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ISOLATION AND MOLECULAR CHARACTERIZATION OF MALAYSIAN MAHSEER (*Tor tambroides*) GROWTH HORMONE (GH) GENE

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Thesis Submitted in Fulfillment of the Requirement for the Degree of Master of Science in the Faculty of Fisheries and Aqua-Industry
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DEDICATION

Bismillahi rahmani rahim
Assalamualaikum warahmatullahi wabarakatuh

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Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Master of Science

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Main Supervisor : Shahreza Md. Sheriff, Ph.D.

Co. Supervisor : Associate Professor Abol Munafi Ambok Bolong, Ph.D.
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A study was conducted to isolate and characterize a cDNA of Malaysian mahseer, *Tor tambroides* (*T. tambroides*) growth hormone (GH) gene. Reverse transcription PCR (RT-PCR) technique was carried out to amplify the GH encoding cDNA of *T. tambroides* using primer designed based on Cypriniformes GH gene sequences. In amplifying strategy, the primers were used to amplify five different target regions: 1) from start codon to highly conserved region (CFKKDMHKVETYL); 2) coding sequence from start to stop codon; 3) 5' to 3' unstranslated region (UTR); 4) highly conserved region to stop codon; 5) highly conserved region to 3' UTR. From this strategy, the one-step RT-PCR produced eight DNA fragments with size ranged between ~100 bp to ~700 bp. The fragments obtained were then purified and cloned into pGEM-T Easy cloning vector. Sequence analysis on the positive clones showed that the GH encoding cDNA of *T. tambroides* consists of 1189 nucleotides (nt) excluding the poly (A) tail. It has a 36 nt untranslated (UTR) at the 5' region and a 520 nt UTR at the 3' region. The GH cDNA contains an ORF of 633 nt and encodes for a polypeptide of 210 amino acids (aa), including a signal peptide 22 aa. Comparison of *T. tambroides* GH gene with other teleost GH gene indicated that *T.*

tambroides GH gene has highest similarity with *C. carpio* GH type 1(GHI) which it share 99% and 98% homology in terms of amino acid and nucleotide sequences respectively.

Molecular characterization of *T. tambroides* GH gene showed it exhibit typical GH features similar with other teleost GH gene. In addition, western blot analysis showed that the size of the expressed *T. tambroides* GH protein was 25 kDa. Therefore, the GH sequence of *T. tambroides* was successfully isolated and the sequence of *T. tambroides* GH gene was subjected into the GenBank database (JF428142). This is the first report on the isolation of GH gene from Malaysian indigenous fish species. The finding would assist in understanding the molecular characteristics of *T. tambroides* GH gene. This could lead to the use of molecular approach to understand the growth of *T. tambroides* which would give an insight on the molecular aspect of its growth performance in aquaculture practices.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan ijazah Sarjana Sains

PEMENCILAN DAN PENCIRIAN MOLEKULAR GEN HORMON TUMBESARAN (GH) KELAH (*Tor tambroides*)

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Mei 2013

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Satu kajian untuk memencilkan dan mencirikan cDNA gen hormon tumbesaran (GH) ikan kelah, *Tor tambroides* (*T. tambroides*) telah dijalankan. Teknik transkripsi berbalik PCR (RT-PCR) telah dijalankan untuk amplifikasi GH cDNA *T. tambroides* menggunakan pencetus yang telah direka berdasarkan jujukan gen GH Cypriniformes. Dalam strategi amplikasi, pencetus-pencetus tersebut telah digunakan untuk amplifikasi lima kawasan sasaran: 1) daripada kodon permulaan sehingga kawasan yang paling terpelihara (CFKKDMHKVETYL);2) jujukan pengkodan daripada kodon permulaan sehingga kodon penamat; 3) 5’ sehingga 3’ kawasan tidak ditranslasi (UTR); 4) kawasan paling terpelihara sehingga kodon penamat; 5) kawasan paling terpelihara sehingga 3’ UTR. Daripada strategi ini, RT-PCR satu langkah menghasilkan lapan fragmen DNA dengan saiz di antara 100~700 bp. Fragmen-fragmen ini kemudiannya telah ditulenkan dan diklon ke dalam vektor pengklonan pGEM-T Easy. Analisis jujukan ke atas klon yang positif mendapati bahawa gen GH cDNA *T. tambroides* mengandungi 1189 nukleotida (nt) tidak termasuk bahagian belakang poly (A). Ia mempunyai 36 nt yang tidak diterjemahkan (UTR) pada kawasan 5’ dan 520 nt UTR pada kawasan 3’. GH cDNA mengandungi

ORF 633 nt dan mengkodkan polipeptida 210 asid amino (aa), termasuk 22 aa peptida signal. Perbandingan gen GH *T. tambroides* dengan gen GH teleost lain menunjukkan gen GH *T. tambroides* mempunyai persamaan yang paling tinggi dengan gen GH jenis I (GHI) *C. carpio* dengan persamaan 99% dan 98% dari segi jujukan asid amino dan nukleotida.

Pencirian molekular gen GH *T. tambroides* menunjukkan bahawa gen GH cDNA *T. tambroides* mempunyai ciri GH yang sama dengan ciri gen GH ikan-ikan lain. Selain itu, analisis pemblotan western menunjukkan bahawa saiz protein GH *T. tambroides* yang diekspreskan adalah 25 kDa. Oleh itu, jujukan gen GH *T. tambroides* telah berjaya dipencilkan dan jujukan gen GH telah dimasukkan ke dalam pangkalan data GenBank (JF428142). Ini merupakan laporan yang pertama ke atas pemencilan gen GH spesis ikan dari Malaysia. Ini boleh membawa kepada penggunaan kaedah molekular bagi memahami tumbesaran *T. tambroides* yang mana dapat memberi pemahaman terhadap aspek molekular ke atas perkembangnya dalam amalan akuakultur.