The effect of salinity on the expression of heat shock protein 70 (hsp70) in the gills and kidneys of srikandi tilapia (Oreochromis niloticus)

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Abstract. Heat Shock Protein 70 (HSP70) is the main stress protein synthesized by fish where there is a stressor, one of which is salinity. This study aims to determine the effect of salinity on the expression of HSP70 in the gills and kidneys of srikandi tilapia using the ELISA method. The parameter observed was the expression of HSP70 in the gills and kidneys of srikandi tilapia. These parameters were analyzed using one way factor ANOVA. The lower HSP70 expression was found in the P3 and the highest was found in P5 treatment. There was no difference in the gills and kidneys in the P1, P2, P3 treatments. However, the three treatments were significantly different from the P4 and P5 treatment.

1. Introduction
There are many strains of tilapia that have been developed in Indonesia. One of the tilapia strains that can live in brackish waters to the sea is Srikandi tilapia (Salinity Resistant Improvement from Sukamandi). Srikandi tilapia was released by the Ministry of Maritime Affairs and Fisheries in 2012. Srikandi tilapia is a hybrid fish that is tolerant of high salinity (10-30 ppt) obtained from the marriage of female tilapia (Oreochromis niloticus) and male blue tilapia (Oreochromis aureus) [1].

Srikandi tilapia can inherit superior genes from its parent, which has a high tolerance in brackish waters and grows fast in fresh waters. High tolerance to brackish water was obtained from the parent gene of blue tilapia and fast growth in fresh water was obtained from the parent gene of nirvana tilapia. Srikandi tilapia has a competitive advantage because it has good growth performance at 10-30 ppt salinity compared to other fish strains [1].

Osmoregulation is carried out by Srikandi tilapia to maintain the osmolarity of the body with its environment. The organs involved in the osmoregulation process are the gills, kidneys and intestines [2]. Tilapia gills have chloride cells located in the gill filament to help the osmoregulation process so that they can live in a wide salinity range. When entering saline water, chloride cells will experience an increase in the Na+/K+ ATPase portion in the blood [3]. In addition to the gills, the kidneys also play a role in the osmoregulation process by pumping water out through the urine. The kidneys of Srikandi tilapia have small gromelurus so that the role of osmoregulation is more in the gill organs [1]. The inability of fish to
maintain body osmolarity can cause fish to experience stress [2]. One of the stress responses of fish is the increased expression of HSP70 [4].

HSP70 is the main stress protein synthesized by prokaryotic and eukaryotic organisms [5]. Based on the synthesis mechanism, HSP70 consists of constitutive HSP70 and inducible HSP70. Constitutive HSP (HSC70) is synthesized in cells under normal conditions and acts as a chaperon, while inducible HSP70 is synthesized in the presence of a stressor [6]. Sensitive tissues in response to HSP70 secretion are the liver, kidneys, and gills [7]. HSP70 can be used as a biomarker (biological response marker) that can be used to indicate stress in fish [6].

Based on the description above, it is necessary to conduct research to determine the effect of changes in salinity on the expression of HSP70 in the gills and kidneys of Srikandi tilapia. so that it can be used to detect stress levels in fish.

2. Material and Method

2.1 Sample Collection

Fish samples were collected from Lamongan Brackish Water Cultivation Installation. The fish samples rearing at different salinity.

2.2 Preparation of maintenance containers

The maintenance container is cleaned with soap. Furthermore, the maintenance container was soaked using a solution of potassium permanganate (KMnO4) at a dose of 2.5 ppm.

2.3 Making Salinity of Media

Calculation of salinity is carried out using the formula:

\[ M_1 \times V_1 = M_2 \times V_2 \]

Description:
M1 : Initial dissolved salt concentration (ppt)
M2 : Desired concentration of dissolved salt (ppt)
V1 : Initial dilution volume
V2 : Final dilution volume

2.4 Fish maintenance in saline media

Srikandi tilapia were reared in different salinity media, namely 15 ppt, 20 ppt, 25 ppt, 30 ppt, and 35 ppt. Fish were kept in different salinity media for 1 week. Fish were fed 3 times a day as much as 5% of the biomass. Dissolved oxygen, pH and temperature were measured every day at 06.30 WIB and 16.30 WIB. After that, 4 fish were randomly sampled from each treatment to take the gills and kidneys and then continued to observe HSP70 expression. Prior to organ harvesting, the fish were stunned using phenoxy ethanol at a dose of 0.3 ml/L. Measurement of HSP70 levels was carried out using fresh organs.

2.5 Elisa Test

Expression of HSP70 in the gills and kidneys of Srikandi tilapia can be determined by the ELISA method. ELISA (Enzym Linked Immuno Sorbent Assay) is a serological test that uses a combination of specific antibodies with enzymes that function to detect the presence of antigens. The binding of antigen to antibody can be determined by adding a substrate that will be decomposed by the marker enzyme and can be seen directly through a color change or using a spectrophotometer.

2.6 Data Analyst
Data analysis used one-factor ANOVA test and if there was a significant difference, then continued with Duncan's further test.

3. Result and Discussion

The results of observations of HSP70 (table 1) on the gills and kidneys of Srikandi tilapia reared at different salinities showed the presence of HSP70 secretion.

Table 1. HSP70 values in the gills and kidneys of Srikandi Tilapia reared at different salinities

| Treatments | Gills          | Kidneys         |
|------------|----------------|-----------------|
| P1         | 356,425 ± 9,70a | 324,675 ± 9,85a |
| P2         | 348,750 ± 11,2a | 320,550 ± 5,12a |
| P3         | 336,775 ± 10,68a | 307,700 ± 23,12a |
| P4         | 385,600 ± 14,21b | 421,975 ± 25,55b |
| P5         | 484,175 ± 27,39b | 446,375 ± 25,57b |

- The data presented are mean
- Different superscript symbols in each column indicate a significant difference in the data in each treatment based on the one-way ANOVA test with Duncan's test (p < 0.05)

Based on the results of the ELISA test to determine the levels of HSP70 in the gills and kidneys of Srikandi tilapia reared at different salinities, it showed that there were differences in the secretion of HSP70 in each organ and in each treatment. The results of data analysis using one factor ANOVA followed by Duncan's further test showed that there were significant differences between treatments P1, P2 and P3 with treatments P4 and P5. The results of the analysis showed that the Srikandi tilapia experienced little stress due to changes in salinity when reared at a salinity of 15-25 ppt. This was indicated by the low secretion of HSP70 in the gills and kidneys of Srikandi tilapia reared in treatment P3 and treatment P3 was not significantly different from treatment P1 and P2.

The low levels of HSP70 in the P1, P2 and P3 treatments in the gills and kidneys were thought to be due to the salinity of 15-25 ppt the body condition was close to isoosmotic conditions with the environment so that fish secreted little HSP70 because they did not experience stress due to differences in body osmolarity with their environment. This opinion is reinforced by the statement of Smith et al. (1999) [8] who stated that the salinity incompatibility of the environment can disrupt the mechanism of protein synthesis and cause stress in aquatic animals. Environmental stress can trigger a response in the form of HSP70 secretion in the gills to protect cells and maintain gill function. This is because HSP70 has the ability to refold proteins and bind abnormal proteins [9].

The results of the HSP70 test on the kidneys of Srikandi tilapia showed that the HSP70 value in the kidneys was lower than the gills. This is presumably because the gills are external organs that are directly exposed to environmental salinity. This opinion is supported by the results of the statement of Deane and Wo (2010) [10] which states that the gills are the first organs to be exposed to and overcome salinity exposure, therefore HSP70 in the gills of fish reared at high salinity shows higher yields than in the kidneys.

Madeira et al. (2012) [11] stated that normal HSP70 values in fish that live in waters with a temperature of 28°C with a salinity of 12-35 ppt range from 150-370 ng/mL. Xing et al. (2013) [12] stated that normal HSP70 values in carp gills ranged from 0-380 and carp kidneys ranged from 0-420 ng/mL.
While in shrimp normal HSP70 values range from 50-200 ng/mL [11]. Based on these two statements, the HSP70 value in Srikandi tilapia maintained at 15-25 ppt salinity was classified as normal, which was below 356 ng/mL for gills and 324 ng/mL for kidneys. The HSP70 value in Srikandi tilapia reared at 30-35 ppt salinity above the normal value. The high value of HSP70 in Srikandi tilapia reared at 30-35 ppt salinity indicates that the fish are under stress. This statement is supported by Basson (2006) [6] which states that the higher the value of HSP70 in an organism, it can indicate a stress condition in the organism.

Increased HSP70 in gills and kidneys was also found in euryhaline milkfish reared at different salinities. The results Madeira et al. (2012) [11] stated that milkfish euryhaline which was originally reared at high salinity (16-20 ppt) experienced an increase in HSP70 secretion when reared in waters with low salinity (0 ppt). This increase in HSP70 secretion can indicate an increase in the stress level of milkfish due to lower salinity changes. The euryhaline milkfish reared in a hypo-salinity environment will experience higher stress than the euryhaline milkfish reared in a hyper-salinity environment so that it requires greater energy to achieve homeostatic conditions at low salinity. The stress condition was indicated by a significant increase in the value of HSP70 in milkfish reared at 0 ppt salinity compared to those reared at 30 ppt salinity.

Increased synthesis of HSP70 in the gills and kidneys of fish can be triggered by environmental stressors such as salinity. Stress conditions can cause the protein fold to open and the multiceparon complex consisting of HSP70, heat shock factor (HSF) to decompose because HSP70 tends to start binding to the unfolded protein fold rather than bind to HSF. binds heat shock elements (HSE) to the cell nucleus. These HSF trimers then bind HSE to become hyper-phosphorylated (P) before being able to carry out transcription. The HSP70 gene at the transcription stage produces HSP70 mRNA which then exits to the ribosome to carry out the HSP70 synthesis process. This molecular mechanism causes an increase in HSP70 in the gills and kidneys in response to exposure to environmental stress [13].

The results of data analysis showed differences in the expression of HSP70 in the gills and kidneys at salinities of 15 ppt, 20 ppt, 25 ppt with salinities of 30 ppt and 35 ppt. This indicated that the salinity of the media affected the stress level of fish. The further the range of media salinity from the optimal salinity where tilapia live, the more stressed the fish will be. This is in accordance with the statement of Royan et al. (2014) [14] which states that sudden and higher salinity shifts can lead to stress and death of fish. Anggoro (1992) [15] stated that the death was caused by symptoms of internal osmolarity, namely the disruption of osmolarity between body fluids and living media. This is also related to changes in the absorption of oxygen. The higher the salinity of the medium, the lower the oxygen content in the water [18].

Based on the results of the study showed that the salinity of 15-25 ppt was the optimal salinity for the maintenance of Srikandi tilapia. This statement is based on the low HSP70 value in the gills and kidneys and the high survival rate compared to maintenance at 30-35 ppt salinity. The low secretion of HSP70 and the high survival rate of Srikandi tilapia reared at a salinity of 15-25 ppt is suspected under these conditions, tilapia releases little energy for the process of adapting to the environment so that the energy contained in the body can be used for growth. This statement is supported by Setiawati et al. (2008) [16] which states that the energy contained in fish feed will be used for the maintenance and growth process, growth can take place if the energy needs for maintenance have been met. Hadie et al. (2011) [1] added
that the Srikandi tilapia can live at a salinity of 10-30 ppt and will experience a decrease in growth when kept at a salinity above 30 ppt.

4. Conclusion
Based on the results of the research that has been done, it can be concluded that there is an effect of salinity on the expression of HSP70 in the gills and kidneys of Srikandi tilapia. The effect that occurs is an increase in the expression of Heat Shock Protein 70 (HSP70) in the gills and kidneys of Srikandi tilapia reared at salinities above 25 ppt.

5. References
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