Test of *Chloramphenicol* (CAP) on shrimp *Vannamei* (*Litopenaeus vannamei*) food products with elisa method

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**Abstract.** This study aims to determine of Chloramphenicol test for Vannamei shrimp conducted of ELISA (Enzyme-Linked Immunosorbent Assay) method with 1 treatment. This method is done by adding the Abenzyme conjugate, and ends with the addition of the substrate and the reaction stop buffer. Each treatment was performed three times. The value of Chloramphenicol (CAP) of Vannamei shrimp (*Litopenaeus vannamei*) which was tested at for 4 weeks with ranged from 0.021 to 0.153 ppb. This result showed for the value of Chloramphenicol (CAP) in shrimp is still in the standard CAP value specified.

1. Introduction

In 2007, fishery product exports reached a value of US $ 2,258 billion and shrimp with a value of US $ 1.029 billion. The quality requirements of fishes must be follow the regulations of the importing country, such as the European Union and the United States. But shrimp exports still experience food security constraints in the global market. Product rejection in the port of entry of the importing country often occurs because the reason for food hazard contamination, such as *Salmonella*, filth, and antibiotics, encourages the development of a shrimp certification system [1]. The used of antibiotics and drugs for animals is usually used at the stage of cultivation or given during storage to reduce microbial attacks or bacteria that can cause disease in the crabs and crabs.

Detection of antibiotics in processed products indicates that antibiotics cannot be easily removed with the current process stages [2]. The use of antibiotics in dealing with health problems in cultivation caused residues in the meat. Antibiotic residues occur due to caused of downtime, the use of antibiotics that exceed the recommended dosage, and animal feed. One of the antibiotics that are often used by farmers is *Chloramphenicol* [3]. The shrimp product certification system was developed in order to anticipate the era of global trade liberalization. Success in free trade is determined by the ability of the state to develop regulations, systems and standards that are equivalent to international regulations, such as guidelines or standards issued by FAO or WHO, and the provisions of trading partner countries, such as the European Union and the United States, science or technology and the ability to access information quickly, as well as the availability of equipment and high production efficiency. This study aims to determine the absorbance value of the Chloramphenicol test on Vannamei shrimp to be exported out of Indonesia.
2. Materials and methods

Three market samples were used for these experiments, the research method in this study were using the ELISA (Enzyme-Linked Immunosorbent Assay) Method by adding the Abenzyme conjugate, and ends with the addition of the substrate and the reaction stop buffer was performed three times. These methods were used of enzymes to detect sample bonds to antigens (Ag) and antibodies (Ab). The enzyme will convert colourless substrate (chromogen) to a coloured product. This change shows the sample bond to Ag: Ab. The colour spectrum is read its absorbance at a certain wavelength. This absorbance value will be inversely proportional to the histamine concentration contained in it. This test was conducted for 4 weeks, namely on July 16, 2018 until August 10, 2018, and was tested at the Fish Quarantine Center Laboratory of Class I Fisheries Product Quality and Safety Control in Medan I.

2.1. Tools and materials

The tools were used of Chloramphenicol test follows of blender, knives, sudip, digital scales, 15 ml tube, 1000 μl micropipette, microtip, vortex, centrifuge, incubator, well, shelves, microplate reader. Toused of Chloramphenicol test and sample of Vannamei shrimp with 10x, Sample extraction buffer, distillate water, ethyl acetate, nitrogen gas n-hexane solution, 1x sample extraction buffer, HRP conjugate, 1x wash solution, TMB substrate and stop buffer.

2.2. Procedures

Preparation of 1x Sample Extraction Buffer Diluted with 1 ml of 10x and Sample Extraction Buffer with 9 ml of distilled water. Next the shrimp must be cut and put in the blender. After the were homogeneous then weighed using 3 grams of digital scales, then mixed with 6 ml of ethyl acetate. The sample in the vortex for 3 minutes then centrifuge the sample 4000 rpm for 5 minutes. Taken 2 ml of ethyl acetate supernatant then dried with gas nitrogen at a temperature of 60-70 °C. Dissolved residue with n-Hexane as much as 2 ml then added 1x sample extraction buffer as much as 1 ml. In the vortex for 2 minutes and centrifuge 4000 rpm for 10 minutes at room temperature. The upper layer (hexane) is disposed of in the sample solution and then 100 μl was used for ELISA testing.

2.3. Preparation of 1x wash solution

Mixed with 1 ml 20x wash solution with 19 ml of distillate water volume ELISA procedure sample of 100 μl were used in the well CAP. The sample was added with 50 μl HRP conjugate. Shaken the sample for 1 minute then incubated the sample with a tissue wrapped for 1 hour at room temperature and dark. Wash the well 3 times with 1x wash solution, then dry it. Added 100 μL of TMB substrate and then shaken for 1 minute and incubated for 20 minutes at room temperature and dark. Added stop buffer as much as 100 μl then read on ELISA reader at 450nm.

3. Results and discussion

Vannamei shrimp can live in a wide range of salinity and are able to adapt as low temperature environments. In addition, Vannamei shrimp has a high appetite, so that in the feed cost cultivation system can be minimized [4]. Vannamei shrimp was more resistant to virus attacks in fact at this time, Vannamei shrimp also often fail due to virus attacks. Also if to pollution in the environment extreme [5]. The problem of the emergence of disease attacks in shrimp farming greatly affects the results of cultivation that is necessary to look for alternative maintenance methods to avoid virus attacks on Vannamei shrimp farming. One effort that needs to be done is cultivation with the administration of Chloramphenicol antibiotics [3]. The method used in the Chloramphenicol test is the ELISA method, where this method uses enzymes and reads the absorbance at a certain length of wave. In addition, the absorbance value read will be inversely proportional to the histamine concentration contained in the shrimp [6].
Table 1. Results of measurement of *chloramphenicol* in Vannamei shrimp in several samples treatment.

| Sample Treatment | Sample Code | Weight (g) | Concentration (μg/L) | Result (mg/kg) |
|------------------|-------------|------------|----------------------|----------------|
| Sample 1         | A(1)        | 1.948      | 1.948                | 0.029          |
|                  | A(2)        | 1.529      | 1.529                | 0.064          |
|                  | A(3)        | 1.992      | 1.992                | 0.026          |
|                  | B (1)       | 1.744      | 1.744                | 0.042          |
|                  | B (2)       | 1.555      | 1.555                | 0.061          |
|                  | B (3)       | 1.688      | 1.688                | 0.047          |
| Sample 2         | C (1)       | 1.745      | 1.745                | 0.050          |
|                  | C (2)       | 1.743      | 1.743                | 0.050          |
|                  | C (3)       | 1.705      | 1.705                | 0.054          |
| Sample 3         | D (1)       | 1.253      | 1.253                | 0.101          |
|                  | D (2)       | 1.267      | 1.267                | 0.099          |
|                  | D (3)       | 1.241      | 1.241                | 0.104          |
|                  | E (1)       | 1.027      | 1.027                | 0.153          |
|                  | E (2)       | 0.984      | 0.984                | 0.165          |
|                  | E (3)       | 1.151      | 1.151                | 0.122          |
| Sample 4         | F (1)       | 2.241      | 2.241                | 0.021          |
|                  | F (2)       | 2.129      | 2.129                | 0.025          |
|                  | F (3)       | 2.016      | 2.016                | 0.030          |
|                  | G (1)       | 1.886      | 1.886                | 0.038          |
|                  | G (2)       | 1.891      | 1.891                | 0.038          |
|                  | G (3)       | 1.977      | 1.977                | 0.033          |

In the *Chloramphenicol* test, the amount of Vannamei shrimp used for counting was found of 4 tails. This is to get as many samples as 15 mg. After the sample was chopped, then put it in a 15 ml tube. Samples that have been chopped are then stored in the freezer. *Chloramphenicol* test and repeated three times. This is because to ensure the results read on the ELISA method are more accurate. The accuracy of the reading can be seen from the range of values on the repetition of 1 to 3, the values read are not much different. In samples with code M / U / VII / 732 in learning 1 to 3 the results was found with value of 0.029; 0.064; and 0.026 ppb.

Figure 1. Results of measurement of *chloramphenicol* in Vannamei shrimp several treatment samples.
The results of Chloramphenicol test with Vannamei shrimp was found that the absorbance value of Vannamei shrimp still below compared the value of international trade standards, with the international trade standards of Chloramphenicol residue of 0.3 ppb. The use of chloramphenicol in fishery commodities (shrimp and fish) has spread in the local, regional and international markets so that it can derail exports, especially shrimp from Indonesia to various countries in the world. Therefore, it is necessary to know the value of international trade standards for Chloramphenicol residues. This is in accordance with [7], who stated that until now Chloramphenicol has not been known for a long time due to lack of research and information sources that use Chloramphenicol as the object of research. This must be considered before the product will exported to the destination country.

4. Conclusions
The value of Chloramphenicol (CAP) on Vannamei shrimp (Litopenaeus vannamei) which was tested at the Class I Medan I KIPM for 4 weeks ranged from 0.021 to 0.153 ppb. This shows that the value of Chloramphenicol (CAP) in shrimp is still in the standard CAP value specified.

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