five times.

The treatments were as follows:

R_0 = Basal low-protein ration (15% crude protein) with no nutrient concentrate
R_1 = Basal ration containing 5% nutrient concentrate
R_2 = Basal ration containing 10% nutrient concentrate
R_3 = Basal ration containing 15% nutrient concentrate
R_4 = Basal ration containing 20% nutrient concentrate
R_S = High-protein ration (18%) with no nutrient concentrate

Ethics approval: Animal procedures were approved by the Faculty of Animal Husbandry of Padjadjaran University vide letter number 963/UN6.J1/LT/2017.

The experiments were conducted using the poultry cages of the Faculty of Animal Husbandry of Padjadjaran University, Jatinangor-Sumedang, West Java, Indonesia and the data were collected and analysed at the Laboratory of Animal Feed Chemicals, Department of Nutrition and Feed Technology, Faculty of Animal Husbandry, Padjadjaran University.

Data analysis: The data were analysed using the Statistical Analysis System (SAS, version 9.1, SAS Institute Inc., Cary, NC, USA). Variables were analysed by one-way analysis of variance (ANOVA) using the statistical programme SPSS version 19 (SPSS Inc., Chicago, IL). Significantly different means were determined by Duncan's multiple comparison test at the level of 0.05.

RESULTS

The average nutrient contents of shrimp products bioprocess with *Bacillus licheniformis*, *Lactobacillus* sp. and *Saccharomyces cereviseae* are presented in Table 1.

The bioprocessed product (nutrient concentrate) with the highest crude protein content was obtained with the W_2 treatment (48.50%) and the lowest resulted from the W_1 treatment (43.50%). Similarly, the highest phosphorus content was obtained from the W_2 treatment (3.14%), while the lowest crude fat (7.42%) and highest calcium contents (7.72%) were obtained with the W_3 treatment. The results showed that bioprocessing with *Bacillus licheniformis* for two days, *Lactobacillus* sp. for two days and *Saccharomyces cerevisiae* for two days (W_2) is an effective method for producing a product (nutrient concentrate) with the best protein content. The contents of crude protein, crude fat, calcium and phosphorus during bioprocessing by *Bacillus licheniformis*, *Lactobacillus* sp. and *Saccharomyces cerevisiae* are presented in Fig. 1.

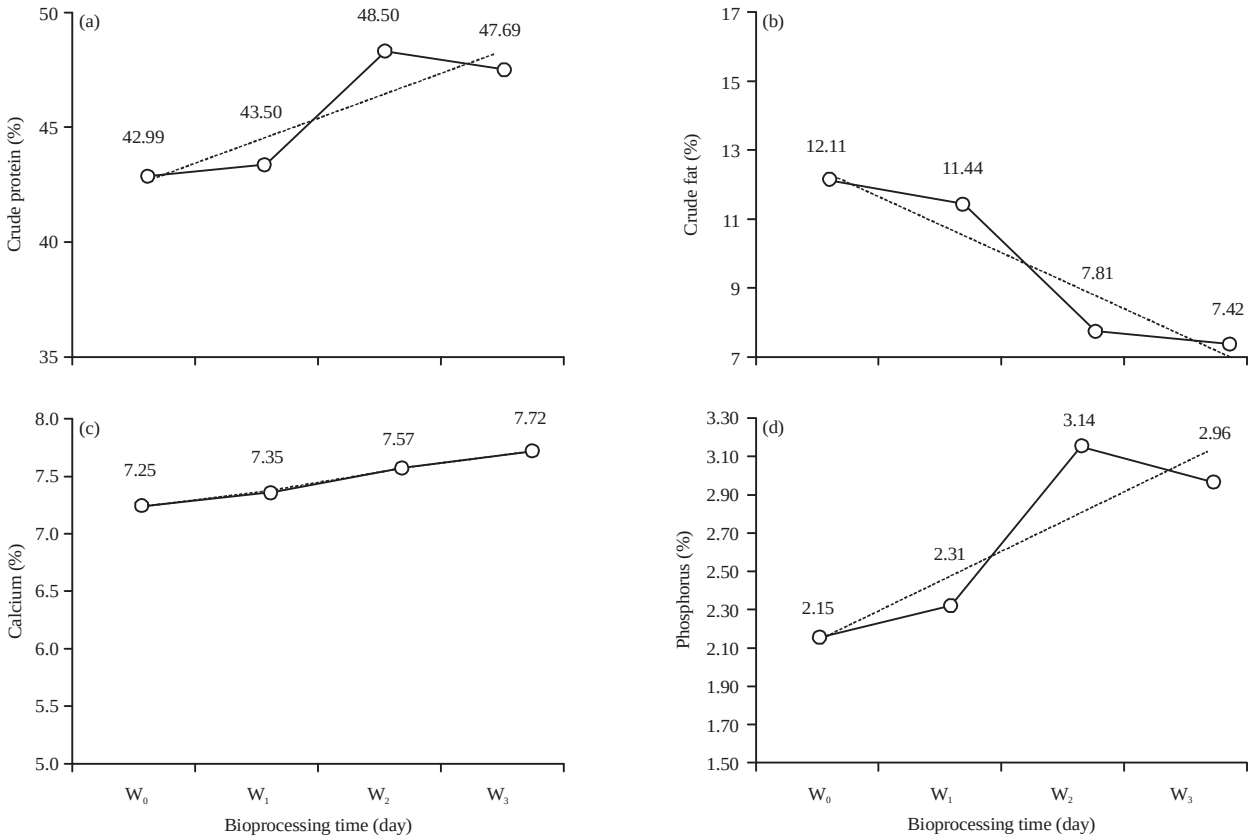

Fig. 1(a-d): The crude protein, crude fat, calcium and phosphorus contents during bioprocessing by *Bacillus licheniformis*, *Lactobacillus* sp. and *Saccharomyces cerevisiae*

Table 1: Crude protein, ether extract, calcium and phosphorus contents of shrimp waste products bioprocess by *Bacillus licheniformis*, *Lactobacillus* sp. and *Saccharomyces cerevisiae*

	Percentage			
Treatment	Crude protein	Ether extract	Calcium	Phosphorus
Bl+Ls+Sc. (W_1)	43.50^b	11.44^a	7.35^b	2.31^b
Bl+Ls +Sc. (W_2)	48.50^a	7.42^b	7.57^a	3.14^a
Bl+Ls +Sc. (W_3)	47.69^a	7.42^b	7.72^a	2.96^a

W_1: One day of fermentation, W_2: Two days of fermentation, W_3: Three days of fermentation. a, b: Means with no common superscript differ significantly based on the standard mean error: $p<0.05$

Table 2: Average metabolizable energy and protein digestibility values of the nutrient concentrate in domestic chickens

Treatments	Metabolizable energy (kcal kg^{-1})	Protein digestibility (%)
Bl+Ls+Sc. (W_1)	2569.24^b	62,90^b
Bl+Ls +Sc.(W_2)	2613.90^a	72,91^a
Bl+Ls +Sc.(W_3)	2629.09^a	71,73^a

W_1: One day of fermentation, W_2: Two days of fermentation, W_3: Three days of fermentation. a, b: Means with no common superscript differ significantly based on the standard mean error: $p<0.05$

The quality of the nutrient concentrate is not only determined by its nutrient contents; its true value can be determined by measuring the metabolizable energy and protein digestibility values. The average metabolizable energy and protein digestibility values of the nutrient concentrate in domestic chickens are shown in Table 2.

The metabolizable energy value of the nutrient concentrate under the W_2 treatment (2613.90 kcal kg^{-1}) was not significantly different from that of the W_3 treatment (2629.09 kcal kg^{-1}) but was significantly ($p<0.05$) higher than that of the treatment under the W_1 treatment (2569.24 kcal kg^{-1}). Similarly, the protein digestibility under the W_2 treatment (72.91%) was not significantly different from that of the W_3 treatment (71.73%) but was significantly ($p<0.05$) higher than that of the treatment under the W_1 treatment (62.90%).

DISCUSSION

Effect of treatment on the contents of the nutrient concentrate: Differences in protein content due to bioprocessing time are caused by the growth of microbes, which can be divided into three phases based on the rate of growth; the slow phase when the cells perform metabolizable and physiological activities to prepare for cleavage, the exponential or accelerated growth phase and the stationary or resting phase[17,18,5]. The duration of fermentation is related to the size of the microbial population, which is likely to determine the speed of microbial development that produces the enzymes necessary to break the substrate that, in turn, affects the final product. The longer the bioprocessing time, the more abundant the microbial populations and the more substrate components that are overhauled[19]. *Bacillus licheniformis* is a bacterium that is capable of producing relatively high amounts of proteases and chitinases[20,6]. According to Ranjhan[6], the enzyme protease can be obtained from proteolytic microbial metabolites, including *Bacillus licheniformis*. It has been suggested that acid-forming microbes, such as *Lactobacillus* sp., lead to the formation of complex salts[12] and it has also been suggested that mineralization can be achieved by dissolving minerals contained in shrimp waste through the process of acid fermentation[8]. The citric acid produced in the fermentation process with *Lactobacillus* sp. reacts with calcium carbonate to form calcium citrate, carbon dioxide and water. The presence of the phosphorus released from the chitin bonds means that the fermentation process using *Lactobacillus* sp. produces an acidic atmosphere that can form mineral deposits. *Saccharomyces cerevisiae* is a yeast that can produce amylase enzymes, lipases, proteases and other enzymes that can facilitate the processing of food substances in the digestive organs[9].

The fermentation time determines the size of the microbial population, which is subsequently connected to the microbial development that produces the enzymes that remodel the substrate and affect the final product. The longer the bioprocessing time, the larger the microbial populations and the more substrate components are overhauled. Microbes experience an increasing rate of growth until the stationary phase and this is consistent with the results of this research that longer bioprocessing does not produce a product with a higher phosphorus content.

The metabolizable energy and protein digestibility values of the nutrient concentrate: The metabolizable energy value is a widely adopted measure of feed quality due to its practical applications, especially in the preparation of poultry rations, such as those for domestic chickens. Measurements of energy are suitable for all purposes including the assessment of overall health, growth and fattening, so metabolizable energy can be applied to various metabolic processes in the body[21,22]. The metabolizable energy value of nutrient concentrate can be improved by microbiological treatment, such as through the process of deproteinization by *Bacillus licheniformis*, *Lactobacillus* sp. and *Saccharomyces cerevisiae*. This is consistent with the results of Wahju[22] that the metabolizable energy value of millet can be increased by immersing it in water before providing it to chickens or that the energy of rations containing barley can be enhanced using enzyme preparations obtained from fermentation with microbes.

The highest nutrient protein digestibility value was obtained from the W_2 treatment (72.91%) and the lowest value resulted from the W_1 treatment (62.90%). Similarly, the highest metabolizable energy value was obtained from the W_2 treatment (2,614 kcal kg^{-1}). Biological tests demonstrated that bioprocessing with *Bacillus licheniformis* for two days, *Lactobacillus* sp. for two days and *Saccharomyces cerevisiae* for two days (W_2) produces the best nutrient concentrate based on the digestibility and metabolizable energy values.

Processed feed products have a higher biological value than their original ingredients, which is consistent with the opinion of Winarno[23] that processing can convert an organic material into another useful product with added value, especially through biosynthesis and biolysis. The products that can be produced include microbial or biomass cells, enzymes, primary and secondary metabolites and chemical compounds derived from bioprocessing by microbes[24]. Chickens face limitations when digesting certain food substances, especially feed ingredients that contain high amounts of chitin and crude fibre compounds, because poultry cannot produce cellulose and chitinase enzymes, so chitin and coarse fibre can bind digestible food substances with faeces[25,22]. This is consistent with the results of a study conducted by Abun[2] who reported that the chitin content of shrimp waste is quite high without processing, i.e., 20.11%.

Fermentation products generated by deproteinization with *Bacillus licheniformis* followed by mineralization with *Lactobacillus* sp. and *Saccharomyces cerevisiae* have better metabolizable energy and protein digestibility values. This is because *Bacillus licheniformis* is a bacterial species capable of producing relatively high quantities of proteases and chitinases[26] and the acidic atmosphere created by *Lactobacillus* sp. allows the minerals bound to decomposed proteins to be shed. Furthermore, fermentation with *Saccharomyces cerevisiae* improves digestibility through the production of the enzymes carbohydrase and protease.

CONCLUSION

The results of the current study showed that optimal shrimp waste bioprocessing was achieved through gradual fermentation using *Bacillus licheniformis* followed by *Lactobacillus* sp. and *Saccharomyces cerevisiae* for two days each; this process yielded the best nutrient concentrate. The metabolizable energy value of the nutrient concentrate for domestic chickens was 2,614 kcal kg^{-1} and the protein digestibility value was 72.91%.

SIGNIFICANCE STATEMENT

This study discovers the synergy between microbial *Bacillus licheniformis*, *Lactobacillus* sp. and *Saccharomyces cerevisiae* with bioconversion time that can be beneficial for digestion and absorption of nutrients in the digestive system of domestic chicken. This study will help the researcher to uncover the critical areas of low efficiency of nutrient metabolism in domestic chickens that many researchers were not able to explore.

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