The quality of the shrimp head meal fermented using *Bacillus* sp. PAS7 isolates at different dosages of inoculum as fish feedstuff

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Abstract. Shrimp head is a feedstuff that potential to be developed as an alternative source of protein to substitute fish meal. It is because the shrimp head contains a high protein and its availability in Indonesia is quite abundant. However, this protein can not be utilized by fish because it contains high crude fiber (chitin) and ash which is difficult to digest. This study aimed to improve the quality of shrimp head meal by fermentation using *Bacillus* sp PAS7 at different inoculum doses and evaluate its nutrient content. The study was conducted in a completely randomized design (CRD) of four treatments and three replications. The treatment was carried out by varying concentrations of 0, 3, 5 and 7% per gram (weight) of shrimp head meal (v/w). Fermentation was done for three days at 28°C. The parameters measured were reducing sugar, soluble protein and proximate analysis (crude protein, crude fiber, ash and crude fat). The results showed that all dosage treatments had crude protein, soluble protein and crude fiber, which were better than control. Where dose of 7% had the highest soluble protein content (6.25 mg mL⁻¹) and the lowest crude fiber content (15.02%) compared to other treatments.

1. Introduction
One of the procedures that determine the success of fish farming is the availability of feed ingredients. In aquaculture, feed plays an essential role because almost 60-80% of production costs come from feed [1]. Intensive fisheries cultivation requires the availability of sufficient and suitable quality feed, relatively inexpensive and sustainable prices. However, the high cost of fish meal as one of the primary raw material sources of protein in fish feed indirectly causes fish feed prices to become expensive. The demand for fish meal which continues to increase every year, is not matched by its availability [2]. It is due to the declining production of capture fisheries in Indonesia so it needs to be imported from other countries. Therefore, efforts should be made to replace fish meal with cheaper alternative protein sources, one of which is shrimp head meal.

Shrimp head is very potential to be used as a food source of animal protein. It happens because its availability is quite abundant in Indonesia, where the shrimp head is one of the byproducts of shrimp processing in cold storage which has a proportion of shrimp head weights around 34% –45% of the total weight of shrimp. Besides, the shrimp head also contains good nutrients. According to Hetrampf and Pascual [3], based on the composition of dry matter, shrimp heads contain 43.2% crude protein,
5.6% fat, 15.8% crude fiber, 33.0% ash, and 2.4% BETN. However, the protein in shrimp head meal can not be used by fish because of its high chitin and ash content [4, 3] reported that the levels of chitin in shrimp head meal could reach 17.6%. Meanwhile, according to Melati et al [5] chitin content in shrimp waste is around 30% of its dry weight.

Due to limitations in the use of this raw material, it is vital to conduct research related to improving the quality of shrimp head meal. So that the content is easier digested and its utilization in fish feed can be optimized. The digestibility of raw material can be improved by the fermentation process. Fermentation made raw materials simpler and easier to digest from its original products [6]. By fermentation technology, the improvement can be conducted and the nutritional value of local feed raw materials can be increased so that they can be optimally utilized for fish feed raw materials [7]. Through the right dose of inoculum, the fermentation process will run optimally and the nutritional value of shrimp head meal will increase. Based on these considerations, a study was conducted to obtain an appropriate dose of inoculum for improving the quality of shrimp head meal through fermentation using the *Bacillus* sp. PAS7 isolate and evaluating its nutrient content.

2. Methods
2.1. Time and place of research
This research was conducted from July 2018 to February 2019 at the Laboratory of Feed Nutrition and Technology, Institute for Freshwater Fisheries Research and Fisheries Extension, Bogor, West Java, Indonesia.

2.2. Tools and materials
The tools used in this study were analytical balance, autoclave, incubator, oven, hotplate, test tubes, Erlenmeyer, magnetic stirrer, aluminum foil containers, water bath, measuring pipettes, vortex, beaker cups, pH meters, measuring cups, bunsen, laminar flow, spatula, centrifuge and measuring flask. While the research materials used include shrimp head meal, Trypticase Soy Broth (TSB) media, Trypticase Soy Agar (TSA) media, 1% Carboxymethylcellulose (CMC) liquid media, 1% CMC solid media, distilled water, dinitro-salicylic acid (DNS), Bradford reagents, H2SO4, NaOH, citric acid buffer, phosphate buffer, filter paper, heat-resistant plastic, rubber band, masking tape, spiritus, pipette tip.

2.3. Inoculum production
One inoculating loop of isolate *Bacillus* sp. PAS7 was inoculated on 10 mL TSB media then incubated for 24 hours at 28°C on static conditions. After that, 1 mL of bacterial culture was taken then inoculated on 9 mL of TSB media containing 1% CMC. Incubated for 24-48 hours at 28°C on static conditions [8]. The bacterial culture is then ready to be used for fermentation.

2.4. Determination of inoculum dosage for shrimp head meal fermentation process
A total of 50 g shrimp heads meal added with 70 mL sterile distilled water in a sterile aluminum foil container and then evenly stirred. Furthermore, *Bacillus* sp. PAS7 isolates were added at a dose of 0, 3, 5, and 7% (w/v) of the volume of the media and covered with plastic. Dosage determination was based on the literature that uses fermentation doses between 3 to 9% [9, 10] but in this study used doses from 3 to 7% because the best results in the literature were at doses below 9%.

The inoculation process was carried out aseptically. Based on our preliminary studies, the temperature of 28°C and three days was the best combination of bacteria in hydrolyzing shrimp head meal. Therefore in this study, the shrimp head was then fermented under static conditions incubated at 28°C for three days. After the fermentation process was complete, the shrimp head meal was dried in an oven at 60 °C for 2-3 days.
2.5. Analysis of shrimp head nutrient content
Analysis of reducing sugar (glucose content) of fermented shrimp head meal refers to Miller [9] with a slight modification. One g of the fermented shrimp head meal was extracted with 20 mL of distilled water, filtered, put into a 100 mL volumetric flask, and then added aqua dest till the boundary mark. After that 1 mL of extract was taken and again diluted to 100 mL. 1 mL of sample in 0.05 M acetate buffer (pH 4.8) was added with 3 mL of DNS reagent and boiled for 5 minutes. After cooling, absorbance was measured with the Eppendorf biophotometer at a wavelength of 550 nm. As a standard, glucose 0.6-4 µmol mL⁻¹ was used in 0.05 M acetate buffer (pH 4.8). Analysis of the soluble protein content of the fermented shrimp head meal was carried out using the Abun et al [10] method. 0.5 g of fermented shrimp head meal doses of 0, 3, 5, and 7% added with 5 mL of Tris HCl pH 6.5 and then centrifuged at a speed of 10,000 rpm for 20 minutes. 0.5 mL samples were taken and combined with 0.5 mL Bradford solution then incubated at room temperature for 15 minutes. Absorbance was measured at a wavelength of 595 nm. As a standard Bovine Serum Albumin (100 mg in 100 mL distilled water) was used. The crude protein content of fermented shrimp head meal (doses of 0, 3, 5, and 7%) was carried out by the Kjeldahl method. The crude fiber content was measured by gravimetric method after heating the washing of samples in acid and base alternately. Ash content was determined by burning the sample in a furnace at 550° C.

2.6. Data analysis
Data were tabulated using Excel 2013 and statistically analyzed using ANOVA (Analysis of Variance) with SPSS software version 20.0.

3. Results and Discussion
Fermentation of shrimp head meal using Bacillus sp. PAS7, with different dosages, affects its glucose content. The highest glucose content was at a dose of 3% (0.028 mg mL⁻¹), and the lowest was at a dose of 7% (0.0207 mg mL⁻¹) (figure 1). The addition of Bacillus sp. PAS7 with a concentration of 3% produced the same soluble glucose levels as the control (dose 0%). However, the higher bacterial concentrations (doses of 5 and 7%), the lower levels of soluble glucose produced. It presumably because the glucose contained in shrimp head meal was used by bacteria for metabolism and growth [11]. Reported the same thing in the fermentation process of vegetables. The results of his research showed that the fermentation process for 80 hours reduced the reducing sugar levels from tomatoes, red chilies and carrot juice. According to him, the decrease occurred because the amylolytic activity of microbial strains used for fermentation was able to convert some of the starch in vegetables into sugar which was then converted again into lactic acid during organic acid metabolism. However, the sugar that was formed was not all converted to lactic acid but was used by microorganisms for metabolic processes.
Figure 1. Reducing sugar levels (mg mL⁻¹) of shrimp heads that fermented using *Bacillus* sp. PAS7 isolate at different doses (the same letter indicates no significant difference at 95% confidence interval).

Figure 2. Soluble protein content (mg g⁻¹) of shrimp head that fermented using *Bacillus* sp. PAS7 isolate at different doses (same letter indicates no significant difference at 95% confidence interval).

Fermentation with different dosage of *Bacillus* sp. PAS7 also affected (P < 0.05) the soluble protein content of shrimp head meal. Dosage of 5 and 7% resulted in higher and significantly different levels of soluble protein (P < 0.05) compared to controls (dose 0%). In this study, the highest levels of dissolved protein were at a dose of 7% (6.25 mg mL⁻¹), and the lowest was at a dose of 0% (2.67 mg mL⁻¹) (figure 2). The soluble protein in the addition of *Bacillus* sp. PAS7 isolate at a dose of 3% was not significantly different from the treatments of 0% and 5% (P > 0.05) but was lower and significantly different with a treatment of 7% (P < 0.05). The level of dissolved protein from shrimp head waste increases with increasing concentration of *Bacillus* sp PAS7 isolates. It happens because the higher the dose the more bacteria that secrete the protease enzyme to hydrolyze proteins into pure compounds such as peptides, amino acids or other nitrogen compounds. It is supported by the statements of Bradford [12] and Thorat et al [13] that bacteria play a role in the process of protein degradation that cause an increase in soluble protein in fermented products.

The effect of inoculum dose on protein, fat, and crude fiber content of shrimp head meal was presented in Table 1. In general, the crude protein level of fermented shrimp head meal increased and was significantly different from controls (P < 0.05), but between treatments, the dose was not significantly different (P > 0.05). According to Reerueangchai et al [14] an increase in crude protein during the fermentation process is thought to be due to the dry matter that is lost. The same was reported by Khairina et al [15] that changes in crude protein are not a reflection of actual changes but are relative changes related to the loss of dry matter as a result of the hydrolysis of carbohydrates and fats by microorganisms that used as energy sources. The levels of crude fat and ash of fermented shrimp heads meal at various doses of inoculum were not significantly different (P > 0.05). It showed that the amount of inoculum does not affect the content of crude fat and ash of fermented shrimp head meal.
Table 1. Proximate content (%) of shrimp heads meal that fermented using isolates of *Bacillus* sp PAS7 at different doses (same letter indicates no significant difference at 95% confidence interval).

| Doses of Inoculum | Crude Protein | Crude Fat | Ash | Crude Fiber |
|-------------------|---------------|-----------|-----|-------------|
| 0%                | 19.38±0.34a   | 0.84±0.12a| 38.45±0.35a| 20.24±0.28a |
| 3%                | 20.29±0.16b   | 0.87±0.10a| 39.06±0.67a| 18.24±0.45b |
| 5%                | 20.22±0.11b   | 0.61±0.01a| 38.7±0.36a | 18.11±0.25b |
| 7%                | 20.51±0.99b   | 0.7±0.15a | 40.06±0.34a| 15.02±0.91c |

The crude fiber content of shrimp head meal in this experiment was significantly (P <0.05) influenced by the addition of various doses of *Bacillus* sp. PAS7. The level of crude fiber at a dose of 0% was the highest (20.24%) while the lowest was at dose of 7% (15.02%). It showed that the fermentation process using *Bacillus* sp. PAS7 was able to reduce levels of shrimp head meal crude fiber. The results of this study are in line with the research of Halid [16] who reported that the inoculum concentration influences the levels of crude fiber of shrimp head waste when fermented for 30 days. The higher the concentration of inoculum given, the lower the crude fiber content of shrimp waste. The crude fiber measured on the shrimp head was most likely derived from chitin. Chitin is a structural polysaccharide that forms shells in crustaceans and other similar animals [17]. These polymers form, cellulose-like fibers, cannot be digested by vertebrates [18]. During the fermentation process, *Bacillus* sp. PAS7 isolates can degrade chitin into simpler compounds such as glucose molecules and N-acetyl glucosamine. Fermentation can reduce chitin as a crude fiber by breaking the glycoside bonds between protein and chitin and converting products to be more easily digested [19]. Besides having protease activity, *Bacillus* sp PAS7 isolate was also suspected of having chitinase activity.

Based on the results of the study, it was seen that the isolate *Bacillus* sp PAS7 was able to improve the quality of shrimp head meal by increasing crude protein, soluble protein and decreasing the crude fiber content of the material. Inoculum dose of 7% has been able to improve the quality of shrimp head flour nutrition. *Bacillus* sp PAS7 at a dose of 7% increased the level of dissolved protein by 126.45%, crude protein by 5.83%, and reduced crude fiber by 25.79%. Significant increase in protein and decrease in crude fiber indicates that shrimp head meal has changed into simpler molecules. This simple form of protein, carbohydrates, and fat in fermented shrimp head meal will make it easier for fish to digest and absorb it. Fermentation can increase the protein digestibility of plant material [20, 21, 22]. However, in this study, the fermentation process was not able to reduce the ash content in shrimp head meal. The content of both fermented and unfermented ash was very high at up 40%, whereas the ash content requirement for fish feed based on the Indonesian National Standard is only around 12-14% [23]. Therefore, further research was needed to overcome this problem such as demineralizing shrimp meals so that the ash or mineral content of the material can be reduced.

4. Conclusion
Fermentation using isolates *Bacillus* sp PAS7 can increase crude protein, soluble protein and can reduce the levels of crude fiber of shrimp head meal. Inoculum concentration of 7% from *Bacillus* sp PAS7 was the best dose in improving the quality of shrimp head meal nutrition. This study provides information that fermentation using proteolytic bacteria such as *Bacillus* sp. PAS7 can enhance the quality of shrimp head meal by increasing its digestibility so that its use in fish feed can be optimized and so can reduce feed cost. Also, the fermentation process of shrimp meal in this study is simple and can be applied in the field by fish farmers or small scale feed producers.
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