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EFFECT OF rGH HORMONE ADMINISTRATION ON PROTEASE ENZYME ACTIVITY AND RNA/DNA RATIO OF STRIPED CATFISH (*Pangasianodon hypophthalmus*) MAINTAINED IN SALINE MEDIA

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ABSTRACT

Striped catfish is a prevalent fish and is in great demand. Striped catfish cultivation generally uses fresh water as a living medium, but several freshwater fish commodities are starting to be developed to adapt to saline media. Harvesting striped catfish usually takes approximately 3 - 8 months. To overcome this, one way is to use growth hormone. The growth hormone currently used is Recombinant Growth Hormone (rGH). GH administration has been reported to have various effects, particularly on protein metabolism. In fish digestion, protein from feed cannot be directly absorbed but is first broken down by protease enzymes into amino acids. The physical appearance, which is the phenotype of an organism, is the result of metabolic processes in each cell that makes up the organism. This research aimed to analyze the effect of administering the rGH hormone on protease enzyme activity, RNA/DNA ratio, and growth of striped catfish maintained in saline media. The method used was experimental in the form of a Completely Randomized Design (CRD) with four treatments and three replications. Based on the results obtained during the research, it shows that Protease Enzyme Activity (U/minute) and the best RNA/DNA Ratio use a dose of 3 mg/kg with respective values of 0.75±0.24 U/minute and3.30±0.49.

Keywords: Striped catfish, Protease enzyme activity, RNA/DNA ratio

1. INTRODUCTION

The striped catfish is a prevalent fish in great demand on the market. Various food menu preparations can be made using catfish meat. This is what increases sales of striped catfish domestically. The price of catfish on the market ranges from IDR 25,000-IDR 30,000/Kg. The high selling value of catfish certainly opens up more significant cultivation business opportunities.

Striped catfish cultivation generally uses fresh water as a living medium, but now, it is starting to be developed to adapt to saline media. This is expected to increase the variety of fish cultivation commodities for coastal communities. Even though they cannot survive in high salinity, striped catfish cultivation can still be done in low salinity ponds. According to Tahe et al.[1], Indonesia has salinity ponds (<3 ppt), generally 2-3 km from the coast.

Cultivating striped catfish using salinity can affect the quality of fish meat in terms of texture, taste, color, and nutritional composition. Fitrani[1,] in his research, stated that as many as 46-60% of panelists preferred the texture and taste of striped catfish in the 3-7 ppt treatment. This is because the texture of striped catfish is more compact, and the flavor of the meat is savorier, while in terms of protein composition, the fish meat also increases by 2% when reared in saline media.

Harvesting striped catfish generally takes a relatively long time, approximately 3-8 months. Fissabela et al.[3] stated that many factors cause the growth of striped

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catfish to be optimum. There are several problems at the farmer level, such as a lack of cultivation technology, the availability of quality feed, and less than optimal water quality at the rearing location, which causes harvesting to increase. Time and increased production costs. To overcome this, one way that can be taken is to use growth hormone. The growth hormone that is currently being used is Recombinant Growth Hormone (rGH).

Zhu et al., in Alimuddin et al.[4] stated that the administration of Growth Hormone (GH) has been reported to have various effects, especially protein metabolism. In fish digestion, protein from feed cannot be directly absorbed but is first broken down by protease enzymes into amino acids or peptides. The ability of the protease enzyme to break down protein can be measured based on its activity in digestion. When protease enzyme activity increases, protein breakdown can become faster.

Salt levels can also influence hormone performance in the environment. The osmoregulation system is a system that regulates the osmotic pressure between the environment and the fluids in the fish's body. According to Pamungkas[5], the Growth hormone is involved in osmoregulation and works synergistically. Taufik & Kusrini[6] added that the growth hormone secreted by the adenohypophysis is also influenced by changes that occur during the adaptation period to water salinity.

The physical appearance, which is the phenotype of an organism, is the result of metabolic processes in each cell that makes up the organism. Apart from phenotype, variations in the genotype of an organism cannot be separated from the effects of metabolism. According to Masuda et al.[7], growth can be achieved through protein biosynthesis, the level of which depends on the quantity of RNA. Because there is some influence from the environment, the amount of RNA will respond quickly to the expression level of the genes responsible for growth. Based on this, it is necessary to test the administration of the rGH hormone to catfish fry reared in saline media to determine the effect of the rGH hormone on protease enzyme activity and to what extent the rGH hormone can influence changes in the RNA/DNA ratio.

2. RESEARCH METHOD

Time and Place

This research was carried out from 17 October to 15 December 2022 at the Fish Seed Center (BBI) Riau Islamic University, and it included testing protease enzyme activity and seed maintenance. Meanwhile, RNA/DNA concentrations were extracted and measured at the Laboratory of Reproduction and Genetics of Aquatic Organisms, Faculty of Fisheries and Marine Sciences, IPB University.

Tools and materials

The tools used in this research are Aerator, Aquarium 60 x 40 x 40 cm, Fiber Tub diameter 1 m, Fiber Tub 162 x 86 x 37 cm, Digital Scales, Ruler, Thermometer, pH Meter, DO meter, Ammonia MR, Sprayer, Freezer, Centrifuge, Refractometer, 1 ml Drop Pipette, Surgical Tools, Incubator, sized Styrofoam Box, Tray, 1.5 ml Tube, Micropipette. Meanwhile, the ingredients used in this research are Patin fish seeds and rGH hormone.

PF-1000 Pellets, ASW Salt, Freshwater, SolutionPBS, Egg yolk, Phosphate Buffer pH 7, Casein Substrate Solution 20 mg/mL pH 7, Tyrosine Standard Solution 5 mmol/L, Aquadest, Folin Ciaocalteau, Ice pack, Alcohol 96%. General, Phosphate buffer (0.05 M, pH 8.0), Casein substrate (20 mg/ml, pH 8.0), $CaCl_2$ (20 mmol/L), Tyrosine standard 5, mmol/L, Aquades, TCA (0.1 M), $CaCl_2$ (2mmol/L), Na_2CO_3 (0.4 M), Folin Ciocalteau.

Preparation of Containers and Media

The container used is an aquarium measuring 60 x 40 x 40 cm. Before use, the aquarium is cleaned and filled with 32 L of fresh water first, then 8 L of seawater. The

seawater used is artificial seawater, where the manufacturing process uses ASW (Artificial Salt Water) salt with the Blue Treasure brand. Seawater is made using ASW salt by dissolving 670 g/19 L of water. ASW salt was used because it is more efficient. The salinity used in this research was six ppt.

Seed Preparation and Feeding

The striped catfish seeds used had an average length of 8.6 cm and came from Mandiri Jaya Farm Jl. Health No. 118, Air Winter Village, Bukit Raya District, Pekanbaru City. The seeds are acclimated to fresh water first to adjust to environmental conditions for one day. Seed acclimation in saline media is carried out by gradually increasing salinity. Increased salinity refers to research by Nirmala et al.[8] where the increase in salinity carried out was one ppt every one day by dissolving salt based on the volume of water filled each day until it reached six ppt and then maintenance was carried out first for one week before being transferred to the aquarium which would later be given treatment.

The stocking density used in this research refers to Riswan et al.[9] Research, namely one fish/2 L of water. Feeding is carried out thrice daily at 08.00, 12.00, and 16.00 WIB ad libitum. The feed used is PF-1000 pellets with a protein content of ±35%. The hormone used is the rGH hormone with the Minagrow brand. The rGH hormone dissolution method refers to the procedure for using the product, and the hormone is dissolved using 2 mL of PBS solution and 50 mL of water, then 2.5 mL of egg yolk is added as an adhesive and then sprayed onto the feed. The feed that has been sprayed is then aired for 3-5 minutes. The test feed and remaining feed containing hormones were then stored in the refrigerator at 4°C. The time interval for administering recombinant growth hormone (rGH) refers to Research by Kurniawan et al.[10] once every three days.

Sampling

The samples for protease enzyme activity in this study were all parts of the fish intestine, which were first made into a crude extract of the enzyme. To know surgery and done starting from the anus hole towards the front until near the pectoral fins using scissors. Cutting is done carefully, then the upper flesh is opened with tweezers, and the intestines are separated from other organs.

The sample weight used was 1 g of the intestine, which was then ground with mortal, and then 10 mL was added phosphate buffer pH 7, which was cooled to a temperature of 4°C and centrifuged at 6,000 rpm for 10 minutes. The supernatant was taken as a crude enzyme extract and used as a sample for enzyme activity testing. Testing was carried out before the research and at the end of the study. The working procedure of protease enzyme activity is presented in the table.

The sample used to determine the RNA/DNA ratio was fish liver. The use of the liver as a test sample is assumed by the treatment given, where relative changes in RNA are analyzed in liver tissue in response to growth hormone administration. Ratnawati et al.[11] stated that rGH that enters the liver would be broken down by the enzyme responsible for initiating protein synthesis, namely aminoacyl tRNA synthetase, mainly concentrated in the liver.

Attempts were made to take the fish liver at a cold temperature, so the surgery was carried out in a styrofoam box measuring 20.5 x 17.5 x 15 cm filled with ice packs in a room with a temperature of 20^{o}C. The liver that has been obtained is then immediately put into a 1.5 ml tube filled with 96% alcohol for DNA samples and 0.1 mL of Genezol for RNA samples. The weight of the liver samples taken ranged from 30–100 mg. The samples were then stored at -20^{o}C.

Protease Enzyme Activity

Protease enzyme activity follows the method of Bergmeyer & Grassi[12] using

casein as a substrate and tyrosine as a standard, namely by measuring the ability of the enzyme to hydrolyze protein, producing tyrosine. Protease activity was calculated according to the equation:

$$U = \frac{Act - Abl}{Ast - Abl} \times \frac{P}{T}$$

Information:
Act = Sample absorbance value
Abl = Blank absorbance value
Ast = Standard absorbance value
P = Dilution factor
Q = Incubation time in minutes

RNA/DNA ratio

To calculate the RNA/DNA ratio, first find out the RNA and DNA concentrations. The formula used refers to research Pamungkas et al.[12]:

$$\text{RNA/DNA Ratio:} \frac{\text{RNA total}}{\text{DNA total}}$$

Information :
Total RNA = RNA concentration(µg/mL)
Total DNA = DNA concentration(µg/mL)

3. RESULT AND DISCUSSION

Protease Enzyme Activity (U/min)

Protease is an enzyme that plays a role in protein breakdown reactions. According to Manik & Jogi[14], the fish's body can metabolize most of the digestible energy in protein. The results of the enzyme activity test at the end of the study are presented in Table 3.

Table 3. Protease Enzyme Activity (U/min)

Hormone Dosage	Protease Enzyme Activity (U/min)	
	Beginning	End
0 mg/kg	0.036	0.39±0.02[a]
1 mg/kg	0.036	0.43±0.11[ab]
2 mg/kg	0.036	0.49±0.02[ab]
3 mg/kg	0.036	0.75±0.24[b]

The One Way Anova Analysis Test Procedure obtained a probability value smaller than the 0.05 significance level ($p<0.05$) used, so it can be stated that administering the rGH hormone to catfish seeds reared in saline media affects the activity of the protease enzyme. ResultsThe highest dose was 3 mg/kg feed, 0.75 ± 0.24 U/minute. As seed metabolism increases, enzymes' performance in seed digestion also grows to break down the protein consumed from feed. Fauziah et al.[15] said that digestive enzyme activity correlates with the number of enzymes found where digestion occurs. Protease enzyme activity can be determined by measuring the number of micromoles of amino acids produced per minute.

An increase in protease enzyme activity can occur because exogenous administration of rGH can increase the metabolic capacity of fish, which plays a role in digestion, absorption, and transport of nutrients[16]. Then, Syahrir et al.[17] added that factors that influence the process of fish digestive enzyme activity are metabolism, use of metabolic energy, and growth hormones.

Feed containing rGH is thought to stimulate the hypothalamus gland to produce more GH in the seed's body, where the GH made is a polypeptide compound. Widiyanto in Manurung[18] states that growth hormone is a polypeptide hormone synthesized and secreted by the pituitary gland, stimulating cell growth and reproduction in other vertebrate animals.

Silalahi et al.[19] stated that feed containing rGH will enter the bloodstream and be captured by the pituitary, triggering the hypothalamus to excrete Growth Hormone hormone-releasing hormone (GHRH) and Somatostatin Hormone (GH). GH produced by the pituitary will be captured and flowed with GHBPs (Growth Hormone Binding Proteins) and delivered directly to several target organs involved in growth. The target organ will later absorb recombinant growth hormone through the growth hormone receptor (GHr) found in target organs such as muscles, bones, and the liver. In this case, it is suspected that the intestine is also one of the target organs that will be supplied with GH.

RNA/DNA ratio

The RNA/DNA ratio is one way to evaluate growth in seeds. To determine the RNA/DNA ratio, first determine the concentration of RNA and DNA in the sample. The average value of the RNA/DNA ratio is presented in Table 4.

Table 4. Average RNA/DNA Ratio

Hormone dosage	RNA/DNA ratio
0 mg/kg	1.19 ± 0.11^{a}
1 mg/kg	2.81 ± 0.04^{b}
2 mg/kg	3.13 ± 0.01^{b}
3 mg/kg	3.30 ± 0.49^{b}

The One Way Anova analysis test obtained a probability value smaller than the significance level of 0.05 ($p<0.05$) used, so it can be stated that there is an effect of giving the rGH hormone to catfish seeds reared in saline media on the RNA/DNA ratio. The highest RNA/DNA ratio value used a dose of 3 mg/kg feed 3.30±0.49; exogenous administration of the rGH hormone is thought to influence changes in RNA concentration. According to Chicharo & Chicharo[20], DNA as a primary carrier of genetic information is stable even under environmental changes. In contrast, the amount of RNA fluctuates depending on the level of protein synthesis.

The changes in RNA that occur are thought to be due to differences in metabolism in the seed body due to the administration of different doses of the rGH hormone. When proteins are broken down into amino acids, this can affect the RNA translation process in ribosomes. According to Campbell in Suhermiati[21], RNA adds an amino acid charge to the growing polypeptide chain when the anticodon forms a hydrogen bond with its complementary codon in the mRNA to translate the codon.

Protein synthesis in cells is generally divided into two processes, namely transcription and translation processes. According to Gusrina[22], Transcription is the process of transferring genetic information from DNA to RNA; all RNA molecules (mRNA, rRNA, and tRNA) are synthesized based on a specific sequence of bases in DNA, which acts as a template. At the same time, translation is translating a sequence of bases. Contained in mRNA into a sequence of amino acids.

Due to differences in doses, there are also differences in metabolism in each treatment. This is also thought to impact the activity of enzymes in the digestion of seeds and in breaking down proteins into amino acids, which will later go through the RNA translation process. After reading the RNA code, the amino acids will bind and re-form polypeptides or proteins, which can be cells or certain enzymes and hormones.

Then, Jamal[23] stated that the RNA/DNA ratio can show protein synthesis activity, which influences the increase in cell number (hyperplasia) and cell size (hypertrophy). It is thought that maintenance using salinity also affects seeds' RNA/DNA ratio because the seeds are in isoosmotic conditions. Based on research by Hasbullah et al.[24], which used different salinities, the highest RNA/DNA ratio was at a salinity of 16 ppt of 1.73 ± 0.08. These results indicate that salinity in culture media can increase RNA synthesis, as shown in the protein content. Several studies state that the higher the RNA/DNA ratio value, the higher the quality of the individuals produced.

According to Faidar et al.[25], a high RNA/DNA ratio will impact individual quality. Research related to the RNA/DNA ratio has previously been carried out on several types of catfish, and when compared with previous research, the value of the RNA/DNA ratio in the research is relatively low. In Sari et al. 26's research using baung fish, the RNA/DNA ratio value obtained was 13.14±0.38. In the research of Pamungkas et al.[12], the RNA/DNA ratio value for fast-growing selected Siamese catfish was 23.75. Meanwhile, Dewi & Tahapari[27] research using pearl strain catfish showed an RNA/DNA ratio of 43.0±3.8.

4. CONCLUSION

The conclusion that can be drawn from this research is that administration of the rGH hormone at a dose of 3 mg/kg feed affects the Protease Enzyme Activity value by 0.75±0.24 and an RNA/DNA ratio of 3.30±0.49.

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