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CLASSIFICATION OF THE MULTIGENE FAMILY OF FATTY ACID BINDING PROTEINS (FABPS) AND TRANSCRIPTION PROFILE OF THE GENES IN STRIPED CATFISH (*PANGASIANODON HYPOPHTHALMUS*)

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SUMMARY

Striped catfish (*Pangasianodon hypophthalmus*) is an economically important fish in Vietnam. The catfish fillets contain high fatty acid composition. The FABP family is involved in lipid transport and metabolism as well as in the regulation of gene expression and cell development. In this study, the catfish genome database was searched for *fabp* gene family; then, gene structure, classification and phylogenetic relationships were analyzed. In striped catfish genome, we found 10 *fabp* genes that are homologous to other fish species and other 5 novel *fabp* genes that have not been clearly annotated. These newly identified *fabp* genes cluster separately from the known members of the *fabp* family on the phylogenetic tree, and further studies are needed to understand their roles and functions. We examined transcriptional gene expression of *fabp3, fabp7* and *fabp10a* genes in muscle, liver and brain tissues of the stripped catfish. The results showed that *fabp10a* gene was not strongly expressed in all 3 types of tissues; *fabp3* gene was most strongly expressed in liver tissue and *fabp7* was highly up-regulated in brain tissue. The results of this study provide a resource for further research on the function of *fabp* genes and their genetic diversity in striped catfish.

Keywords: *FABPs, fatty acid binding proteins, Pangasianodon hypophthalmus, striped catfish*

INTRODUCTION

The striped catfish (*Pangasianodon hypophthalmus*), which belongs to the Asian catfish family Pangasiidae, is native to Mekong river and successfully cultured in the river delta. Vietnam is the world's largest producer of *P. hypophthalmus*. According to Vietnam Association of Seafood Exporters and Producers (VASEP), the pangasius products were exported to over 140 markets including USA, EU, China, ASEAN, Mexico, and Brazil.

Recently, with the development of next generation sequencing (NGS) technology, a draft genome of *P. hypophthalmus* has been reported, which has developed genomic resources for genetic improvement of the striped catfish (Kim *et a*l., 2018). The available of genomic information will enhance opportunities for fundamental researches and commercial applications. In order to develop molecular markers, identification of the genes linked with traits of interest is an effective approach. Based on *P. hypophthalmus* genome, several gene families related to growth and development have been analyzed, such as members of the insulin-like growth factor (IGF) system (IGFs, IGFRs, IGFBPs) (Kim *et al*., 2018, Le *et al*., 2019).

Fatty Acid Binding Proteins (FABPs) belong to a family of 14-16 kDa molecules and long-chain fatty acid bonds in both vertebrates and invertebrates (Alvite *et al*., 2008; Borchers *et al*., 1989; Kanda, 1989). FABPs can mediate the transport of free fatty acids for specific metabolic pathways, protecting cells from the cytotoxic effects of free fats, acids and modifying lipid metabolizing enzymes (Besnard *et al*., 2002; Lowe *et al*., 1987; Storch and McDermott, 2009). A number of studies have indicated the role of FABPs in a multitude of cellular processes including: (1) Binding and isolating long-chain fats, acids, bile salts and other hydrophobic ligands; (2) Transporting these ligands to the intracellular compartments for metabolism and energy production; (3) Interacting with other enzyme systems and transport proteins; and (4) Transporting fatty acids (FA) to the nucleus for the regulation of gene transcription through the activation of nuclear receptors, peroxisome proliferator activation receptors (PPARs) (Denovan-Wright *et al*., 2000; Sharma *et al*., 2006; Storch *et al*., 2008, Leaver *et al*., 2005; Judith *et al*., 2010, Angel *et al*., 2010). FABPs participate in the regulation of gene expression and cell growth (Haunerland, Spene, 2004). In addition, FABPs also play an important role in resilience to environmental temperatures and extreme nutritional conditions in vertebrates (Syamsunarno *et al*., 2014; Furuhashi *et al*., 2008). The expression of FABPs in various tissues such as intestinal tissue, heart tissue, and liver; and fat fulfill specific roles associated with histological structure and physiological function of these tissues have been confirmed (Banaszak *et al*., 1994; Veerkamp *et al*., 1991; Veerkamp *et al*., 1993; Judith *et al*., 2010, Angel *et al*., 2010).

FABPs are encoded by a group of *fabp* genes. Atotal of 12 *fabp* genes have been identified in vertebrates so far, but not all members of *fabp* genes occur in the same species (Lucke *et al*., 2003). For example, *fabp10* and *fabp11* have only been proposed in nonmammalian vertebrates, like teleost fishes (Smather *et al*., 2011), while *fabp12* appears restricted to mammals, such as human (Parma *et al*., 2012). Venkatachalam described 12 *fabp* genes in zebra fish, based on results of cDNA sequence synthesis, gene structure, and conservative gene regions the steady-state levels of *fabp* mRNA and heterogeneous nuclear RNA (hnRNA) transcripts in liver, intestine, muscle, brain and heart for four sets of duplicated *fabp* genes, *fabp1a/fabp1b.1/fabp1b.2, fabp7a/fabp7b, fabp10a/fabp10b* and *fabp11a/fabp11b* in zebrafish fed with different concentrations of clofibrate (Venkatachalam, 2012). The *fabp* genes expressed differently, but their tertiary structure and genetic makeup were highly conservative (Storch *et al*., 2008; Glatz *et al*., 1996; Ong *et al*., 1994). Almost all *fabp* genes comprise four exons and three different sized introns between the isomorphic and orthogonal *fabp* genes in different species (Schaap *et al*, 2002), except for the *fabp3* gene in the desert grasshopper (Wu *et al*., 2001) the *fabp1a* gene from zebrafish (Sharma *et al*., 2004) and the *fabp11a* gene from anchovies (Parma *et al*., 2012).

This study aims to identify and classify the *fabp* gene family from genome database; and analyse transcriptional expression of *fabp* genes in various tissues in striped catfish (*P. hypophthalmus*). Since protein FABPs participate in the regulation of gene expression and cell growth, members of *fabp* gene family are candidates for studying genetic variations of the genes and their association with growth traits. The results of this study would provide material for applying in further research to develop molecular markers toward growth.

MATERIALS AND METHODS

Identification of *fabp* genes from striped catfish genome data

FABP genes were surveyed based on previous reports of teleost FABP genes (Venkatachalam *et al*., 2017). The teleost FABP genes were used as queries for BLAST searches of FABP genes in the *P. hypophthalmus* genome (Kim *et al*., 2018). NCBI assembly record for *P. hypophthalmus* genome is GCF_003671635.1 (BioProject: PRJNA501861; BioSample:

SAMN08866743). The nucleotide/protein sequences with annotation of FABP family are also available in NCBI database.

Comparative analysis of *fabp* genes

T he identified *fabp* genes from *P. hypophthalmus* and *fabp* genes from different taxa (157 genes) available in the NCBI database were used for phylogenetic analysis. Multiple alignment of the deduced amino acid sequences was performed using the ClustalW web-based tool with default parameters. A phylogenetic tree was constructed with MEGA7.0 (Kumar *et al*., 2016) using neighbor-joining methods (Saitou, Nei, 1987). The tree topology was evaluated with a bootstrap probability calculated on 1000 resamplings.

Sampling

The catfish samples used in the study were collected directly from the Research Institute of Aquaculture No.2, Ho Chi Minh City. Liver, brain, and muscle tissue samples were cut into small pieces and immediately immersed in RNA later solution, and subsequently stored at -80°C until RNA extraction.

Total RNA extraction and cDNA synthesis

Catfish tissues were homogenized and used for RNA extraction with RNeasy Mini Kit (Qiagen) according to the manufacture's protocol and stored at -80ºC. The quantity and quality of total RNAs were checked by gel electrophoresis on a 1% agarose gel and NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific). The cDNA libraries were constructed using RevertAid First Strand cDNA SynthesisKit (Thermo Fisher) according to the manufacturer's procedure.

Primer design

The primer pairs for qRT-PCR were designed by Primer 5.0 software based on the annotated nucleotide sequences from striped catfish genome. The 18S ribosomal RNA was used as internal control for qRT-PCR analyses.

Among *fabp* genes identified in the striped catfish, three genes (*fabp10a, fabp3, fabp7*) were chosen for further examination of transcriptional gene expression profiles. The gene structures and primer positions were shown in Figure 1. Information about the designed primers used in this study is shown in Table 1.

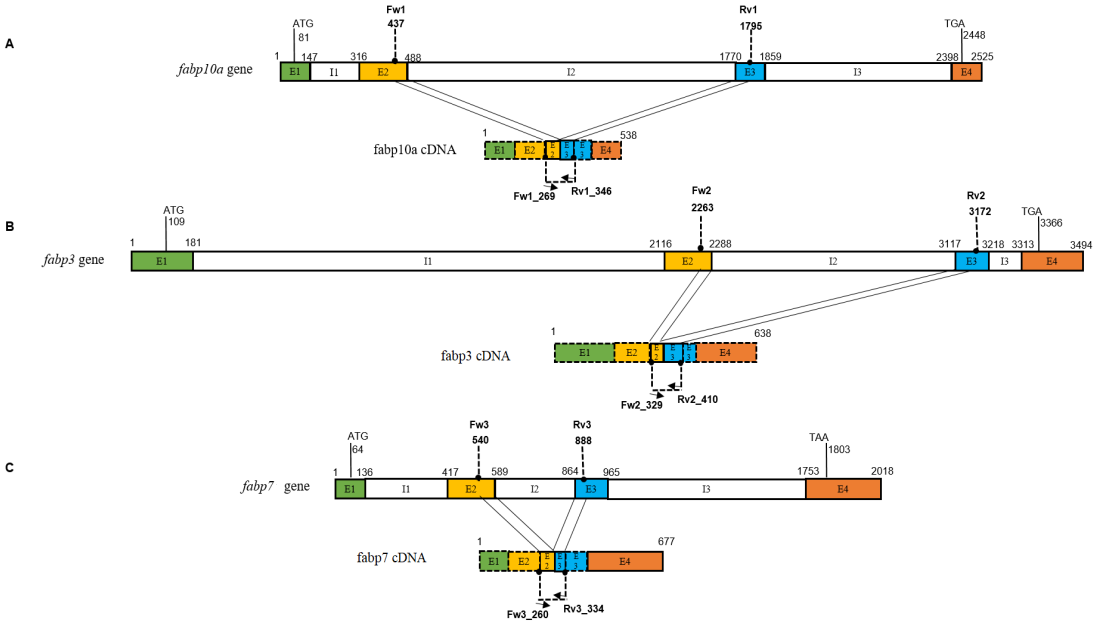

Figure 1. Structure of *fabp* genes and position of designed primer pairs for qRT- PCR. **A**. *fabp10a* gene; **B**. *fabp3* gene; and **C**. *fabp7* gene. The nucleotide of each gene was numbered based on the annotation for the gene in scaffold. The boxes denoted "E" and "I" are exons and introns, numbered in ascending order.

Table 1. Primers for qRT-PCR used in this study.

Gene (Accession	Primer name	Nucleotide sequences (5'-3')	Amplicon length	Exon jucntion
fabp10a	*fabp10a* -	CACCATCGGCAAAGAAGCAG	78 bp	Exon2-Exon3
(XM_026924089.1	*fabp10a* -	TTTCCTCCTTCCAGCCTCAC		
fabp 3	*fabp3* -Fw	CCACAGCAGACGACCGTAAAG	82 bp	
(XM_026929677.1	*fabp3* -Rv	TTGCCGTCCCATTTCTGAACG		Exon2-Exon3
fabp 7	*fabp7* -Fw	AACTGGGAGAGGAGTTTGACG	75 bp	Exon2-Exon3
(XM_026925940.1	*fabp7* -Rv	TGTCTTCGTCCAAGGTCACTG		
18S rRNA	*18S*-Fw	TGACTCAACACGGGAAACCTC	122 bp	
(XR_004577708)	*18S*-Rv	CAGACAAATCGCTCCACCAAC		

Transcriptional gene expression analysis by qRT-PCR method

The cDNA was synthesized from total RNA by reverse transcription using the First-Strand cDNA Synthesis Kit for qRT-PCR (Sigma Aldrich). The cDNA product was quantified using the NanoDrop™ 1000 Spectrophotometer and then diluted to a 20 ng/µl working concentration.

Primer pairs were checked by performing regular PCR reaction and agarose gel electrophoresis. Quantitative RT-PCR reaction was performed using FastStart Essential DNA Green Master kit (Roche) and LightCycler® 96 Instrument as follow: pre-incubation 95°C for 1 min, 40 cycles of denaturation at 94°C for 15 sec, annealing at 60°C for 20 sec, extension at 72°C for 30 sec and final elongation at 72°C for 30 sec. Each reaction was performed thrice simultaneously and with a negative control (without cDNA).

Analysis of qPCR Data

Relative quantification of RT-PCR data is based on the expression ratio of the *fabp* gene versus the reference *18S* gene. Analysis of qRT-PCR results using the relative quantitative $2^{-\Delta\Delta CT}$ method where $\Delta Ct = Ct_{target\ gene} - Ct_{18S}$. (Livak, 1997; Livak, Schmittgen, 2001). Differences were considered significant when $p<0.05$.

RESULTS AND DISCUSSION

Catfish genome data mining for *fabp* gene family

Fatty acid-binding proteins belong to the conserved multigene family of the intracellular lipid binding proteins. Twelve zebrafish *fabp* genes have been identified in the zebrafish (*Danio rerio*) genome based on their sequence similarity, phylogeny and conserved gene synteny with their human and chicken orthologs (Venkatachalam *et al*., 2017). In this study, we used *fabp* genes from zebrafish and other teleost fish asqueries to search the catfish genome sequence database (Kim *et al*., 2018) by BLAST to identify catfish *fabp* genes. The identified *fabp* genes in the *P. hypophthalmus* genome are listed in Table 2.

Table 2 showed that 15 genes related to the FABPs family were found in the *P. hypophthalmus* genome. Among these genes, 10 *fabp* genes were annotated based on their sequence similarity with that of other teleost fish, including *fabp 1, fabp2, fabp3, fabp 6, fabp7, fabp 10* and*fabp11*. Five genes, namely *fabp_brainlike*, were not clearly annotated.

Classification of the FABPs multigene family

All of the catfish *fabp* genes found in the BLAST searches (Table 2) were annotated based on sequence identity/similarity and phylogenetic analyses. A neighbor-joining tree shows the phylogenetic relationship of *fabps* from the

stripped catfish and other vertebrate species (Figure 2).

Figure 2 showed that the genomes of the striped catfish (*P. hypophthalmus*) contained at least one copy of fatty acid-binding protein genes: *fabp1*, *fabp2*, *fabp3*, *fabp6*, *fabp7*, *fabp10* and *fabp11* as same as other teleost fish. *P. hypophthalmus* genome retains duplicates of *fabp2, fabp10* and *fabp11* while zebrafish (*D. rerio*) contains duplicates in *fabp1a/b, fabp7a/b*, *fabp10a/b* and *fabp11a/b* (Venkatachalam *et al*., 2017).

Table 2. Genes related to the FABPs family in the *P. hypophthalmus* genome.

No	Description	*P. hypothalmus* 2018 Genome/ scaffold ID	NCBI- Nucleotide ID	NCBI- ProteinID
1	*fabp1*	NW_020824196.1/sc0000001	XM_026915289.1	XP_026771090.1
2	*fabp2*	NW_020824279.1/sc0000084	XM_026910795.1	XP_026766596.1
3	*fabp2_intestinal-like*	NW_020824240.1/sc0000045	XM_026945385.1	XP_026801186.1
4	*fabp3*	NW_020824213.1/sc0000018	XM_026929677.1	XP_026785478.1
5	*fabp6*	NW_020824225.1/sc0000030	XM_026938834.1	XP_026794635.1
6	*fabp7*	NW_020824209.1/sc0000014	XM_026925940.1	XP_026781741.1
7	*fabp10a*	NW_020824207.1/sc0000012	XM_026924089.1	XP_026779890.1
8	*fabp10b*	NW_020824330.1/sc0000135	XM_026911873.1	XP_026767674.1
9	*fabp11a*	NW_020824213.1/sc0000018	XM_026929359.1	XP_026785160.1
10	*fabp11b*	NW_020824227.1/sc0000032	XM_026939973.1	XP_026795774.1
11	*fabp_brainlike*	NW_020824206.1/sc0000011	XM_026922912.1	XP_026778713.1
12	*fabp_brainlike*	NW_020824238.1/sc0000043	XM_026944702.1	XP_026800503.1
13	*fabp_brainlike*	NW_020824206.1/sc0000011	XM_026922911.1	XP_026778712.1
14	*fabp_brainlike*	NW_020824290.1/sc0000095	XM_026911183.1	XP_026766984.1
15	*fabp_brainlike*	NW_020824290.1/sc0000095	XM_026911177.1	XP_026766978.1

The phylogenetic tree showed close relationship between stripped catfish (*P. hypophthalmus*) and channel catfish (*Ictalurus punctatus*). However, only single *fabp2* was found in *I. punctatus* genome while two *fabp2* genes were found in *P. hypophthalmus*. Among 15 genes related to the FABPs family in *P. hypophthalmus* genome, five ambiguously annotated *fabp* genes clustered together in the same clade which was distantly related to the currently known members of the *fabp* family. Future research will be needed to investigate function of these members of the *fabp* family.

Transcriptional gene expression of several *fabp* genes in various catfish tissues

In our study, transcriptional gene expression of *fabp10a, fabp3* and *fabp7* were examined in brain, muscle, and liver tissues from catfish *P. hypophthalmus*. Firstly, total RNAs were extracted from tissue samples. The quantity and quality of total RNA were checked by 1.5% agarose gel electrophoresis and NanoDrop spectrophotometer. The data showed that all RNA samples had a ratio of A260/280 of circa 2 and contain ≥ 50 ng RNA.

The cDNA was synthesized from total RNA by reverse transcription using the First-Strand cDNA Synthesis Kit for qRT-PCR (Sigma Aldrich).

Primers designed for qRT-PCR were shown in Figure 1 and Table 1. To check the primer specificity and quality of cDNA product, regular PCR reactions were performed. The products were checked by 1% agarose gel electrophoresis (Figure 3). Figure 3 showed that each PCR

product gave a single band on the gel with expected product size. This result confirmed primer specificity and cDNAs could be further used as templates of qRT-PCR reactions.

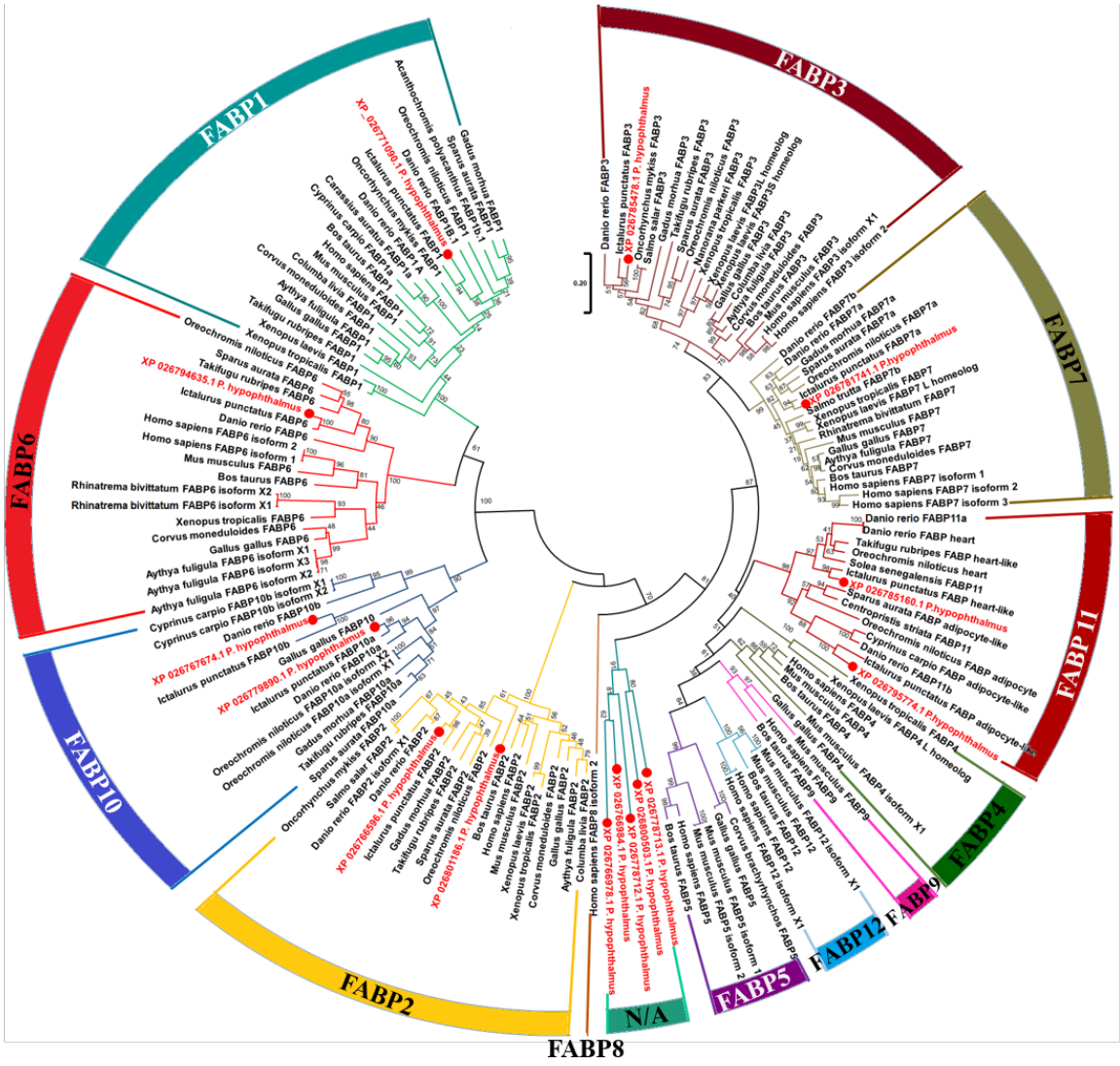

Figure 2. Molecular phylogenetic analysis of FABPs showing classification and duplication of *fabp* genes.

After qRT-PCR run, specific amplifications were also confirmed by the presence of a single peak in the melting curve (Figure 4). The melting curves presented in Figure 4 showed that all amplicons for *fabp10a, fabp3* and *fabp7* have the same melting peaks at circa 84°C. The single peak observed for each amplification verified the single, specific product.

Based on the qRT-PCR performance, transcriptional gene expression of *fabp10a*, *fabp3* and *fabp7* genes in stripped catfish *P. hypophthalmus* was examined in brain, muscle, and liver tissues. The results of the transcriptional gene expression were shown in Figure 5. The qRT-PCR results showed that for the *fabp10a* gene, minimal mRNA expression

was detected in all tissues. In the case of *fabp3*, it had a very high expression level, up to more than 80 times in liver tissue and also had a significantly up-regulation, more than 7 times, in brain tissue. For the *fabp7* gene, the highest expression was observed in brain tissue and followed by liver tissue, but only minimal expression was showed in muscle tissue.

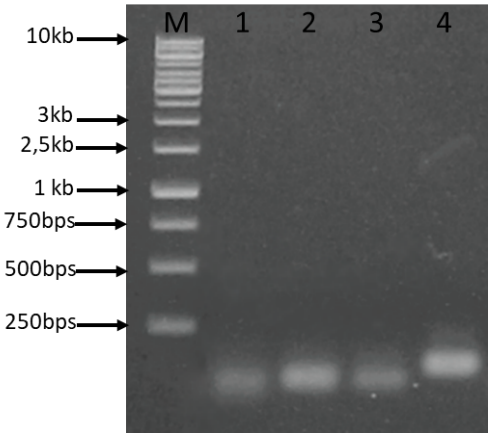

Figure 3. Electrophoresis of PCR products on 1% agarose gel. M. 1kb DNA marker; 1. *fabp10a*; 2. *fabp3*; 3. *fabp7;* 4.*18S*

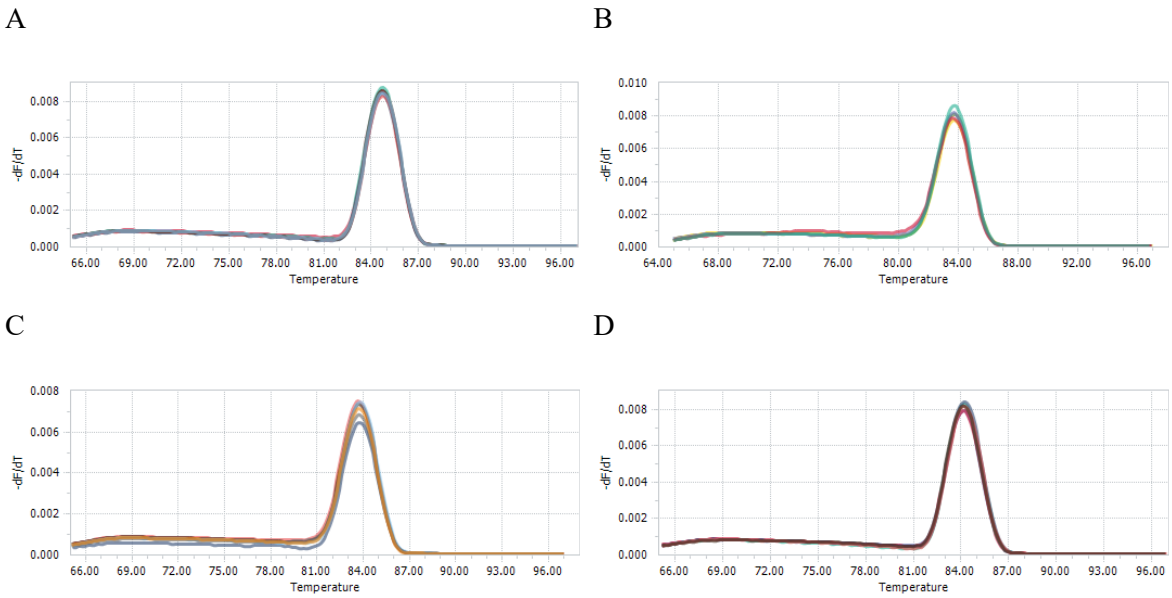

Figure 4. Melting curves for determination of specificity and efficiency of qRT-PCR amplification of *fabp* genes. A.*18S rRNA*; B. *fabp10a*; C. *fabp3*; D. *fabp7.*

The *fabp10* gene has been found in the liver of vertebrates such as chickens (Ceciliani *et al*., 1994), iguanas, toads (Schleicher, Santome, 1996; Di Pietro *et al*., 2003), frogs (Baba *et al*., 1999) and in many fish species such as shark (Cordoba *et al*., 1999), zebrafish (Denovan-Wright *et al*., 2000; Sharma *et al*., 2006; Venkatachalam *et al*., 2009), lungfish (Di Pietro. Santome, 2001) and rainbow trout (Kim 2006; Bayir *et al*., 2015). In our study, *fabp10a* only

showed expression at minor level in all examined tissues (liver, brain and muscle) of the catfish *P. hypophthalmus*. On the contrary, previous study in zebrafish showed that the steady-state level of *fabp10a* increased 6-fold in intestine and > 5-fold in muscle (Ventakachalam *et al*., 2012).

The study of Wang and colleagues have reported expression profile of nine separate *fabp* genes in different chicken tissues. Among them, *fabp7* and *fabp10* were also showed to be highly expressed in the liver tissue (Wang *et al*., 2019). In pufferfish (*Tetraodon nigroviridis*), duplicated *fabp7* and *fabp10* genes was found in the genome and differently distributed in diffenrent tissues (Parmar, Wright, 2013, Thirumaran, 2014). In gold pompanos (*Trachinotus ovatus*), the expression of *fabp7* gene in brain tissue was significantly higher than that of other *fabp* genes (Lei *et al*., 2020), which is very similar to our case. For the *fabp3* gene, a study in Japanese seabass (*Lateolabrax japonicus*) has showed that although *fabp3* was widely distributed in many tissues, but muscle and liver tissues have much higher *fabp3* expressions compared with other tissues (Xu *et al*., 2017). Our study in the catfish *P. hypophthalmus* showed the remarkable up-regulation of *fabp3* gene in liver tissue and followed by brain tissue. Our results further indicated the important roles of fish liver in fatty acid and lipid metabolism, including synthesis, oxidation and storage of fatty acid and lipid. The tissue expression patterns of stripped catfish *fabp* genes were different with those of some other fish to some extent that may indicate specific evolutionary *fabp* functions in stripped catfish. The function of *fabp* gene family in stripped catfish needs to be elucidated by further studies.

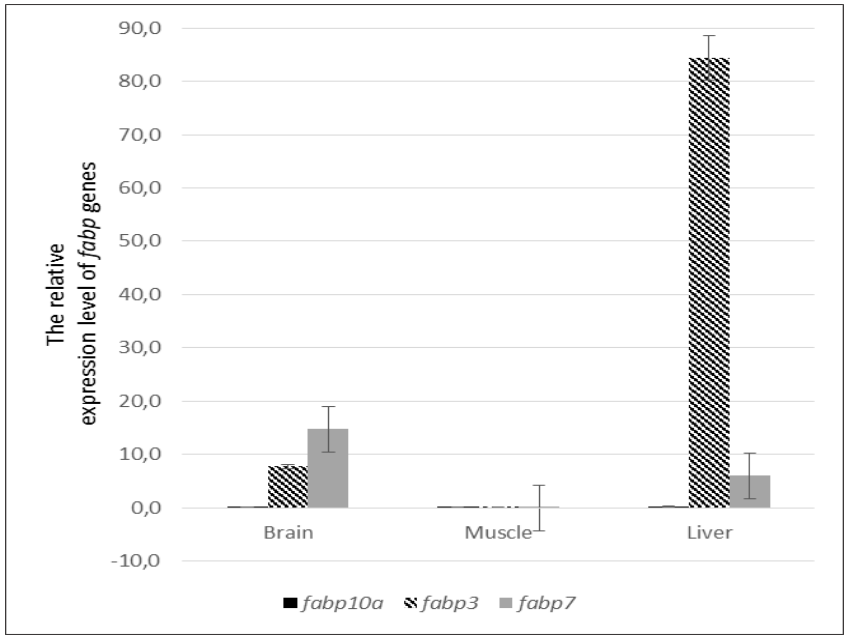

Figure 5. Expression levels of transcripts belonging to the *fabp* gene family.

CONCLUSION

The available of *P. hypophthalmus* genome database enables us to analyze particularly the *fabp* gene family. Totally, 15 genes related to the FABPs family in *P. hypophthalmus* were annotated and classified based on sequence identity/similarity and phylogenetic analyses. Among them, a cluster of 5 novel FABP related genes was identified. Moreover, transcriptional

gene expression patterns of *fabp3, fabp7* and *fabp10a* genes in muscle, liver and brain tissues were firstly examined in *P. hypophthalmus*. This study provides a fundamental understanding of *fabp* gene family in *P. hypophthalmus*, which promotes further studies to clarify the function and genetic diversity of the *fabp* gene family.

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PHÂN LOẠI HỌ ĐA GEN MÃ HÓA PROTEIN LIÊN KẾT ACID BÉO (FABPS) VÀ NGHIÊN CỨU SỰ BIỂU HIỆN CỦA MỘT SỐ GEN THUỘC HỌ NÀY Ở CÁ TRA NUÔI (*PANGASIANODON HYPOPHTHALMUS*)

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TÓM TẮT

Cá tra nuôi *(Pangasianodon hypophthalmus*) là một loài cá kinh tế quan trọng ở Việt Nam. Thịt cá tra có chứa thành phần axit béo cao. Protein liên kết acid béo (FABPs) tham gia vào quá trình vận chuyển và chuyển hóa lipid cũng như điều hòa biểu hiện gen và phát triển tế bào. Trong nghiên cứu này, họ gen *fabp* được khai thác từ cơ sở dữ liệu hệ gen cá tra. Tiếp đó cấu trúc gen, phân loại gen và các mối quan hệ phát sinh loài được tiến hành phân tích. Trong dữ liệu hệ gen cá tra, chúng tôi tìm thấy 10 gen *fabp* tương đồng với các loài cá khác và 5 gen *fabp* mới được xác định. Các gen mới xác định này tập trung thành một nhóm riêng trên cây phát sinh chủng loại của họ gen *fabp*, và cần có các nghiên cứu sâu hơn để hiểu thêm về vai trò và chức năng của chúng. Chúng tôi đã kiểm tra sự biểu hiện gen của các gen *fab*p3, *fabp7* và *fabp10a* ở các mô cơ, gan và não của cá tra nuôi. Kết quả cho thấy gen *fabp10a* không biểu hiện mạnh ở cả 3 loại mô, gen *fabp3* biểu hiện mạnh nhất ở mô gan và *fabp7* biểu hiện mạnh ở mô não. Những kết quả này có thể làm nguyên liệu cho các nghiên cứu sâu hơn về chức năng của gen *fabp* và sự đa dạng di truyền của chúng ở cá tra nuôi.

Từ khoá: *cá Tra, FABPs, Pangasianodon hypophthalmus, protein liên kết acid béo.*