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Modification of Carp (*Osphronemus Gouramy*) Growth with Insulin-Like Growth Factor-I *Recombinant Mouse* in Vivo

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Abstract

Osphronemus gouramy consumption is increasing every year, which causes a high number of inquiries for this fish, which cannot be offset sufficiently by the amount of production. The growth rate of O. gouramy is relatively slow, so achieving the required consumption is relatively long. Quality improvement of productivity by improving the ability of cell metabolism to intake fish feed through hormone injection recombinant mouse insulin-like growth factor-I (IGF-I) is expected to spur growth so that fish production can be increased. This study aims to determine the effect of the hormone IGF-I recombinant mouse on the increased growth of O. gouramy. The benefits of this research are expected to deliver the benefits of science in the form of scientific information to the public in general and fish farmers, especially about the effect of the hormone recombinant mouse IGF-I on the growth of O. gouramy. The research method is carried out experimentally by using a completely randomized design (RAL); this study uses 80 O. gouramy was divided into 4 treatments P0: without hormone injections of IGF-I, P1: injected with the hormone IGF-I at a dose of 10 ng/ml, P2: injected with the hormone IGF-I at a dose of 20 ng/ml and P3: injected with the hormone at a dose of 40 ng/ml each of 5 replicates each test 4 tail O. gouramy. The hormone IGF-I recombinant mouse is done by injection. The treatment with hormone injection of recombinant mouse IGF-I. The parameters measured were growth. The results showed that the treatment hormone injection of recombinant mouse IGF-I with different doses gave significant differences (p <0.05); weight growth (P0: 0 ng/ml = 7,23 gram ± 0,37-gram dan P3: 40 ng/ml = 26,35 gram ± 1,89 gram.); length growth (P0 : 0 ng/ml = 5,76 cm ± 0,38 cm dan P3: 40 ng/ml = 7,93 cm ± 0,07 cm). The conclusion is that recombinant mouse IGF-I increased the growth by increasing in weight and length of O. gouramy, and improvement of the growth occurred in the treatment injection of the dose: 40 ng/ml.

Keywords: *Osphronemus. Gouramy, Insulin-Like Growth factor-I (IGF-I), Growth*

1. Introduction

Gouramy (Osphronemus gouramy) is a freshwater fish that has long been known in Indonesia and has quite a lot of demand. The savoury taste and the texture of the meat could be mushy, making carp very popular with the people of Indonesia, especially the people of the island of Java (Arfah & Carman, 2006). Carp are also known as freshwater cultured fish with slow growth. The Ministry of Maritime Affairs and Fisheries (KKP) establishes policies. It implements several programs in the form of development activities that are adapted to changes in the national and international environment. Meeting the economic needs of the community that focuses on the welfare of the people requires an effort, one of which is in the form of increasing the productivity of aquaculture (Hidayat et al., 2023). During 2014-2015, the market in East Java Province experienced an increase in fish production from 8,425 to 9,525 tons, but only needed a supply of carp of 12-13 tons per week for the local market and the rest of the production was distributed throughout Indonesia. East Java Province is the central producer of carp, which contributes 60-70% of total production in Indonesia. Nationally, the need for carp still needs to be improved (Center for Statistics and Information of KKP, 2015).

The consumption of carp that increases every year results in a high number of requests; this situation has not been able to be balanced with an adequate amount of production. The growth rate of carp is known to be relatively slow; reaching the size of consumption takes a relatively long maintenance time. Efforts to increase fish production are implemented in various ways, for example choosing good seeds through increasing production quality (Mulyati et al., 2002; Sakdiah et al., 2003), improving feed (Djarijah, 1995; Suprayudi and Setiawati. 2003), and applying technology. Fish farming (Arfah & Carman, 2006). Improving the quality of production by increasing the ability of cell metabolism to intake fish feed ingredients through the administration of the hormone insulin-like growth factor-I is expected to stimulate growth so that the amount of fish production can be increased (Poppinga et al., 2007; Maggio et al., 2013).

Insuline-like growth factor-I (IGF-I) or somatomedin is a polypeptide growth factor secreted by the liver and various tissues in response to growth hormone (GH) stimulation. IGF-I mediates stimulating growth hormones (Hafez et al., 2000). Growth hormone stimulates the production of IGF-I in the liver (Bjornsson et al., 2004), which plays an important role in regulating several physiological processes such as growth, metabolism, development (Pozios et al., 2001), reproduction (Weber & Sullivan, 2000), and osmoregulation (McCormick, 2001). Insulin and insulin-like growth factor I (IGF-I) are structurally closely related to peptides. We are related to the major insulin family, with the biological function of binding to certain cell surface receptors. Insulin bound in the liver, white muscle and heart, in the brain or reproductive organs has been studied in different fish species (Gutiérrez et al., 1995; Leibush et al., 1996). The main function of IGF-I is to stimulate growth and differentiation (Le Roith et al., 1995). Insulin and IGF-I closely interact in the regulation of metabolism and growth. In mammals, IGF-I affects glucose uptake and metabolism (Dohm et al., 1990; Boulware et al., 1992) and protein synthesis in skeletal muscle (Fuller et al., 1992). IGF-I is hypoglycemic in fish (Plisetskaya et al., 1994).

Research on insulin-like growth factor-I is rarely done in fish, especially in Indonesia. In contrast, the application of IGF-I to increase growth in fish in Indonesia has never been made. This study aimed to determine the effect of the hormone IGF-I recombinant mouse on increasing the growth of carp (Osphronemus gouramy).

2. Materials And Methods

Place and time

The research was conducted at Griya Kartika Sedati Housing, Sidoarjo, in July-September 2021.

Materials and Tools

Research materials: carp with an initial length of 10-15 cm and an initial weight of 150-200 grams in as many as 80 individuals, well water, factory-made fish feed, hormone IGF-I (recombinant mouse, Cat#591406, Biolegend, San Diego USA), and adjuvants.

Research equipment: 4 glass aquariums with dimensions of 150 cm x 50 cm x 50 cm; each aquarium is insulated with glass into 5 parts so that each part measures 30 cm x 50 cm x 50 cm with accessories such as an aerator, aeration hose and ram wire cover above the aquarium, disposable tuberculin syringe 1 ml, rubber gloves (rubber glove), centimetre (cm) length gauge or ruler, gram analytical scale.

Research design

The study was conducted experimentally using a completely randomized design (CRD). 80 carp fish were randomly divided into 1 aquarium divided into 5 parts, each partition filled with 4 fish as control (P0), in 2,3 and 4 aquariums the same as 1 aquarium. Each aquarium partition contained 4 fish as treatment (P1, P2, P3). Each treatment was repeated 5 times. The administration of IGF-I hormone by injection per im, namely:

P0: Carp were injected with 0.1 ml physiological NaCl per im. Each fish was injected once.

P1: The carp were once injected with the IGF-I recombinant mouse hormone 0.1 ml per im. in physiological NaCl solution (10 ng/ml dose) per fish.

P2: Carp were injected with recombinant mouse IGF-I hormone 0.1 ml (per im) in physiological NaCl solution (dose of 20 ng/ml) once per fish.

P3: Carp were injected with recombinant mouse IGF-I hormone 0.1 ml (per im) in physiological NaCl solution (dose of 40 ng/ml) once per fish.

Preparation of Containers and Carp

The rearing container uses 4 glass aquariums which have been divided into 20 parts/bulks with dimensions: length x width x height 30 cm x 50 cm x 50 cm, filled with water as much as the volume of the aquarium so that each partition (part) containing 56.25 of well water and allowed to stand overnight.

As many as 80 carp fish were distributed randomly into 20 sections/bulk, with each section/section filled with 4 carp. The carp in the aquarium was adapted for 7 days to avoid stress with environmental conditions of temperature, feed and the atmosphere of a new place. After the carp were adapted, the initial length and weight were measured, and then the IGF-I hormone injection was given at the appropriate dose for each control group and the treatment group.

Feeding

The carp feed given during the study period used feed made by a fish feed factory in the form of pellets, given three times a day, namely at 06.00 in the morning, 13.00 in the afternoon, and 20.00 in the evening. Feeding was adjusted based on the body weight of the fish, which was 3% of the fish's body weight. IGF-I.

Hormone Injection

The recombinant mouse IGF-I hormone was diluted by adding 0.9 ml of adjuvant solvent, diluted again according to the dose given to the treatment, namely; 10 ng, 20 ng and 40 ng, were given by injection using a 1 ml injection syringe on the back muscle of the fish with the injection direction from back to front with a dose corresponding to the treatment of 0.1 ml. The dose of IGF-I given follows Reinecke's (2010) suggestion from a review of various studies of IGF-I in fish.

Data analysis

The data obtained in the form of weight and body length of carp were tabulated in a table and then analyzed using ANOVA to determine the effect of IGF-I from each treatment. To see the difference between treatments, then continued with Tuckey B. Statistical data processing using program facilities: IBM SPSS Statistics Version 20.

3. Results and Discussion

A The results of the study on the growth of body weight and body length of carp (Osphronemus gouramy) are presented in Table 1 and Table 2 below.

Table 1. Weight growth of carp in treatment with the injection of the hormone IGF-I recombinant mouse

Treatment	N	Weight growth (G) Average ± SD (grams)
P0 (Control) 0 ng/ml	5	7,23 ± 0,37 [a]
P1: IGF-I *rec mouse* 10 ng/ml	5	17,01 ± 0,42 [b]
P2: IGF-I *rec mouse* 20 ng/ml	5	18,22 ± 0,35 [b]
P3: IGF-I *rec mouse* 40 ng/ml	5	26,35 ± 1,89 [c]

Note: different superscript marks indicate significant differences ($p<0.05$)

From Table 1. the statistical analysis using one-way ANOVA between treatments P0: IGF-I at a dose of 0 ng/ml and P1: IGF-I recombinant mouse at a dose of 10 ng/ml was significantly different ($p<0.05$), P1: IGF-I recombinant mouse dose of 10 ng/ml and P2: IGF-I recombinant mouse dose of 20 ng/ml were not significantly different ($p>0.05$), P2: IGF-I recombinant mouse dose of 20 ng/ml and P3 : IGF-I recombinant mouse dose of 40 ng/ml was significantly different ($p<0.05$), in increasing the weight of carp (Osphronemus gouramy).

Table 2. Length growth of carp in treatment with the injection of the hormone IGF-I recombinant mouse

Treatment	N	Growth in length (L) Average ± SD (cm)
P0 (Control) 0 ng/ml	5	5,76 ± 0,38 [a]
P1: IGF-I *rec mouse* 10 ng/ml	5	5,88 ± 0,17 [a]
P2: IGF-I *rec mouse* 20 ng/ml	5	6,42 ± 0,08 [b]
P3: IGF-I *rec mouse* 40 ng/ml	5	7,93 ± 0,07 [c]

Note: different superscript marks indicate significant differences ($p<0.05$)

The growth hormone and insulin-like growth factor (GH-IGF) axis affects somatic growth and metabolism regulation in teleost fish. The GH-IGF axis begins in the anterior pituitary gland under the control of several hypothalamic hormones, including growth hormone-releasing hormone (GHRH) (Vong et al., 2003; Peterson et al., 2005). Insulin-like growth factor binding protein (IGFBP) plays a central role in prolonging the half-lives of IGF, which coordinates and transports circulating IGF (Moriyama et al., 2000). This extended family of IGFBPs includes IGFBP-related protein (IGFBP-rP), which also regulates IGF activity (Rodgers et al., 2008). through receptors on target tissues resulting in increased growth (Peterson et al., 2009). Serum concentrations of IGF-I stimulate or suppress GH release from the anterior pituitary via feedback on m mammals and lower vertebrates, as has been demonstrated especially in bony fish. In mammals and bony fish, the pituitary GH/liver IGF-I axis involved in the endocrine regulation of important physiological processes appears to be present (Eppler, 2011).

In fish, as in other vertebrates, energy ingestion and accumulation are the key to survival, growth and reproduction, with normal body fat as an important energy reserve. Growth hormone (GH) displays pluripotency which includes a wide range of effects apart from its growth-stimulating action in mammals and fish. However, most of GH's subsequent action is through the production and stimulation of insulin-like growth factor-I (IGF-I) secretion (Kling et al., 2011).

Insulin-like growth factor-I is structurally and functionally related to insulin, and its biological actions in fish include growth regulation, stimulation of tissue differentiation, reproduction and osmoregulation (Duan, 1998). IGF-I is more effective than insulin in stimulating glucose and amino acid uptake in muscle cells in rainbow trout (Oncorhynchus mykiss), suggesting that this hormone is also involved in the regulation of carbohydrate metabolism and may even exceed its relevance for insulin (Enes, 2011).

Insulin-like growth factor-I is an important member of IGF signaling and regulates skeletal muscle growth and development in vertebrates (Duan et al., 2010). For example, the body weight (mass) born with IGF-1 or IGF-2 in knockout mice is about 60% heavier than in wild-type mice (Liu et al., 1993). Over-administration of IGF-I in rats increased body mass by 30% (Mathews et al., 1988). In most fish species, circulating IGF-I or tissue IGF-I mRNA levels positively correlate with diet, protein content and growth rate (Carnevali et al., 2006; Beckman et al., 2004). Implanted IGF-I injection contributes to accelerated fish growth (McCormick et al., 1992), as Yan et al. (2012) summarized. In many fish species, circulating blood or tissue IGF-I levels of mRNA are positively correlated with food rations, dietary protein content, and body growth rate (Beckman et al., 2004). In vertebrates, the IGF system is critical in the formation and maintenance of skeletal muscle (Le Roith et al., 2001; Stewart & Rotwein, 1996; Benito et al., 1996) because it acts as a potent positive switch for regulating muscle growth (Florini et al., 1996; Le Roith et al., 2001), cited by Montserrat et al. (2007).

Circulating insulin-like growth factor-I has been proposed as a reliable index for fish growth recently related to individual growth rates under different and/or altered nutritional conditions (Beckman et al., 2004a, b, c; Picha et al., 2008b; Beckman, 2011). Usually, circulating levels of IGF-I are decreased when fish are restricted in their feed or are not fed and are fed back either by increasing the feed ratio or being fed again (Picha et al., 2008b; Beckman, 2011). Serum IGF-I levels in year-old carp salmon showed a similar pattern to other types of fish. Namely, there was a positive correlation between serum IGF-I levels and growth rates of both fish length and weight, which supports the idea that circulating IGF-I I is a good growth index in various fish species, which is summarized by several researchers by Kawaguchi et al. (2013) in his research.

The main factors that regulate protein synthesis are IGFs (insulin-like growth factors), together with their receptors/ IGFR (insulin-like growth factor receptor) and binding proteins/ IGFBP (insulin-like growth factor-binding protein) (Johnston et al., 2011). The IGFs are circulating peptides that comprise mostly two variants, IGF1 and IGF2, with roles in muscle cell viability, the proliferation and differentiation of myoblasts, and hypertrophy and repair after muscle injury and exercise [8–10] (Jiao et al., 2013; Lawlor et al., 2000; Zanou & Gailly, 2013). IGF1 is one of the most studied and characterized growth factors that promote muscle growth. When binding to its receptor (IGF1R).

4. Conclusion

The better understanding and new insights into fish muscle growth regulation by important pro-growth inputs. Together, these findings may support future research and contribute to improvements in aquaculture programs, aiming to increase muscle mass, enhance growth rate, and/or better feed conversion efficiency, as well as background information for developing and advancing in vitro meat production. Administration of the hormone insulin-like growth factor-I (IGF-I) by injection can increase carp weight and length growth. The best dose for increasing weight and length growth is 40 ng/ml. IGF-I hormone injection can increase weight and length growth in carp. IGF-I hormone can be given at a dose of 40 ng/ml.

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