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Effectiveness of Sea Cucumber Viscera Waste to Improve Reproductive Performance of Male Tiger Shrimp (*Penaeus monodon*)

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Abstract

Tiger shrimp has received attention because of the high prices. However, sustainable cultivation of tiger shrimp is still hampered by the low quality of spermatozoa in male broodstock. Sea cucumbers are a type of invertebrate that is very useful because they have many viscera with almost the same nutritional content as fresh food. However, it has other advantages compared to other fresh feeds, namely it has a very high testosterone content so that it can be used to improve the quality and quantity of spermatozoa of male tiger prawns. The study aims to compare the influence between sea cucumber viscera and fresh squid as well as the combination of sea cucumber and squid viscera against the density of tiger shrimp spermatozoa (*Penaeus monodon*). Test animals used were 18 male tiger shrimp with an average weight of 80.93 g. Shrimp are kept in tanks with a volume of 1 ton, with a density of 3 broodstock/tank and 3 replications. Feeding was carried out with a frequency of 3 times a day and at a dose of 20% of biomass for 30 days. The test feed used consisted of 100% fresh squid feed as a control, a combination of 50% squid and 50% of sea cucumber viscera, and 100% of sea cucumber viscera. The administration of sea cucumber visceral waste in tiger shrimp tend to increase the quantity of spermatozoa tiger shrimp.

***Keywords**:* Male shrimp reproduction, testosterone, sea cucumber visceral, HUFA fatty acids

1. Introduction

In general, research related to the reproductive appearance of Penaeid shrimp broodstock focuses more on female broods than males (Harlıoğlu *et al*., 2018; Braga *et al*., 2010). However, in order to produce high-quality seeds, it is necessary not only to rely on the quality and quantity of eggs produced by female brooders, but also pay attention to the reproductive appearance of the male brooders (Ceballos-Vázquez *et al*., 2004). Currently, fertilization success rates in controlled ponds and tanks are declining due to low sperm quality (Lante *et al*., 2016). Previous research carried out to address low sperm quality have focused on improving nutrition (Harlıoğlu *et al*., 2018; Leelatanawit *et al*., 2014). In addition, common fresh feeds that are often used to improve the reproductive performance of tiger shrimps are clams, squid, polychaete worms, oysters, and Artemia. This is because these feeds contain high levels of protein and lipids (HUFA), namely AA, EPA, and DHA (Chimsung, 2014; Leelatanawit *et al*., 2014). However, the fresh feed used has an economic value and competes with human needs, and therefore has a relatively high price. Sea cucumber waste is an alternative food that can be used as a substitute for fresh food.

Sea cucumber is a highly cultured and traded marine products, with a global production (both capture and aquaculture) amounting to 6,018 t in 2018 and among the largest contributing countries were Indonesia (5,097 t) and Malaysia (513 t), respectively (FAO, 2020). Pangkey *et al*. (2012) cites FAO data showing that the exploitation of sea cucumbers in 2001 reached 18,900 tons, while Wirawati *et al*. (2021) also stated that the average production of sea cucumbers in Indonesia between 1992 and 2002 was 2,556 tons dry weight. In general, sea cucumbers are low in fat and cholesterol, but high in protein content. Out of approximately 1,500 species of sea cucumber found globally, about 100 species are known for human consumption, including those of the Family Holothuroidea (Hossain *et al*., 2020). Fresh sea cucumbers are often eviscerated, followed by a series of boiling, salting, drying and soaking processes to

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achieve the final product, i.e., dried or salted sea cucumbers (Li *et al*., 2019). The process of evisceration inevitably results in the production of unwanted waste and often being discarded. However, studies have shown that sea cucumber viscera contains high level of essential and non-essential amino acids, especially glutamic acid, aspartic acid, and arginine (Hossain *et al*., 2020).

2. Material and Methods

2.1.Time and Place of Research

This research was conducted at Tiger Shrimp Hatchery Installation (IPUW), Brackish Water Cultivation Research Centre, and Fisheries Extension (BRPBAPP) in Lawallu Village, Soppengriaja District, Barru Regency using adult male tiger shrimps. Before rearing, the tiger shrimp from wild were first measured for weight and size and then tagged on the eyestalk. The average weight of the male tiger shrimps obtained was 80.93 g with 18 organisms. The spermatophores of the tiger shrimps were removed before rearing to have an effective treatment. The rearing container used was a fiber tub with a capacity of 1 ton with a RAS system of 9 pieces.

2.2.Test Feed

The test feed used consisted of 100% fresh squid feed as a control (treatment I), a combination of 50% squid and 50% of sea cucumber viscera (treatment II), and 100% of sea cucumber viscera (treatment III). This research was conducted using a completely randomized design (CRD) with 3 treatments and 3 replications. Before being used as feed, the viscera and the squid were cut into small pieces in such a way that it can be easily consumed by shrimp. The feeding frequency was carried out three times a day in the morning, afternoon, and evening with a dose of 20% of the biomass every day for 30 days.

2.3.Spermatophores weight and the total density of spermatozoa

After the rearing period, the spermatophore was removed by providing an electric shock using an electric transformer equipped with two electrodes placed near the 5th swimming leg. In the presence of an electric shock of 15 volts/7 mA regularly for 1-2 minutes, male brooders slowly release spermatophores (Lante *et al*., 2014). After the spermatophores exited, the spermatophores were weighed using an analytical balance and then placed in 1.5 ml Eppendorf microtubes, supplemented with a calcium-free salt solution (Jiang *et al*., 2009). This solution is useful for spermatozoa to survive during observation. Furthermore, the spermatozoa were removed from the spermatophore by grinding, then diluted 10 times by adding 100 µl of spermatozoa into an Eppendorf microtube containing 900 µl of saline solution using an Eppendorf micropipette. In addition, 20 µl of spermatozoa were collected and then deposited on a haemocytometer for observation under a microscope with a magnification of 100 times. The total density of spermatozoa was calculated using the formula developed by Gomes and Honculada-Primavera (1993):

$$\text{The total density of spermatozoa} = \frac{\Sigma(box1+\cdots+box5)}{5}\, x\, \frac{25}{0{,}1\, mm^3}\, 10$$

2.4. Percentage of normal and abnormal spermatozoa

The percentage of normal and abnormal spermatozoa was determined by calculating the number of normal or abnormal spermatozoa compared to the total spermatozoa count. Normal spermatozoa have spherical bodies and long tails, while abnormal spermatozoa are characterized by deformed bodies such as bent, short and missing tails (Lante *et al*., 2014). The percentage of normal and abnormal spermatozoa was calculated using the formula developed by Pérez-Rodríguez *et al*. (2019), namely:

$$\text{Normal or abnormal sperm percentage} = \frac{\text{The total of normal or abnormal cell}}{\text{The total of sperm cell}}\, x\, 100\%$$

2.5.Data Analysis

The data were analysed using Analysis of Variance (ANOVA) one-factor to determine the effect of treatment on the observed parameters. The treatment of the test feed was considered as a factor, and the response variables were the weight of the spermatophore, the number of spermatozoa as well as the percentage of normal and abnormal spermatozoa. A p-value of 0.05 was considered statistically significant. In cases where significant differences were reported, a posterior analysis of the mean comparisons was performed, using the LSD test. All analyses were performed using SPSS statistical software version 23.0.

3. Results and Discussion

3.1. Results

The weight of spermatophores obtained is no significantly different among treatments as shown in Table 1. Unlike the case of the total density of spermatozoa produced, the results of the analysis on the number of spermatozoa in treatment I showed a significant difference ($P<0.05$) with respect to treatments II and III (Figure 1). The analysis results on the percentages of normal and abnormal spermatozoa also showed no significant differences for all feed treatments (Table 2), but descriptively showed that the lowest percentage of abnormal spermatozoa was obtained in tiger shrimps fed fresh sea cucumber viscera, while the highest was found in tiger shrimp fed squid.

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3.2. Discussion

This research shows that spermatophore weight is not positively correlated with the number of spermatozoa cells. Spermatophores are not only composed of spermatozoa cells, but also of an outer mucopolysaccharide tissue (basophilic and acidophilic substance) (Shen *et al*., 2020), in addition, it plays an important role in the transfer and storage of spermatozoa in shrimp, and also protects spermatozoa as long as it is stored in the telicum of the females before fertilization of the egg (Lante *et al*., 2014). Besides body weight, spermatophore weight is also influenced by its nutrition, improving its quality, especially in the maturation process (Shailender *et al*., 2012). Similar to spermatophore quality, the number of spermatozoa is also influenced by the nutritional content of the feed, especially fatty acids play an important role in the quantity and quality of spermatozoa. Shailender *et al*. (2012) mentioned that the concentration of n-3 and n-6 HUFAs is closely related to the reproductive performance of tiger shrimp. The n-6 HUFA fatty acid is a precursor of the hormone prostaglandin and plays an important role in the reproduction process of tiger shrimps. In this research, the total spermatozoa of tiger shrimps fed with sea cucumber visceral, differed significantly from squid, even though both contain the nutrients needed to increase the number of spermatozoa. However, sea cucumbers have one advantage over squid, namely the presence of steroids.

According to Dewi (2008), sea cucumbers contain unsaturated omega-3 fatty acids, namely DHA (Docosahexaenoic Acid) and EPA (Eicosapentaenoic Acid), as well as omega-6, namely linoleate and arachidonic. The content of EPA in sea cucumbers from the extraction results using PBS is 25.69%, while DHA from the extraction results using water is 57.55% (Dewi, 2008). Unsaturated n-3 fatty acids (n-3 HUFA) such as DHA and EPA are important components of cell membrane phospholipids with an important role in cell membrane fluidity, lipid metabolism and performance, reproductive development and immune function in aquatic animals (Jin *et al*., 2017; Lim *et al*., 1997). However, high concentrations of HUFA n-3 can accelerate the occurrence of oxidative reactions. The ease with which this molecule undergoes an oxidation reaction (oxidative stress) leads to an increase in free radicals and damages cells. The formation of an unstable lipid hydroperoxide molecule causes a secondary reaction that produces hydroxide and a breakdown of the carbonyl group, therefore the aldehyde group easily reacts with amino acids causing the formation of irreversible covalent modification of the protein molecule (Aldini *et al*., 2015; Jaya Ram *et al*., 2008). Some of the nutrients of sea cucumbers and squid are shown in Table 3.

Moreover, sea cucumbers are thought to contain testosterone dye, which has the ability to increase male vitality, they are in great demand as dietary food ingredients good for the health (Dewi *et al*. 2010). The testosterone can also improve the performance of spermatozoa production because it is a hormone that plays an important role in the sperm formation process (Akbar *et al*., 2015). Several researches have been conducted and found that testosterone from the steroid extract of sea cucumberscan also be used for sex reversal in organisms whose male is more economically valuable than the female, such as giant shrimps (Riani *et al*. 2010), guppy fish (Emilda, 2015; Saputra *et al*., 2018) and freshwater crayfish (Susanto *et al*., 2018). Furthermore, it has been mentioned that fresh sea cucumbers contain more testosterone than dried sea cucumbers.

According to Arisandi (2012), 1 kg of fresh sea cucumber viscera yielded an extract with a percentage of 2.128% of steroid compounds. While dried visceral, wet meat, and dried meat of sea cucumbers yielded 1.796%, 1.296% and 0.816% of steroid compounds, respectively. The results of GC-MS and NMR analysis showed that the molecular weight of the steroid extracted from the sea cucumber was 288.42, which was a type of testosterone (Triajie, 2010).

The analysis results on the percentages of normal and abnormal spermatozoa also showed no significant differences for all feed treatments (Table 2), but descriptively showed that the lowest percentage of abnormal spermatozoa was obtained in tiger shrimps fed fresh sea cucumber viscera, while the highest was reported in squid feed treatment. Lelatanaawit *et al*. (2014) suggested that maximal nutrition is required to reduce the percentage of abnormal spermatozoa. A good feed should not only contain essential nutrients, but also the right balance of certain nutrients, and as an example we have fatty acids, if they are not present in adequate concentrations, there can be a negative impact on reproduction (Shailender *et al*., 2012). In this research, it was shown that the percentage of abnormal spermatozoa was higher than the normal one (Table 2). The difference between normal and abnormal spermatozoa can be seen from their morphology. According to Lante *et al*. (2014) that normal sperm cells are characterized by spherical bodies and long tails, while abnormal sperm cells are characterized by body defects such as bent, short and missing tails. It is estimated that the high percentage of abnormal spermatozoa is due to the environmental conditions where the temperature of the rearing media ranges from 30.2°C - 32.8°C in this research.

In addition to the nutritional level of food, according to Anwar *et al*. (2007) sperm quality is also determined by the temperature of the maintenance medium. The difference in temperature between day and night and the length of time shrimp are exposed to high temperatures can cause an increase in the number of abnormal and dead sperm cells. An increase in temperature causes shrimp metabolism to increase which results in an increase in energy intake. High

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temperatures can change the permeability of the membrane to ions causing loss of water and/or influx of ions that affect the osmotic balance. The feed given as an energy source is used to control the internal ion concentration through pumping which results in a decrease in osmotic pressure which triggers an increase in osmotic capacity (OC).

Although this temperature is the level that shrimp can tolerate, this range is quite high, the optimum temperature for the development of *P. monodon* is 26-32°C, when it is subjected to high environmental temperature it can increase the abnormalities of its spermatozoa. This is supported by Pascual *et al*. (2003) who reported a decrease in spermatophore melanization, which had an impact on the formation of normal spermatozoa cells from 1.64 × 106 cell/spermatophore at day 0 to 0 cells at days 10 at 33°C compared to 27°C sperm counts around 2.06 × 106 cell/spermatophore (75%-90% of normal cells, and 10%-25% of abnormal cells) in reared *L. setiferus* shrimp. The same was also reported by Pérez-Velázquez *et al*. (2001), for the white shrimp *P. vannameii*, where the highest number of spermatozoa produced was 18.6 million cells, with the lowest percentage of abnormal spermatozoa (36.7%), at a temperature of 26 °C. Whereas at 29 °C, only 0.1 million spermatozoa were produced and the percentage of abnormality reached 99.7%.

According to Lante *et al*. (2014), a low level of spermatozoa abnormalities indicates good performance. The high percentage of normal spermatozoa with slight abnormalities is one of the criteria used to determine the quality of shrimp sperm (Alfaro-Montoya, 2010). Therefore, it is expected to improve the quality of fertilization by increasing the chances of male spermatozoa to fertilize the egg, therefore it will affect the quality and number of nauplius (Perez-Jar, 2007).

In shrimp farming activities, one of the efforts made to improve the reproductive performance of male shrimp is by the administration of the testosterone hormone. Maheswarudu *et al*. (2015) explained that the testosterone hormone induces spermatozoa development in tiger shrimps. The most commonly used testosterone hormone is 17α-methyltestosterone (Mulyaningrum *et al*., 2021; Rosmiati *et al*., 2022). . According to Susanto (2018), sea cucumber can produce a higher ratio of natural steroid hormone extract compared to the use of 17α-methyltestosterone. Therefore, viscera extract can be an alternative option to replace this hormone in tiger shrimp farming activities.

4. Conclusion

The treatment of sea cucumber viscera waste did not affect the weight of the spermatophores, but affect for spermatozoa density where it showed a higher number of spermatozoa compared to other feed treatments. The treatment of sea cucumber viscera waste also had no effect on the number of normal and abnormal spermatozoa, but descriptively showed a higher number of normal spermatozoa and a lower number of abnormal. The inclusion of sea cucumber viscera waste in the diet of tiger shrimp can tend to increase the quantity of their spermatozoa in males.

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