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ISOLATION AND IDENTIFICATION OF PARTIAL GROWTH HORMONE (GH) mRNA OF TIGER SHOVELNOSE CATFISH *Pseudoplatystoma fasciatum* (LINNAEUS, 1766)

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ABSTRACT

The growth of fish is regulated by growth hormones secreted in a limited amount by its pituitary glands. Tiger shovelnose catfish has a relatively faster growth indicating that some growth hormones suspectedly play roles in the process and could be used in improving other farmed fish species. This study aimed to isolate and identify the growth hormone (GH) mRNA gene in Tiger shovelnose catfish (*Pseudoplatystoma fasciatum* Linnaeus, 1766). The sample was isolated from the pituitary, the fish measuring 602 g and 43 cm body length. Total RNA was extracted using the Tri Reagent Kit, followed with cDNA synthesis. The success of the extraction was confirmed by quantification and PCR using the β-actin gene as an internal control. GH mRNA gene was isolated by RT-PCR method, with degenerated primers from seven catfish species sequence data in the NCBI gene bank. The single band from GH gene was cloned and sequenced. Total RNA quantification with a concentration of 227 ng/µL and purity of 1.821. The successful isolation of mRNA from the pituitary gland was confirmed by amplifying the β-actin gene generated at 300 bp. This isolation of the GH mRNA gene had a sequence length of 234 bp. Tiger shovelnose catfish GH gene consists of 17 amino acid residues. The GH gene of Tiger shovelnose catfish was close to that of striped catfish (*P. hypophthalmus*) and Indian catfish (*Clarias batrachus*) with almost similar homology value of 90.6%. Partial GH gene from tiger shovelnose catfish can be used a molecular marker in revealing the role of growth hormone on fish development, fish biology, growth gene expression, selective breeding and other mechanisms related to the aquaculture.

KEYWORDS: degenerate primer; gene expression; pituitary; PsGH; RT-PCR

INTRODUCTION

Growth in fish culture is one of the keys to increase the productivity of cultured fish. Genetic engineering in increasing growth has been through various methods such as transgenesis and the administration of recombinant growth hormone (rGH). Growth hormone (GH) is a hormone secreted by the pituitary gland of an individual. GH in an individual has a limited amount and is only used for himself. Growth hormone functions to improve and stimulate individual fish growth through fat conversion and increase protein biosynthesis, metabolic system, osmoregulation, reproduction, immune function, and improve feed conversion (Buwono *et al*., 2012; Buwono & Suparta, 2009; Moriyama *et al*., 2000; Pinheiro *et al*., 2008). To get growth hormone, it is necessary to isolate the GH gene. GH gene can be isolated from the fish pituitary to obtain genomic DNA or mRNA (Alimuddin *et al*., 2007; Buwono *et al*., 2012).

The utilization of growth hormone in fish culture has been widely applied through the transgenesis method. Transgenics in carp have been carried out by inserting the TiGH gene (GH tilapia), which can increase the growth of carp higher than control (Faridah, 2012; Kurdianto *et al*., 2016). In Siamese catfish, the insertion of the PhGH gene through sperm has also been successfully conducted through the electroporation method (Dewi *et al*., 2012). The PhGH gene from catfish has also been successfully applied to betta fish (*Betta imbellis*) through the transfection method. It can increase the growth with an

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average gene transmission in F1 betta fish of 62.5 % (Kusrini *et al*., 2018).The utilization of growth hormone is not only through the transgenesis method but can also be applied by recombinant growth hormone (rGH). The rGH method is safer in producing aquaculture products because it is not passed on to the next generation (Apriliana *et al*., 2017). One of the growth hormones that has been successfully commercialized is "Mina Grow". "Mina Grow" is rGH from the giant grouper (rEIGH). rEIGH began to be assembled after discovering three types of rGH vectors from grouper, gourami, and carp (Alimuddin *et al*., 2010; Lesmana, 2010). "Mina Grow" has been widely tested to accelerate fish growth, both consumption fish such as sangkuriang catfish (Triwinarso *et al*., 2014), eel (Alimuddin *et al*., 2014), larasati tilapia (Setyawan *et al*., 2014), Nile tilapia (Safir *et al*., 2017), as well as ornamental fish such as botia fish (Permana *et al*., 2018), betta fish (Hayuningtyas & Kusrini, 2016), and black ghost fish (Sembiring *et al*., 2018).

Tiger shovelnose catfish (*P. fasciatum* Linnaeus, 1766). is one of the introduced fish that has been widely cultivated in Indonesia and is an ornamental fish export commodity (Kusrini *et al*., 2015). Tiger shovelnose catfish have an aggressive and cannibalistic nature and fast growth, especially in the early stages. The growth of Tiger shovelnose catfish can reach a size of 2 m (Britz & Pienaar, 1992; Hseu *et al*., 2007; Nuñez *et al*., 2008). This fast-growing potential can be utilized in obtaining growth hormone genes. There is not much information regarding the isolation of the GH gene from ornamental fish, and there is no data on GH sequences from Tiger shovelnose catfish in the NCBI Gene Bank. Therefore, it is necessary to isolate the growth hormone gene in Tiger shovelnose catfish through mRNA isolation. The purpose of this study was to isolate the partial sequence and identify growth hormone (GH) mRNA in Tiger shovelnose catfish (*P. fasciatum* Linnaeus, 1766).

MATERIALS AND METHODS

Preparation Sampling of Fish

The fish used is the Tiger shovelnose catfish, measuring 602 g and a total length of 43 cm. This fish was obtained from the private collection of hobbyists traded online. This research was conducted at the Testing Laboratory–Genetics, Research Institute for Ornamental Fish Culture, Depok, West Java, Indonesia. Fish samples were anesthetized with 400 ml/L phenoxyethanol and waited until unconscious. Fish were weighed and measured in length, and the fish was dissected on the head to take the pituitary gland. This is located at the bottom of the brain. The pituitary was then weighed and put in an RNA Latter solution to stabilize the condition of the RNA so that it is not easily damaged. The following steps include RNA extraction, cDNA synthesis, RT-PCR amplification performed with the b-actin gene as an internal control (housekeeping gene) and the GH gene. The single band of GH gene amplification was cloned at the Genetics Lab, PT. Genetics Science and continued sequencing at the 1st Base Laboratory.

RNA Extraction

RNA extraction was carried out using the Tri Reagent (MRC) RNA isolation kit. Pituitary tissue was put in 100 mL of Tri Reagent solution, and ground until smooth using a pestle. After being crushed, 900 mL of Tri Reagent solution was added and mixed by vortex, then followed the Tri reagent kit (MRC) protocol. Total RNA elution was carried out by adding 50 mL of nuclease free water, then mixed by vortex. Total RNA concentration to be calculated using a UV-Vis spectrophotometer (Gene-Quant, GE). After measuring, the sample can be stored at -80°C until used.

cDNA Synthesis

cDNA was synthesized using the NZY First-Strand cDNA Synthesis Kit (Nzytech). The premix composition for cDNA synthesis includes NZYRT 2x Master mix 10 mL, NZYRT Enzyme Mix 2 mL, total RNA concentration of 1 ng.μL^{-1}, and H_2O up to a total volume of 20 µL. The mixed premix solution was then incubated at 25°C for 10 min. The samples were incubated again at 50°C for 30 min. Subsequently, the reaction was inactivated by heating at 85°C for 5 min. 1 mL of NZY RNase *E. coli* was added and was incubated at 37°C for 20 min. After the cDNA was obtained, it can be directly used for the PCR step or stored in the refrigerator at -20°C.

Detection of β-actin Gene

Detection of the b-actin gene as a housekeeping gene was done through the PCR method. This uses the DreamTaq Master Green PCR Master Mix (2x) Kit (Thermo Scientific). Reaction composition consists of 12.5 mL master mix, 1 mL universal b-actin primer F (5'- GGT CGA GCT GGA CGG CGA CG –'3), 1 mL universal b- primer R (5'- ACG AAC TCC AGC AGG ACC AT –'3) each primer with a concentration of 10 rmol, 1 mL cDNA, and nuclease-free water up to a volume of 25 mL. The conditions of PCR amplification include consisting of initial denaturation temperature of 94°C for 3 min, denaturation temperature of 94°C for 30 sec, the annealing temperature of 58°C for 30 sec, and extension temperature of 72°C

for 30 sec. This cycle was repeated 35 times, was terminated with a final extension at 72 °C for 3 min and final conditioning at 4 °C. PCR samples can be stored in a refrigerator or directly electrophoresed on the 1.5 % agarose gel with 1´ TBE then immersed in 0.5´ TBE solution. The target gene resulting from the amplification was 300 bp DNA fragment.

Primer Design, Optimization, Primer Selection and Detection of GH Genes

The degenerate primer was designed based on seven sequence data in the Gene Bank (NCBI). The primer was designed from 5 GH catfish gene species, namely, *Heteropneustes fossilis* (AF147792.1), *Pangasianodon gigas* (L27835.1), *Pelteobagrus fulvidraco* (DQ112163.1), *Ictalurus punctatus* (NM_001200245.1), and three sequence data for *Clarias gariepinus* species (FJ823972.1, MN249238.1, EF411172.1) (Figure 1). Sequence data were aligned using BioEdit software, then forward and reverse primers were designed. The resulting forward and reverse primer designs are shown in Table 1. The resulting primer has 21 bases on the forward primer and 24 bases on the reverse primer. There are three types of primers designed, consisting of 1 forward primer and two reference primers. The forward primer (F1) was paired with both reverse primers (R1 and R2), resulting in 2 pairs of primers with different target gene sizes (Table 1). Optimization of melting PCR temperature was at 61°C and 62 °C.

The GH gene was detected using the DreamTaq Master Green PCR Master Mix (2x) Kit (Thermo Scientific). Reaction composition consists of 12.5 mL master mix, 1 mL primer GH F, 1 mL primer GH Reach with a concentration of 10 rmol, 1 mL cDNA, and nuclease-free water up to a volume of 25 mL. The conditions of PCR amplification include consisting of initial denaturation temperature of 94°C for 3 min, denaturation temperature of 94°C for 20 sec, the annealing temperature of 61°C for 30 sec, and extension temperature of 72°C for 30 sec. This cycle was repeated 40 times and was terminated with a final extension of 72°C for 7 min and the final conditioning at 4°C. PCR samples can be stored in a refrigerator or directly electrophoresed on the 1.5% agarose gel with 1x TBE.

Electrophoresis and Visualization

The PCR results were then electrophoresed using horizontal electrophoresis (Biorad). Electrophoresis was carried out on 1.5% agarose gel with 1´ TBE then immersed in 0.5xTBE solution. PCR product was inserted 2 mL of into the electrophoresis well, parallel to the DNA leader 100 bp plus (Vivantis) as a marker. Electrophoresis was run at 100 volts for 30 min. Electrophoresis results were visualized and documented on a Gel Doc UV transilluminator (Simple Protein). The amplification results of the b-actin gene were used as an internal control to confirm the success of mRNA isolation.

Cloning and Sequencing

The results of GH gene amplification showing a single fragment were then cloned in the private companies PT. Genetika Science, Indonesia, and sequencing at the 1st Base Laboratory, Malaysia. The PCR product was cloned with stages including purification, ligation, transformation, and colony PCR using pTA2 vector (Toyobo) a pair of primers, namely T3 Primer: 5'- ATTAACCCTCACTAAAG–3' and T7 Promoter Primer: 5'-TAATACGACTCACTATAGGG – 3'. PCR colony program: initial denaturation of 95°C for 5 min, denaturation temperature of 94°C for 15 sec, the annealing temperature of 45°C for 30 sec, and extension temperature of 72°C for 45 sec. This cycle was repeated 35 times, and final conditioning was at 4°C. The DNA sequencing process was conducted using the Sanger sequencing method.

Data Analysis

The analyzed data are descriptive. The chromatogram image was generated using the Finch TV software. Sequencing results were analyzed using BioEdit software, MEGA X, to produce a phylogenetic tree and amino acid reduction. The amino acid composition was tabulated. BLASTN software was applied to

Table 1. Primer sequence, length, GC content, melting temperature (TM), and size target of GH Catfish (GHC) for Tiger shovelnose catfishv

No.	GH Primer	Sequence (5'-3')	Length	GC (%)	TM (°C)	Size (bp)
1	F1	CAG GCA AGG ACG AGA CCC AGA	21	61.90	61.20	234
	R1	**CTG GTA GAA ATC CTC GAA GGG CGG**	24	58.33	61.10	
2	F1	CAG GCA AGG ACG AGA CCC AGA	21	61.90	61.20	148
	R2	**CCG ATG CCC ATT TTC AGG TCA GCC**	24	58.33	62. 5	

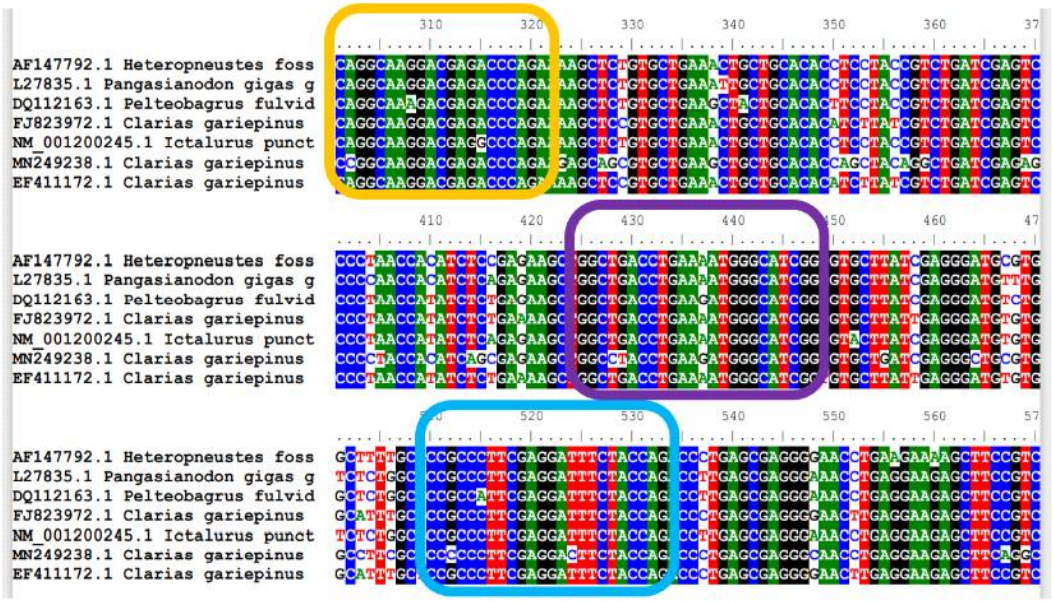


Figure 1. Alignment of GH gene of 7 catfish species (Ordo Siluriformes) from the NCBI gene bank data. Note: Yellow box = sequence area for primer forward (F1); Blue box = sequence area for primer reverse 1 (R1); Purple square = sequence area for primer reverse 2 (R2).

check the similarity of the GH gene with the closer species.

RESULTS AND DISCUSSION

The concentration of the total RNA from the pituitary gland (4.6 mg) was 227 ng/µL with a purity of A260/A280 was 1.821. The visualization of the total RNA electrophoresis results is shown in Fig 2a. The amplification results using β-actin primer as an internal control are shown in Figure 2b. The amplification results show a fragment in the pituitary sample parallel to the positive control of the β-actin gene, which was at a size of 300 bp. The amplification of cDNA from tiger shovelnose catfish samples using b-actin primers indicates that the gene was successfully isolated from the pituitary gland and producing a single band. This is in line with Dewi *et al*. (2009), using the b-actin gene as an internal control to confirm GH gene isolation in catfish (*P. hypophthalmus*).

The success of GH gene isolation was strengthened by the amplification results using two types of GH degenerate primers, namely with a size of 234 bp at annealing temperature of 61°C and at 148 bp with an annealing temperature of 62°C. Both pairs of GH degenerate primers could produce a clear single band. The amplification results in the optimization of GH primers are shown in Figure 3a. The F1 and R1 primer pairs produced the appropriate target gene at 234 bp. The bands that appeared were seen at 61°C and 62°C, but the thickest bands that occurred were at 61°C. The F1 and R2 primer pairs produced the appropriate target gene at 148 bp. The resulting band was at both temperatures of 61°C and 62°C, but the thickest appearance was at 62°C. The results of the annealing temperature were determined from the thickness and consistency of the band. The primer with a target of 234 bp had an annealing temperature of 61°C, while the target of 148 bp had an annealing temperature of 62°C. Primer pairs F1 and R1 are primers with clearer results and have better consistency than pairs F1 and R2. Therefore, we determined the primer pair used for sequencing is F1 and R1, whose final product of the primer pair for sequencing is 234 bp. Primers with a target of 148 bp are suggested as GH detection primers for gene expression using the Real-Time PCR technique because they have targets with lower molecular weights.

The GH gene in Siberian Sturgeon (*Acipenser bareii*) was analyzed for gene expression using real-time PCR with a target sequence of 159 bp (Abdolahnejad *et al*., 2015). Target genes used for semi-quantitative detection of GH genes can use higher molecular weights. Detection of GH genes in giant gourami (Og-mGH), common carp (Cc-mGH), and giant grouper (El-mGH) resulted in PCR products of target genes was 576 bp, 579 bp, and 576 bp, respectively (Alimuddin *et al*., 2010). In the results of the PCR product with a lower size of 148 bp, it is more appropriate to use as

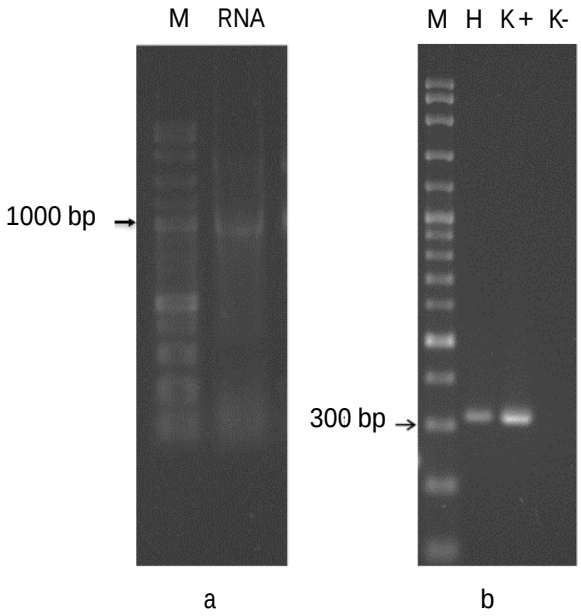


Figure 2. Result of (a)Total RNA electrophoresis, and (b) amplification β-actin gene from pituitary by Tiger shovelnose catfish. Note: M= Marker (100 bp plus DNA Ladder), RNA= Total RNA, H= Pituitary by Tiger shovelnose catfish, K+ = Positive control was obtained from the pituitary gland of Tiger shovelnose catfish which was first isolated and PCR using degenerate primers, K-= negative control without cDNA template.

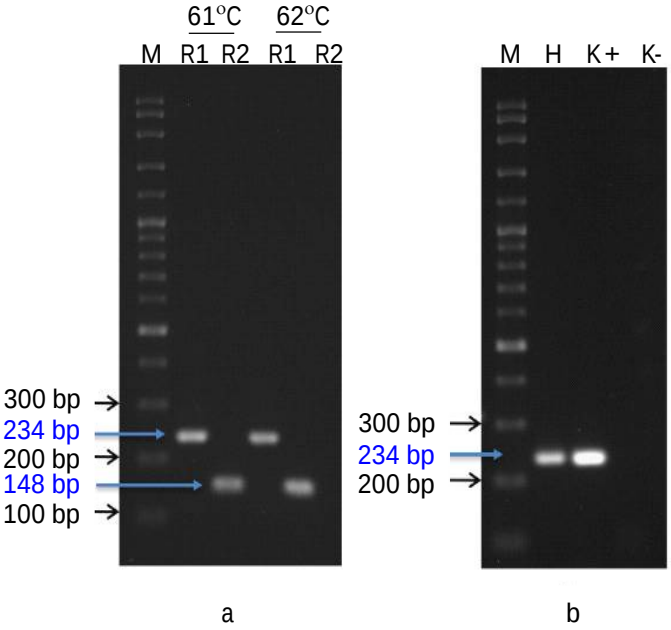


Figure 3. a) The results of optimization GH degenerate primers, b) amplification growth hormone (GH) from Tiger shovelnose catfish using degenerate primers. Note: M = Marker (100 bp plus DNA Ladder); (a) R1= Primer design with pair forward 1 and reverse 1; R2= Primer design with forward 1 and reverse two pairs. Samples used are from the pituitary organ. (b) H= Pituitary by Tiger shovelnose catfish, K+ = Positive control was obtained from the pituitary gland of Tiger shovelnose catfish which was first isolated and PCR using degenerate primers, K-= negative control without cDNA template.

a marker using the real-time PCR method. The PCR product with a size of 234 bp is a GH gene partially isolated from tiger shovelnose catfish. It also shows GH gene expression in tiger shovelnose catfish.

Figure 3b shows the growth hormone (GH) gene of Tiger shovelnose catfish pituitary. The GH gene band was at 234 bp, which was in line with the positive control. This shows that the GH gene was isolated using degenerate primers, which have been designed from several fish belonging to the same order as Tiger shovelnose catfish. The PCR product shows a single and clear band. According to Slater *et al*. (1986), the fragments carried the important information not only for coding certain structural domains in hormones but also for controlling gene expression.

Figure 4 shows the chromatograms generated from the sequencing performed on the GH gene of the Tiger shovelnose catfish. The total length of the resulting sequence is 393 bp. The result GH target gene sequence of 234 bp was a blocked image. The resulting chromatogram shows a good quality because it has a high and single curve shape. However, one overlap occurs in area 244, namely between bases T and G, which was read as T. The open reading frame (ORF) of the GH gene sequence is shown in Figure 5. The initial sequence in the ORF shows the target GH gene starting with the R1 primer sequence consisting of 24 bases. The sequences were also translated into amino acids to produce 78 amino acid residues. Three of which are TGA sequences, stop codons (end) that are not translated into amino acids. The characteristics of the GH gene from various species have different amino acid compositions produced. The growth hormone peptide of *P. hypophthalamus* has about 200 amino acid residues with 20 types contained therein (Dewi *et al*., 2009).

The GH gene that was partially isolated in Tiger Shovelnose Catfish produced only 75 amino acid residues (Figure 5.), consisting of 17 types of amino acid (aa) residues in it (Figure 6). Although 234 nucleotides were initially supposed to be translated into 78 aa residues, three of them could not be translated into aa because they were TAG sequences. The TAG sequence was a stop codon (end), so it was not translated into amino acids but served as a signal sequence for the last translational activity (Dewi *et al*., 2009). GH genes between fish species have different lengths. The length of the GH gene in *P. gigas* was 1.176 bp (Lemaire *et al*., 1994); while in *P. hypophthalmus,* it was 1.151 bp (Dewi *et al*., 2009). The GH gene in yellow catfish (*Pelteobagrus fulvidraco*) has 615 bp ORF translated into a polypeptide amino acid code to 204 (Tang *et al*., 2021). Tiger shovelnose catfish GH gene has 17 types of amino acids in it. GH grouper duck (*Cromileptes altivelis*) has 618 bp of GH gene sequence translated into amino acids into 205 amino acid codes consisting of 20 types of amino acids (Syarifudin *et al*., 2007). The composition of amino acids in Tiger shovelnose catfish was than giant grouper and striped catfish with 20 types of amino acids (Dewi *et al*., 2009; Syarifudin *et al*., 2007).

Figure 6 shows the percentage composition of the amino acids without the TAG sequence. The amino acid composition of GH Tiger shovelnose catfish was divided into four category amino acids based on the R group. The first group, i.e., non-polar R, consists of Leucine (16.0%), Valine (13.3%), Isoleucine (4.0%), Alanine (6.6%), Phenylalanine (4.0%), Methionine (1.3%), Proline (2.6%), so that a total of 48.0% of amino acids

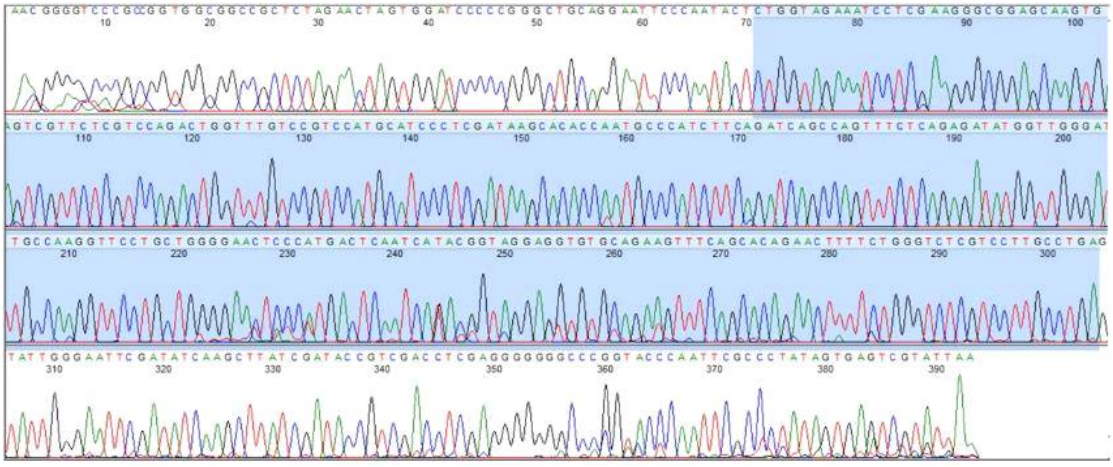


Figure 4. Chromatogram of GH partial gene sequencing of Tiger shovelnose catfish (*P. fasciatum* Linnaeus, 1766).

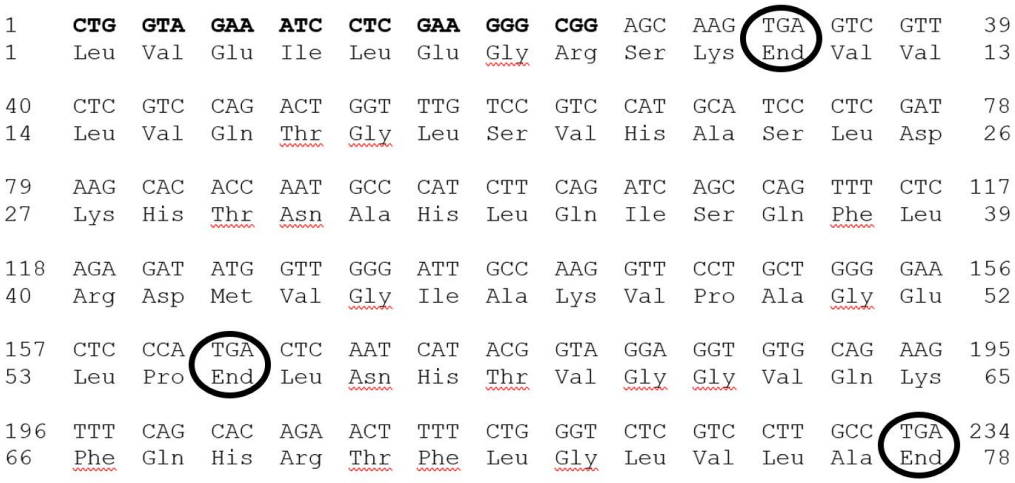


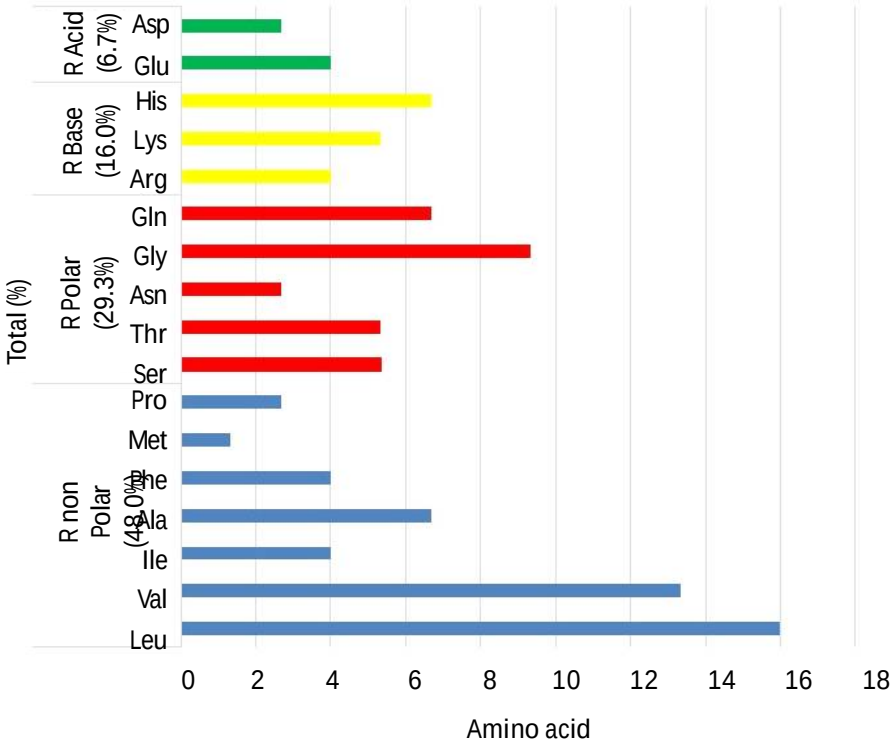
Figure 5. Open reading frame (ORF) sequence and amino acid residues of a GH gene encoding isolated from Tiger shovelnose catfish. (Note: Bold: R1 primer sequence, circle = stop codon, A = adenine, G = guanine, T = thymine, C = cytosine).

are in this group. The second group is R polar, which consists of Serine (5.3%), Threonine (5.3%), Asparagine (2.6%), Glycine (9.3%), and Glutamine (6.6%); with a total of 29.3%. The third group, R Base, consists of Arginine (4.0%), Lysine (5.3%), and Histidine (6.6%), making a total of 16.0%. The fourth group is R Acid with a total of 6,6%, consisting of glutamic acid (4.0%) and aspartic acid (2.6%). The amino acid composition of catfish was more complete than Tiger shovelnose catfish because it has 20 types of amino acids. The difference lies in Tryptophan, Cysteine, and Tyrosine types, although the percentage of occurrence is small, ranging from 0.5-4.0% (Dewi *et al*., 2009). Sequences that do not code for amino acids are called untranslated regions (Nugroho *et al*., 2016). Each amino acid has a different R group or side chain; depending on the size, shape, charge, hydrogen-binding capacity, and chemical reactivity (Hanum, 2017).

The composition of amino acids in the GH gene of each species was different. In grouper, there were 20 types of amino acids of GH gene. The non-polar R group had 40.5% (Syarifudin *et al*., 2007). The highest group was the GH of Tiger shovelnose catfish. Percentage of amino acid composition in grouper, based on other R groups, was polar R group consisting of seven types of amino acids (36.1%), basic R composed of three types of amino acids (12.2%), and acidic R of two types of amino acids (10.7%) (Syarifudin *et al*., 2007). The pattern of amino acid composition based on the R group has similarities between grouper and Tiger shovelnose catfish, but the types and percentages are different.

The amino acid with the highest percentage is leucine, both in catfish (14.0%) (Dewi *et al*., 2009), giant grouper (14.6%) (Syarifudin *et al*., 2007), and Tiger shovelnose catfish (16.0%). The lowest composition of striped catfish and giant grouper has the same amino acid, namely in tryptophan 0.5% and 0.49%, respectively, while the lowest GH in Tiger shovelnose catfish was methionine (1.3%). The amino acid composition of tiger shovelnose catfish is not as complete as that of striped catfish and giant grouper because it does not contain cysteine and tyrosine in R non-polar groups. Cysteine is an amino acid residue conserved in grouper GH (Dewi *et al*., 2009; Syarifudin *et al*., 2007). It is necessary to know the composition of amino acids in the GH gene because this can be used as a reference in the preparation of feed formulations in tiger shovelnose catfish aquaculture. According to Dewi *et al*. (2009), fish cannot synthesize essential amino acids, so their needs must be supported by protein in feed containing essential amino acids. Under normal physiological conditions, GH is secreted by the pituitary, thereby triggering the production of IGF-1 in the liver and other tissues. It can promote growth and especially metabolism by stimulating IGF-1 receptors (Chan *et al*., 2021; Wood *et al*., 2005).

The phylogenetic relationship between the evolutionary history of the GH gene was analyzed using the Neighbor-Joining method. This analysis involved 20 nucleotide sequences representing the GH gene in 20 species consisting of 4 orders, namely Siluriformes, Cypriniformes, Perciformes, and



Note: Leu = Leucine, Val = Valine, Ile = Isoleucine, Ala = Alanine, Phe = Phenylalanine, Met = Methionine, Pro = Proline, Ser = Serine, Thr = Threonine, Asn = Asparagine, Gly = Glycine, Gln = Glutamine, Arg = Arginine, Lys = Lysine, His = Histidine, Glu = Glutamate, Asp = Aspartate

Figure 6. Amino acid composition of GH gene from Tiger shovelnose catfish and its division by amino acid group (R Non-Polar, R Polar, R Base, and R Acid).

Tetraodontiformes as outgroups. The percentage of replicate trees in which related taxa clustered together in the bootstrap test (500 replicates) is shown next to the branch. The evolutionary distance was calculated using Kimura's 2-parameter method and in base substitutions per location units.

Phylogenetic analysis is to compare the GH gene of Tiger shovelnose catfish with several fish species from several order groups, with the furthest order being GH from the Order Tetraodontiformes as the outgroup. The analysis results show that the GH gene in Tiger shovelnose catfish was between two clades of orders, namely Siluriformes and Cypriniformes. The closest relationship between the two clades of the order was with Siluriformes, a type of catfish. The Tiger shovelnose catfish is still a catfish of the order Siluriformes. The link between the GH gene of the Tiger shovelnose catfish and the GH gene of other Siluriformes was not seen in the same clade but separated by the presence of a clade from the order Cypriniformes. The relationship between the Tiger shovelnose catfish GH gene and the order Perciformes was further seen because it does not belong to the same clade. This can be observed more deeply by looking at the nucleotide sequence alignment of several species of GH genes in the order Siluriformes with the GH of Tiger shovelnose catfish (named psGH) (Figure 8).

Sequence alignment results from 6 species of the order Siluriformes, including Tiger shovelnose catfish, 241 bp and 98 bp have similar sequences. The alignment was also compared with the GH gene from the carp (*Cyprinus carpio*) with the code ccGH, compared to the order Cypriniformes. After adding the ccGH gene (the GH *Cyprinus carpio* gene), there were 271 bp alignments with 78 nucleotide sequences similar. This indicates that between GH genes in both orders, the psGH gene is more closely related to the clade in the Siluriformes group with a nucleotide similarity of 40.6%. On the contrary, with the order Cypriniformes, the nucleotide similarity was 28.7%. The psGH gene does have a low resemblance to other GH genes, especially goldfish, which have different orders so that the phylogeny tree does not belong to the clade Siluriformes. This is due to divergent growth hormone genes, which is explained by the

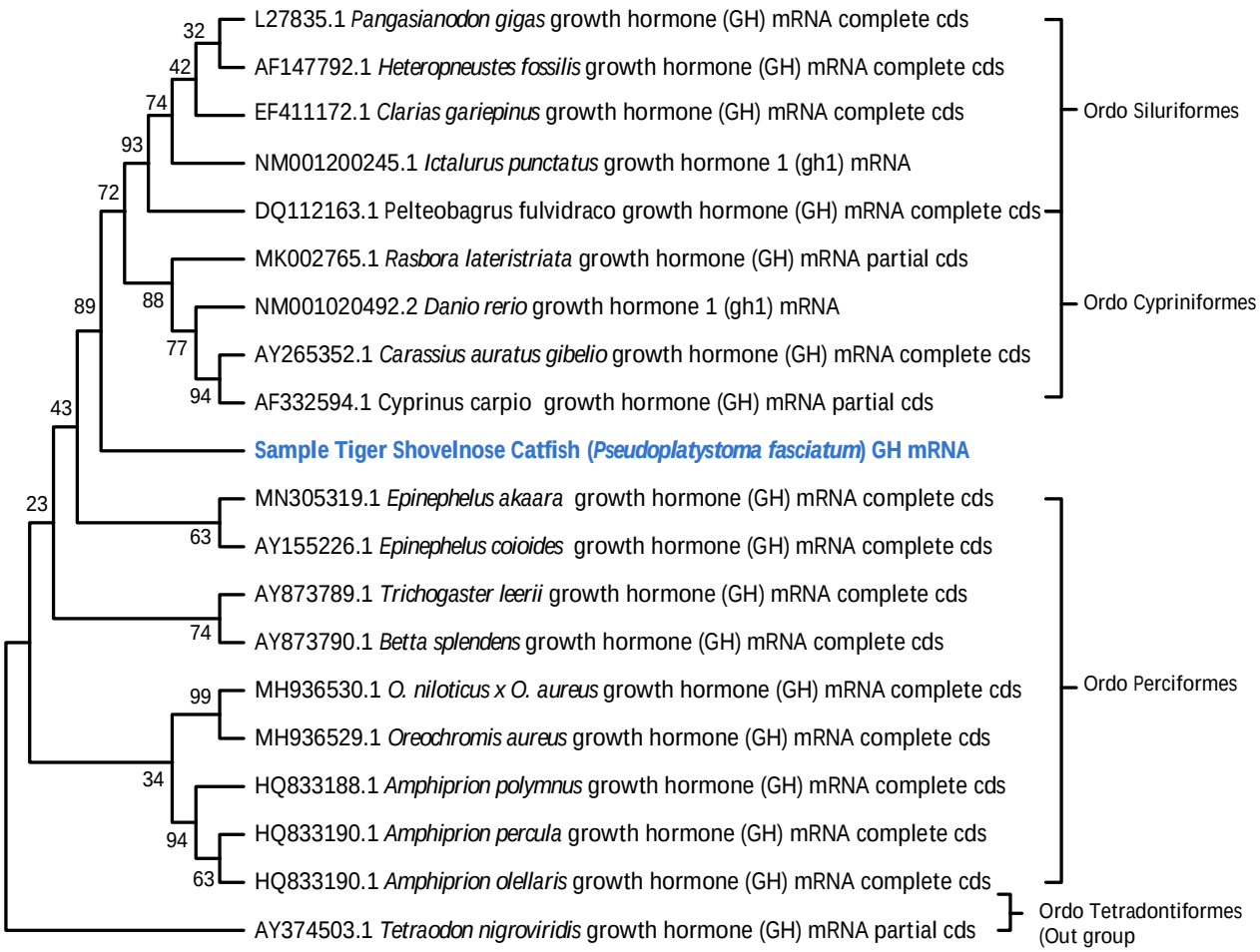
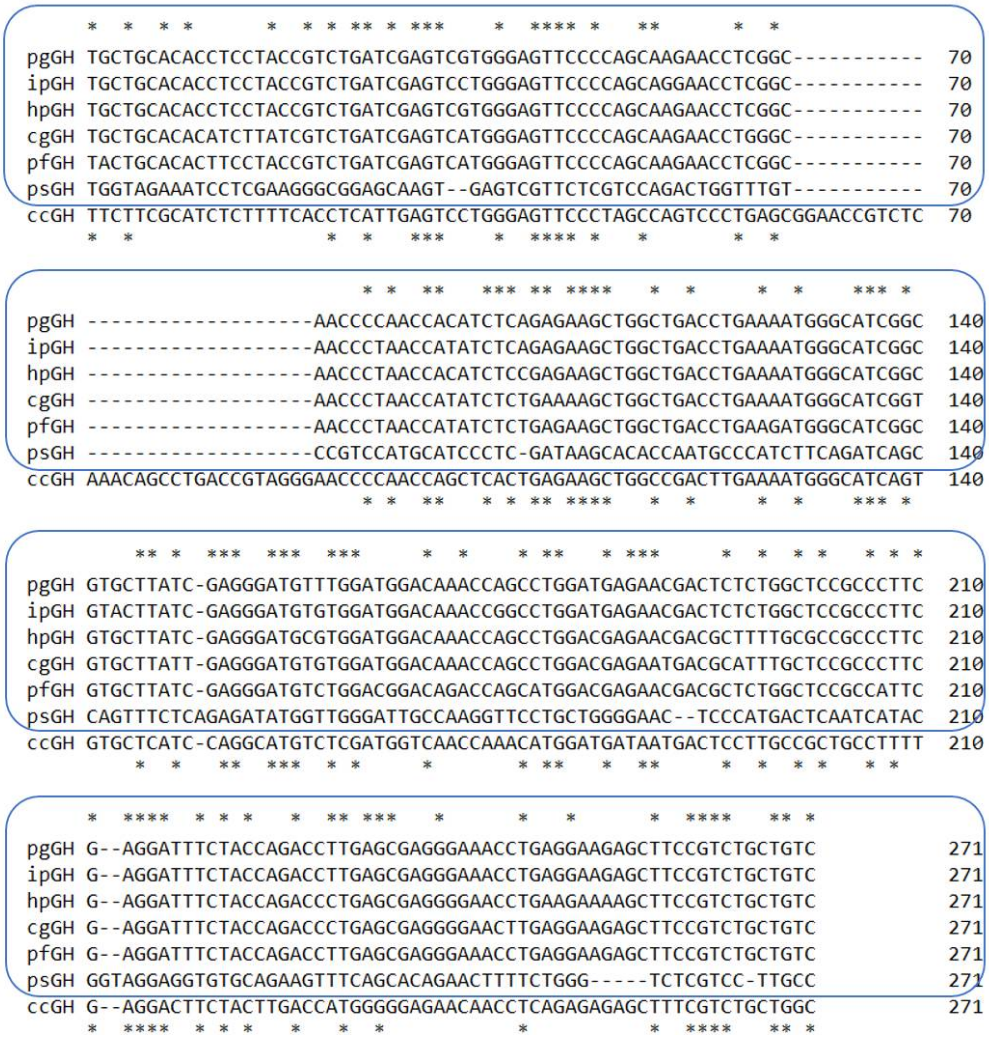


Figure 7. Phylogenetic relationship between Tiger shovelnose catfish GH and all species in one order or outgroup using the Neighbor-Joining method, the number shows the bootstrap value.

high conservation and, accordingly, weak phylogenetic signal and low resolution between closely related species (Kamenskaya & Brykov, 2020).

BLAST analysis was performed to determine the similarity of the sequences obtained with the GH gene data in the Gene Bank. Based on the results of the BLAST analysis on NCBI, homology was between the GH gene of Tiger shovelnose catfish and the GH gene in several fish species that were still in the same order (Table 2.). The homology level was ranged from 89.3% to 90.6%. The highest similarity of the GH gene of the Tiger shovelnose catfish was with the catfish species *P. hypophthalmus* and catfish (*Clarias batrachus*).

Homology analysis in rainbow trout of the gh1 and gh2 genes had 78.6 % and 89.2 % homology at 32 - and 52 -UTR, respectively, compared to 96.8 % homology in the coding region (Chen *et al*., 1989). The two genes' non-coding regions were significantly different, which can be explained by the lack of gene conversion between the two genes. In many cases, identical proteins were also coded by GH gene (Kamenskaya & Brykov, 2020). Partial GH gene sequences in Tiger shovelnose catfish can be used as biomarkers in studies of detection and expression of GH genes in Tiger Shovelnose Catfish and related fish. Information related to the isolation of the full-length GH gene can be done through the primary design of several GH catfish complete sequence data that are aligned with exons and introns of the GH gene in it using sanger sequencing. Another method that can be done is RNA sequencing from the pituitary gland using Next Generation Sequencing (Kamenskaya & Brykov, 2020; Qian *et al.*, 2014). Partial GH gene from tiger shovelnose catfish can be used in aquaculture science as a molecular marker in revealing the role of growth hormone on fish development, fish biology, growth gene expression, selective breeding and other mechanisms related to the cultivation of tiger shovelnose catfish and other fish (Hayuningtyas *et al*., 2022).



Note: The sign (*) above is the similarity of five species of catfish to the psGH gene; The sign (*) below represents the similarity of 5 species of catfish plus ccGH to the psGH gene. (pgGH = *Pangasianodon gigas* GH, ipGH = *Ictalurus punctatus* GH, hpGH = *Heteropneustes fossilis* GH, cgGH = *Clarias gariepinus* GH, pfGH = *Pelteobagrus fulvidraco* GH, psGH = (*Pseudoplatystoma fasciatum* Linnaeus, 1766) GH, and ccGH = *Cyprinus carpinus*).

Figure 8. Alignment of the GH gene sequences of 5 catfish species with the GH of the Tiger shovelnose catfish (psGH) in the Order Siluriformes, and the GH gene (ccGH) of the Cyprinus carpio species (out group representing the order Cypriniformes).

Table 2. Percentage of homology of Tiger shovelnose catfish against several species that are still in the same order

Name of species	Gen GH	Accession number	Query cover (%)	Homology (%)
Pangasianodon gigas	mRNA	L27835.1	55	90.17
Ictalurus puntatus	mRNA	NM_001200245.1	55	89.74
Clarias gariepinus	mRNA	FJ823972.1	55	89.32
Pangasianodon hypophthalmus	mRNA	XM_026942413.2	55	90.6
Clarias batrachus	mRNA	AF416485.1	55	90.6

CONCLUSION

The growth hormone gene in Tiger shovelnose catfish (*P. fasciatum* Linnaeus, 1766) was successfully isolated from the pituitary gland mRNA with a molecular weight of 234 bp. The GH gene of Tiger shovelnose catfish has 234 nucleotides and contains 17 types of amino acids. The GH gene of Tiger shovelnose catfish was closely related to the striped catfish (*P. hypophthalmus*) and Indian catfish (*Clarias batrachus*) with a homology value of 90.6 %. The GH gene has been submitted to the GenBank with accession number OL439543. Information about partial tiger shovelnose fish GH gene can be used as a biomarker in the study of GH gene expression in other catfish species.

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