Identification andExpression ofvitellogenin gene in the Gouramy (Osphronemous Gourammy) under photoperiods manipulation

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Abstract . Vitellogenin was protein hormone where controlled gonad development in fish. Expression of this gene regulated from the external and internal factor.Photoperiods are the external factor that regulated endocrine gland activities in gonadal development, gametogenesis and reproductive cycles. Aim of the research to find out the effect of photoperiod on gouramy reproductive performance by manipulating photoperiod. Design experimental with three photoperiod treatments, namely 14L: 10D (control), 8L: 16D (short photoperiods) and 18L: 6D (long photoperiods). Four aquaria consisting of nine fishes each were served as replicates. Fishes were kept under these photoperiods for 8 weeks. The observed variable was the liver activities. Liver activity was evaluated by measuring gene expression of Vitellogenin. The normalized data were subjected to ANOVA followed by Tukey's multiple-comparison tests. The length of Vitellogenin cDNA was 1136 bp. The vitellogenin precursors encoded cDNA consisted of 378 amino acids. The average of vitellogenin gene in each experimental group significantly increased according to longer photoperiods ($P<0.05$). These results indicated that photoperiods had a stimulatory effect in improving gouramy reproductive performance

1 Introduction

Vitellogenin (Vg) is the precursor of vitellin (Vn), the major egg yolk protein of oviparous animals. The vg gene is generally expressed in a female-specific manner. In vertebrates, Vg is synthesized in the liver, secreted into blood, and accumulated in growing oocytes in the ovaries (3). The synthesis of Vg is induced by estrogens in females and is inducible in immature females by a synthetic estrogen, estradiol-17β (E_2). In fishes, Vgs are produced only in females, not in males due to the absence of endogenous estrogen; however, males begin synthesizing Vgs when they are exposed to exogenous estrogens (29). The expression of vitellogenin gene in fish regulated from environmental factor like photoperiods.

Photoperiod was external factor that controlled fish reproduction. In many study found that photoperiods in primary environmental factor that regulated pituitary activities via

melatonin (5,7,16). Study for the effect of photoperiods timing of spawning in many fish species as European sea bass, *Dicentrarchus labrax* (17), Atlantic cod, *Gadus morhua L* (19), dan Chinook salmon, Oncorhynchus *tshawytscha* (6). The majority of studies were conducted on temperate-zone fishes in which photoperiod strictly differ between seasons. Studies on influence of photoperiod on tropical fishes are still limited. We had already research with hard-lipped barb (*Osteochilus hasselti* C.V) tropical fishes. We found that manipulations with longer photoperiods 18L:6D (18 hour light and 6 hour dark) were significantly decreased melatonin level and increased gene expression cGnRH-II and also incread the vitelogenin gene (Prayogo et al., 2012; Prayogo et al., 2017). Conduct in that study in this research, we wants to evaluated another economically tropical fish like gouramy (Osphronemous Gouramy) in Indonesia. This research was to examined that long photoperiods will increasing gene expression of vitellogenin altered ovarium development in gouramy.

Gouramy (*Osphronemusgourami*) is one of the local freshwater fish species with very potential for propagation by fish farmers, especially in the area around Banyumas District, Central Java, Indonesia. Gourami have high economic value, easily cultivated, and favored by consumers in Indonesia. Many studies were conduct for increasing and mantain fish production in Indonesia (Prayogo et al 2012; Prayogo et al 2016a; Prayogo et al 2016b). Osphronemous Gouramy is a synchronous batch spawner fish (Prayogo et al., 2011) capable of spawning several time during the peak of the spawning period. Osphronemous Gouramy has been adapted to a photoperiod of 12L: 12D to 14L:10D. The present study was examined the effect of different photoperiods on gene expression vitellogenin of the Gouramy. We also identified of vitellogenin genes in the gourami for the first time. This study will inform photoperiods effect to increasing gouramy reproduction.

2 Material and Methods

2.1 Treatment and sampling of fish

In this research we used144 sexually female hard-lipped barb weighing of 100 g in average and maintained inResearch Laboratory of Fisheries and Marine, Jenderal Soedirman University. The female'sgourami was divided into 3 groups photoperiods treatment. Each group consisted of 4 aquaria with 9 fish/50 L water.

In this study, three types of photoperiods namely 6L: 18D, 12L:12D and 18L:6D have been tested toward vitelogenin gene expression. The aquaria were covered with lightproof black polybag. The light source provided from 25-Watt (*Phillips*) bulb regulated by automatic timers 24 hours cycles which were placed at the top of each aquarium. In photoperiod of 14L: 10D, light was turned on since 06.00 am until 08.00 pm and in 8L:16D light was turned on since 06.00 am until 02.00 pm and in 18L:6D light was turned on since 06.00 am until 12.00 pm local time.

The fish were reared photoperiods treatment for four months.During the research, fish were fed on commercial pellet (protein 37% and fat 10%) as much as 3% of total body weight daily. The water was siphoned regularly to maintain water quality. The water temperature, dissolved oxygen, pH and carbon dioxide were monitored every month. Every sampling time,thepituitary was collected from 3 fish of each group and was snap-freeze on liquid nitrogen for vitelogenin expression study. The expression of vitelogenin geneswas evaluated using Real Time PCR applying primers derived from vitelogenin genes. Real Time PCRwas conducted at the Research Laboratory Jenderal Soedirman University.

2.2. mRNA Isolation and DNAse Treatment

Total mRNA was extracted from whole liver using blue Sepasol R-RNA super-1 reagent, based on Ethanol-phenol-chloroform extraction method. The integrity of the RNA was verified in a denaturing agarose gel, stained with ethidium bromide. The RNA samples were treatment with DNAse free RNAse (Takara). The quality and concentrations of total RNA were determined by agarose gel electrophoresis and optical density reading at 260 and 280 nm (Figure 1), the RNA were aliquoted in batches and frozen at -70°C.

2.3 RT-PCR

Total mRNA samples (1µl) were reverse transcript using cDNA synthesis kit (PrimeScriptTM Reverse Transcriptase, Takara Bio.Inc) using Random 6mers (sequence pd (N)6, 50µM) primers and prime script R-tase with manufacture instruction.

2.4 cDNA Amplification

The Degenerated primer pairs, vitelogenin gene were designed from cDNA *cyprinidae* like *cyprinus carpio* and *carrasius auratus*. The forward primer was 5-CGTGGATCHYTGMARTACGAGTT-3 and reverse was 5-ATGGTGGCRTCATTGAT-3. All sequence was aligned with multalin to identify the conserve region in ORF (Open Reading Frame) region. The primers to amplify the vitelogenin gene were designed using primer 3 software.

Thirty five cycles of PCR for hard lipped barb vitelogenin were carried out using a thermal cycler (Robocycler, Stratagene) according to the following cycle, 95°C for 2 min, 35 cycles to 95°C for 30 s, 55°C for 30 s, 72°C for 60 s, followed by a 5 min extension at 72°C . After amplification, the PCR products was electrophoretic ally separated on a 1.5% agarose gel and stained with ethidium bromide.

2.5 Cloning and Sequencing of PCR Products.

PCR products amplified from cDNA were separated by agarose gel electrophoresis, and the incised gels were purified using the DNA gel extraction procedure. The desired DNA fragments from mRNA vitelogenin were sub cloned into T vector (10 ng) (Takara) and ligated with T4 ligase. The plasmid were transfected into *E. coli* and were spread into LB medium. The recombinant positive colonies were screened using ampicilin. Positive colonies were treated with mini scale plasmid preparation for sequencing. DNA sequences of these fragments were determined using the Big Dye version 3.1 sequencing method with specific primers (Table 1). The data were automatically collected on the ABI PRISM 3100 Genetic Analyzer (PE Applied Bio-systems).

2.6 Sequence analysis

The cDNA sequences for vitelogenin gene were checked using BLASTN searches (http://www.ncbi.nlm.nih.gov/ BLAST/) were performed with default settings on the complete, non redundant GenBank database nucleotide sequences.

2.7 Phylogenetic analysis

For phylogenetic analyses, gourammy cDNA vitelogenin genes were compared to

cDNA vitelogenin sequences from ten fish species. All sequences were retrieved from NCBI GenBank. The relationship between gourammyvitelogenin and other teleost vitelogenin was generated with CLUSTAL W with scoring method percent and the unrooted tree was generated using Treeview version 1.5.2.

2.8 Quantitative Real Time Analysis

The primers were designed based on vitelogenin(submit number: 1995561), using the Primer 3.0 software. For the actin used from gouramy actin, used as endogenous control, was amplified by the following primers-actin forward 5-GAGCTATGAGCTCCCTGACGG- 3, actin reverse 5- AAACGCTCATTGCCAATGGT-3-and were used to normalize variations in RNA. After optimization, PCR reactions were performed in a 10 µl volume containing 2 µl cDNA, 5 µl SYBR mix (Applied Biosystem), 0.3 µl forward primer, 0.3 µl reverse primer and 2.4 µl DDW using the following condition: 95°C for 45 s, (45 cycles of 95°C for 15 s and 60°C for 1min), then95°Cfor15s, 60°Cfor15sand95°Cfor15s. The results were analyzed using the standard curve mode, according to the manufacturer's recommendations (Applied Biosystems).

3 RESULTS

3.1 Identification of vitelogenin genes in gourammy

The vitelogenin genes of the gourammy were successfully amplified from cDNA. The agarose gel electrophoresis of the cDNA vitelogenin showed a specific band, approximately 360 bp (submit number:1995561). The corresponding cDNA sequences were called Vitelogenin. The cDNA sequences were check with BLAST and we found there wasnt 100% identity with another vitelogenin gene. The nucleotide sequence identity of vitelogeninmRNA was 82% with mud crap(cirrhinus moliterella, *GU324313.1*), 81 % with grasscarp (*ctenophyringodon idella*, KT984759.1), 81 % chinesse minnow (Gobiocypris rarus, EU623080.1), and 81% with fathead minnow (Pimephales promelas ,AF130354.1).

3.2 Gene and amino acid Structure vitelogenin

Vitellogenin genes in gouramy successfully amplified and contain 361 basepair nucleotide. Based on alignment to another teleost, this region includesopening reading frame in vitellogenin gene. (Figure. 1)

CATTCAACTCATGAGATCAGTGATGCACCAGCCCAGAGATTATTGAAGTTCTTACGCAC
CTTGTTACAAACCATGTGGCCATGGTCGATGATGATGCTCCTCTCATGTTTATTCAGCTCA
TTCAACTCCTGCCTGTTGCCACCCTTGAGAATGATGAGTCTATCTGGGCTCACTACAAGGA
CAAACCAGTTTACAGGGCGCTGGCTTCTGGATGCTCTTCCTGCTGTGGGCACACCAGTAAT
GGTGAAAATGTACAAGGAGAAATGCCTGGCTGTCGATCTTACCCTTTCTGAGAGTCATTCT
GACTGTTGTGGGGCTCTGCAAATGCTTTTGCTGACATCGAAAACATCCGGTGACCGCT

Fig 1. Vitelogenin nucleotide and amino acid sequence of gouramy

The connection vitellogenin sequence in gourami with anotherfishcan be seen as the distance at the phylogenetic tree (Figure.2, 3,4). The vitellogenin sequences in gourami had conserved area with another fish and The striking contrast between conservation of the

vitelogenin coding sequence and lack thereof in the GAP coding sequence is evidence of differential selective pressure within the gene. This is evident in cases where the identity and similarity of vitelogenin and GAP coding sequences have been compared for mRNAs of different vitelogenin genes within a species.

3.3 Phylogenetic analyses

Phylogenetic analyses were performed to establish an evolutionary context for the vitellogenin gene. Genetic distances (measured as substitutions per site) showed moderate low values, and the topology was well supported by strong bootstrap values. As expected, vitellogenin in gouramy was included within a sub-cluster of the *carp* (*ctenopharynodon idella)* and minnowwith high bootstrap values (Figure 5).

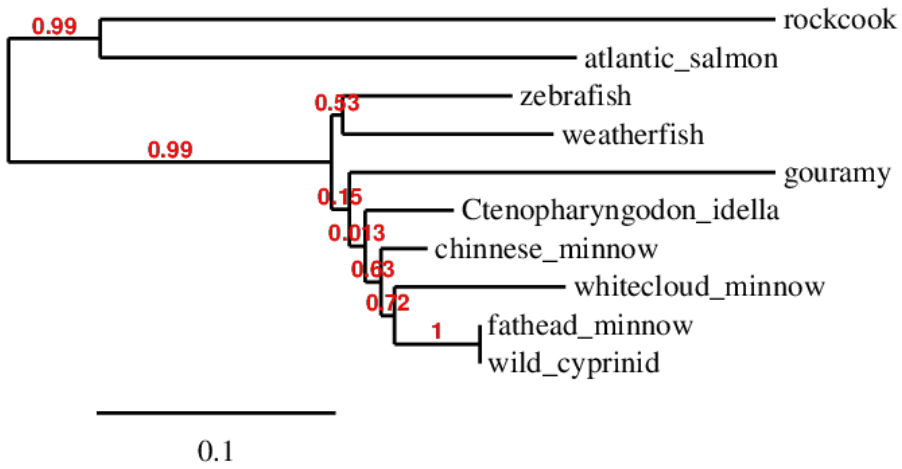
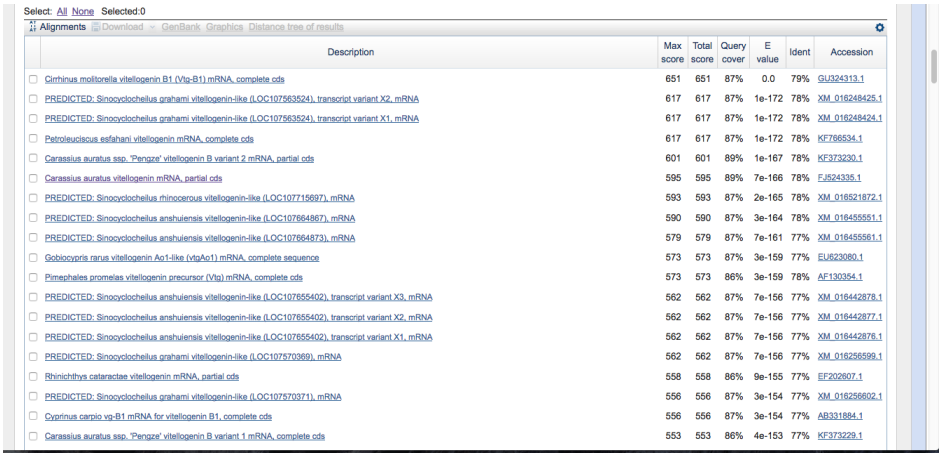


Fig 4. Phylogenetic relationship of precursors derived from known nucleotide encoding vitelogenin. The relationship was generated with CLUSTAL W and the unrooted tree was generated using Treeview version 1.5.2. The scale bar represents the estimated evolutionary distance as 0.1 amino acid substitutions per site.

4 DISCUSSION

This paper reports for the first time the clone of differing vitelogenin genes from pituitary tissues of gouramy. Comparison the vitelogenin gene structure with previously reported gene structures of other fish species shows a high conservation. The nucleotide sequence identity of vitelogenin mRNA also very similar with another variant vitelogenin, from BLAST result showed was 82% with mud crap (cirrhinus moliterella, *GU324313.1*), 81 % with grasscarp (*ctenophyringodon idella*, KT984759.1), 81 % chinesse minnow (Gobiocypris rarus, EU623080.1), and 81% with fathead minnow (Pimephales promelas , AF130354.1) (Figure 2).



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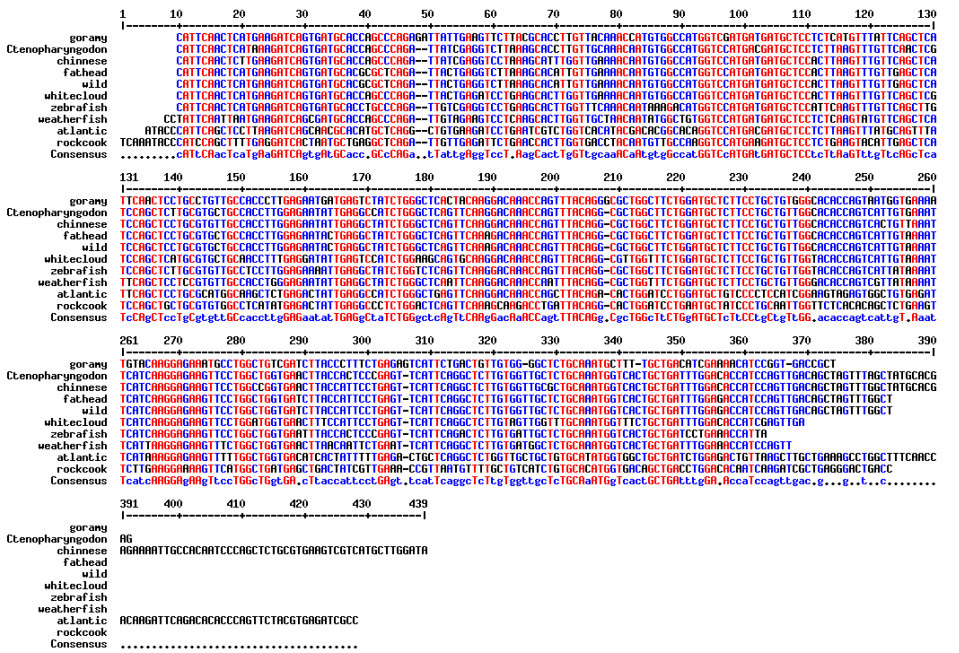
Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Cirrhinus mollorella vitellogenin B1 (Vtg-B1) mRNA, complete cds	651	651	87%	0.0	79%	GU324313.1
PREDICTED: Sinocyclocheilus grahami vitellogenin-like (LOC107563524), transcript variant X2, mRNA	617	617	87%	1e-172	78%	XM_016248425.1
PREDICTED: Sinocyclocheilus grahami vitellogenin-like (LOC107563524), transcript variant X1, mRNA	617	617	87%	1e-172	78%	XM_016248424.1
Petroleuciscus esfahani vitellogenin mRNA, complete cds	617	617	87%	1e-172	78%	KF766534.1
Carassius auratus ssp. 'Pengze' vitellogenin B variant 2 mRNA, partial cds	601	601	89%	1e-167	78%	KF373230.1
Carassius auratus vitellogenin mRNA, partial cds	595	595	89%	7e-166	78%	FJ524335.1
PREDICTED: Sinocyclocheilus rhinocerous vitellogenin-like (LOC107715697), mRNA	593	593	87%	2e-165	78%	XM_016521872.1
PREDICTED: Sinocyclocheilus anshuiensis vitellogenin-like (LOC107664867), mRNA	590	590	87%	3e-164	78%	XM_016455551.1
PREDICTED: Sinocyclocheilus anshuiensis vitellogenin-like (LOC107664873), mRNA	579	579	87%	7e-161	77%	XM_016455561.1
Gobiocypris rarus vitellogenin Ao1-like (vtgAo1) mRNA, complete sequence	573	573	87%	3e-159	77%	EU623080.1
Pimephales promelas vitellogenin precursor (Vtg) mRNA, complete cds	573	573	86%	3e-159	78%	AF130354.1
PREDICTED: Sinocyclocheilus anshuiensis vitellogenin-like (LOC107655402), transcript variant X3, mRNA	562	562	87%	7e-156	77%	XM_016442878.1
PREDICTED: Sinocyclocheilus anshuiensis vitellogenin-like (LOC107655402), transcript variant X2, mRNA	562	562	87%	7e-156	77%	XM_016442877.1
PREDICTED: Sinocyclocheilus anshuiensis vitellogenin-like (LOC107655402), transcript variant X1, mRNA	562	562	87%	7e-156	77%	XM_016442876.1
PREDICTED: Sinocyclocheilus grahami vitellogenin-like (LOC107570369), mRNA	562	562	87%	7e-156	77%	XM_016256599.1
Rhinichthys cataractae vitellogenin mRNA, partial cds	558	558	86%	9e-155	77%	EF202607.1
PREDICTED: Sinocyclocheilus grahami vitellogenin-like (LOC107570371), mRNA	556	556	87%	3e-154	77%	XM_016256602.1
Cyprinus carpio vg-B1 mRNA for vitellogenin B1, complete cds	556	556	87%	3e-154	77%	AB331884.1
Carassius auratus ssp. 'Pengze' vitellogenin B variant 1 mRNA, complete cds	553	553	86%	4e-153	77%	KF373229.1

Fig 2. Comparison of vitelogenin nucleotide between gouramyand another species.



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                1      10        20        30        40        50        60        70        80        90       100       110       120       130
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
gorami                        CATTCAACTCATGAAGATCAGTGATGCACCAGCCCAGAGATTATTGAAGTTCTTACGCACCTTGTTACAAACCATGTGCCATGGTCGATGATGATGCTCCTCTCATGCTTATTCAGCTCA
Ctenopharyngodon              CATTCAACTCATAAAGATCAGTGATGCACCAGCCCAGA---TTATCGAGGTCTTAAAGCACCTTGTTGCAAACAATGTGCCATGGTCCATGACGATGCTCCTCTTAAGTTTGTTCAACTCG
chinnese                      CATTCAACTCTTGAAGATCAGTGATGCACCAGCCCAGA---TTATCGAGGTCCTAAAGCATTTGGTTGAAAACAATGTGCCATGGTCCATGATGATGCTCCACTTAAGTTTGTTCAGCTCA
fathead                       CATTCAACTCATGAAGATCAGTGATGCACGCGCTCAGA---TTACTGAGGTCTTAAAGCACATTGTTGAAAACAATGTGCCATGGTCCATGATGATGCTCCACTTAAGTTTGTTGAGCTCA
wild                          CATTCAACTCATGAAGATCAGTGATGCACGCGCTCAGA---TTACTGAGGTCTTAAAGCACATTGTTGAAAACAATGTGCCATGGTCCATGATGATGCTCCACTTAAGTTTGTTGAGCTCA
whitecloud                    CATTCAACTCATGAAGATCAGTGATGCACCAGCCCAGA---TTACTGAGATCCTGAAGCACTTGGTTGAAAACAATGTGCCATGGTCCATGATGATGCTCCACTTAAGTTTGTTCAGCTCG
zebrafish                     CATTCAACTCATGAAGATCAGTGATGCACCTGCCCAGA---TTGTCGAGGTCCTGAAGCACTTGGTTTCAAACAATAAAGCATGGTCCATGATGATGCTCCATTCAAGTTTGTTCAGCTTG
weatherfish             CCTATTCAATTAATGAAGATCAGCGATGCACCAGCCCAGA---TTGTAGAAGTCCTCAAGCACTTGGTTGCTAACAATATGGCTGTGGTCCATGATGATGCTCCTCTCAAGTATGTTCAGCTCA
atlantic          ATACCCATTCAGCTCCTTAAGATCAGCAACGCACATGCTCAGG---CTGTGAAGATCCTGAATCGTCTGGTCACATACGACACGGCACAGGTCCATGACGATGCTCCTCTTAAGTTTATGCAGTTTA
rockcook    TCAAATACCCATTCCAGCTTTTGAAGATCACTAATGCTGAAGCTCAGA---TTGTTGAGATTCTGAACCACTTGGTGACCTACAATGTTGCCAAGGTCCATGAAGATGCTCCTCTGAAGTACATTGAGCTCA
Consensus   .........cATtCAacTcaTgAaGATCAgtgatGcGCacc.GCcCAGa..tTattgAggTccT.AagCactTgGTtgcaaACaAtgtgGccatGGTCcATGAtGATGCTCCtcTtAaGTtTgTcaAGctca

                131     140       150       160       170       180       190       200       210       220       230       240       250       260
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
gorami          TTCAACTCCTGCCTGTTGCCACCCTTGAGGAATGATGAGTCTATCTGGGCTCACTACAAGGACAAACCAGTTTACAGGGCGCTGGCTTCTGGATGCTCTTCCTGCTGTGGGCACACCAGTAATGGTGAAAAA
Ctenopharyngodon TCCAGCTCTTGCGTGCTGCCACCTTGGAGAATATTGAGGCCATCTGGGCTCAGTTCAAGGACAAACCAGTTTACAGG-CGCTGGCTTCTGGATGCTCTTCCTGCTGTTGGCACACCAGTCATTGTGAAAAT
chinnese        TCCAGCTCCTGCGTGTTGCCACCTTGGAGAATATTGAGGCTATCTGGGCTCAGTTCAAGGACAAACCAGTTTACAGG-CGCTGGCTTCTGGATGCTCTTCCTGCTGTTGGCACACCAGTCACTGTTAAAT
fathead         TCCAGCTCCTGCGTGCTGCCACCTTGGAGAATACTGAGGCTATCTGGGCTCAGTTCAAGGACAAACCAGTTTACAGG-CGCTGGCTTCTGGATGCTCTTCCTGCTGTTGGCACACCAGTCATTGTAAAAT
wild            TCCAGCTCCTGCGTGCTGCCACCTTGGAGAATACTGAGGCTATCTGGGCTCAGTTCAAGGACAAACCAGTTTACAGG-CGCTGGCTTCTGGATGCTCTTCCTGCTGTTGGCACACCAGTCATTGTAAAAT
whitecloud      TCCAGCTCATGCGTGCTGCAACCTTTGAGGATATTGAGTCCATCTGGGCTCAGTTCAAGGACAAACCAGTTTACAGG-CGTTGGTTTCTGGATGCTCTTCCTGCTGTTGGTACACCAGTCATTGTAAAAT
zebrafish       TCCAGCTCTTGCGTGTTGCCTCCTTGGAGAAAATTGAGGCTATCTGGCTCTAGTTCAAGGACAAACCAGTTTACAGG-CGCTGGCTTCTGGATGCTCTTCCTGCTGTTGGTACACCAGTCATTATAAAAT
weatherfish     TTCAGCTCCTCCGTGTTGCCACCTGGGAGAATATTGAGCTATCTGGGCTCAATTCAAGGACAAACCAATTTACAGG-CGCTGGTTTCTGGATGCTCTTCCTGCTGTTGGAGCACCAGTCGTTATAAAAT
atlantic        TTCAGCTCCTGCGATGGCAAGCTCTGAAGCTATTGAGCCATCTGGGCTAGTTCAAGGACAAACCAGCTTACAGA-CACTGGATCTGGATGCTCCCTCTATCGGAAGTAGAGTGGCTGTGAGAT
rockcook        TCCAGCTGCTGCGTGTGGCCTCATATGAGACTATTGAGGCCCTCTGGACTCAGTTCAAGCAAGACCTGATTACAGG-CACTGGATCCTGAATGCTATCCCTGCAATTGGTTCTCACACAGCTCTGAAGT
Consensus   TcCAgCTccTgCgtgttGCcacctgGAGaatatTGAGgCtaTCTGGgctcAgTtCAAgGacAaACCagtTTACAGg.CgcTGGcTtCTGgATGCTcTtCCtgCtgTtGG.acaccagtcattgT.Aaat

                261     270       280       290       300       310       320       330       340       350       360       370       380       390
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
gorami          TGTACAAGGAGAAATGCCTGGCTGTCAATCTTACCCTTCTGAGAGTCATTCTGACTGTTGTGG-GGCTCTGCAAATGCTTT-TGCTGACATCGAAAACATCCGGT-GACCGCT
Ctenopharyngodon TCATCAAGGAGAAGTTCCTGGCTGGTGAAACTTACCACTCCCGAGT-TCATTCAGGCTCTTGTGGTTGCTCTGCAAATGGTCACTGCTGATTTGGACACCATCCAGTTGACAGCTAGTTTAGCTATGCACG
chinnese        TCATCAAGGAGAAGTTCCTGGCCGGTGAACTTACCATTCCTCGAGT-TCATTCAGGCTCTTGTGGTTGCGCTGCAAATGGTCACTGCTGATTTGGACACCATCCAGTTGACAGCTAGTTTGGCTATGCACG
fathead         TCATCAAGGAGAAGTTCCTGGCTGGTGATCTTACCATTCCTCGAGT-TCATTCAGGCTCTTGTGGTTGCTCTGCAAATGGTCACTGCTGATTTGGAGACCATCCAGTTGACAGCTAGTTTGGCT
wild            TCATCAAGGAGAAGTTCCTGGCTGGTGATCTTACCATTCCTCGAGT-TCATTCAGGCTCTTGTGGTTGCTCTGCAAATGGTCACTGCTGATTTGGAGACCATCCAGTTGACAGCTAGTTTGGCT
whitecloud      TCATCAAGGAGAAGTTCCTGGATGGTGAAACTTTCCATTCCTCGAGT-TCATTCAGGCTCTTGTAGTTGGTTTGCAAATGGTTTCTGCTGATTTGGACACCATCGAGTTGA
zebrafish       TCATCAAGGAGAAGTTCCTGGCTGGTGAATTTACCACTCCCGAGT-TCATTCAGACTCTTGTGATTGCTCTGCAAATGGTCACTGCTGATCCTGAAACCATTA
weatherfish     TCATTAAGGAGAAGTTTCTGGCTGGTGAAACTTAACAATTCTGAAT-TCATTCAGGCTCTTGTGATGGCTCTGCAAATGGTCACTGCTGATTTGGAAACCATCCAGTT
atlantic        TCATAAAGGAGAAGTTTTTGGCTGGTGAACATCACTATTTTTGAGA-CTGCTCAGGCTCTGGTTGCTGCTGTGCATATGGTGGCTGCTGATCTGGAAGACTGTTAAGCTTGCTGAAAGCCTGGCTTTCAACC
rockcook        TCTTGAAGGAAAAGTTCATGGCTGATGAGCTGACTATCGTTGAAA-CCGTTAATGTTTTGCTGTCATCTGTGCACATGGTGACAGCTGACCTGGACACACAATCAAGATCGCTGAGGGACTGACC
Consensus   TcatcAAGGAgAAgTtccTGGcTGgtGA.cTtaccattcctGAgt.tcatTcaggcTcTtgTggttgcTcTGCAaATGgTcactGCTGAtttgGA.AccaTccagttgac.g...g..t..c.....

                391     400       410       420       430       439
                |--------+---------+---------+---------+--------|
gorami
Ctenopharyngodon AG
chinnese        AGAAAAATTGCCACAATCCCAGCTCTGCGTGAAGTCGTCATGCTTGGATA
fathead
wild
whitecloud
zebrafish
weatherfish
atlantic        ACAAGATTCAGACACACCCAGTTCTACGTGAGATCGCC
rockcook
Consensus   ......................................
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Fig 3. Multalin of Vitelogenin Sequence of gouramy with another fish

These results showed that gouramy vitelogenin genes had a high similarity with other teleost. The highest similarity was identified between gouramy and *grasscrap* (82%). Based on these results we suggested that goramy had structure of vitellogenin gene similar to teleost (figure 3).

The present study is the first to describe the vitelogenin genes in gouramy, providing new information on this gene family in the gouramy. The vitelogenin in gouramy were grouped together with other teleost in the phylogenetic tree, suggesting a common ancestor for both groups of genes. Phylogenetic analysis shows that vitellogenin can be separated into 2 major groups. Subgroup I contains vitelogenin from rockcook and salmon, sub group II fromzebrafish until minnow. (Figure.4).

4.1Expression of vitelogenin mRNA gouramy under photoperiods manipulation

In gouramy, relative vitellogenin mRNA expression levels in eight weeks were 0.5-2.35 (Figure 5). The highest vitellogenin mRNA expression (2.35) was observed LP group in eight month significantly different ($P<0.05$). mRNA expression for 18L:6D group increased with post spawning periods. The vitelogenin mRNA expression for other treatment photoperiods in second weeks, fourth weeks and sixted weeks had non significantly different ($P>0.05$), but in eighted month long photoperiods (LP) had higher gene vitellogenin than short photoperiods (SP) and control ($P<0.05$).

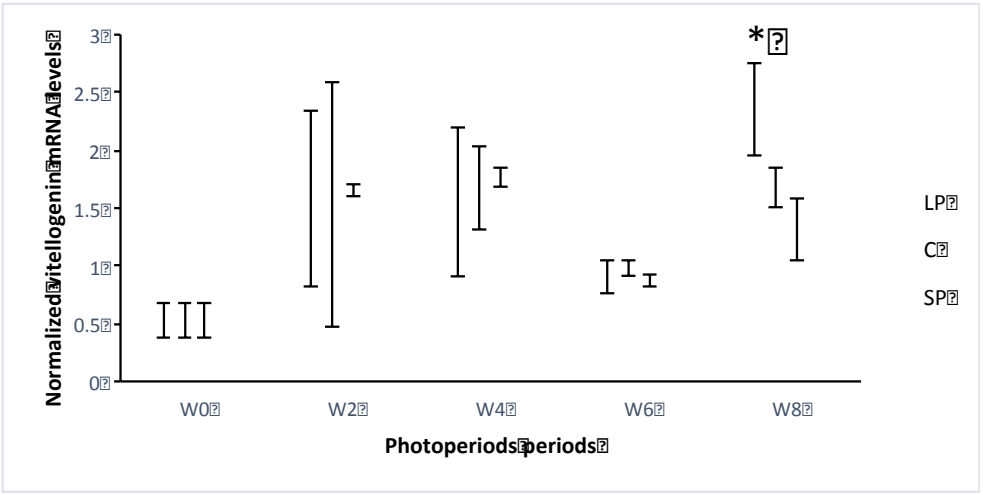

Fig 5. Vitelogenin gene expression of gouramy kept under different photoperiod for 8 weeks. C=control, LP=18L:6D, SP=6L:8D). (*: Significantly different)

In this study, changes in, gene expression of vitellogenin in gouramy was analyzed to characterize the role of neuropeptides in the control of reproduction under photoperiods manipulation. This study confirms previous results from fisheries laboratory showing increased in vitelogeningenes levels during photoperiods manipulation in gouramy. In addition, we report for the first time, changes in the gene expression levels of vitellogenin genesin correlation with photoperiods manipulation. Although we are aware that mRNA levels do not always match with protein levels and/or the physiological effects of the protein products, the regulation of mRNA levels provides an indication of the activity of a particular peptide neuronal system.

In this study showed that vitelogenin level increased equivalent with the long photoperiod increased. This is proved that photoperiod exert it role on reproduction through hipotamaus-pituitary-gonad, that integrates and conveys input from external and internal cues to the pituitary organs [4,5]. Photoperiods regulated melatonin production and melatonin mediated cyclical regulation of GnRH mRNA expression involve the protein kinase C and the extracellular signal-regulated kinase 1 and 2 pathways. Melatonin regulated act through membrane receptors to trigger the protein kinase C pathway and 12-O- tetradecanoyl phorbol-13-acetate (TPA), a modulator of this pathway, has been shown to suppress GnRH gene expression through the promoter (16). GnRH binds to GnRH receptor and active G protein mediated phosphorilation to protein kinase C and shyntezis Gonadotrophin (GtH-I and GtH-II). GtH-II secreted into blood vessel, to reseptor in theca cell actived G protein and adenilat cyclase to phosphorilation cAMP and activation staR

protein. staR protein regulated cholesterol. Steroid hormone will produce vitelogenin in liver.

5 CONCLUSIONS

In summary, the present work has reported for the first time sequence of vitelogenin an gouramy, the phylogenetic results presented in this work support the idea that vitellogenin genes share the same basic structure. That was meaning vitellogenin in gouramy very conserve, that assumed had a same function with another teleost. Photoperiod affected regulation of gene expression of vitellogenin, in the gouramy. The longer photoperiod increased gene expression of vitellogenin, via HPG axis.

REFERENCES

1. M.J. Bayyari, L. Rodrıguez, S. Zanuy, J.A. Madrid, F.J. Sanchez-vazquez, H. Kagawa, K. Okuzawa, and M. Carrillo. General and comparative endocrinology. **136**, 72–81 (2004)

2. S. Bhattacaraya. Current science. **63**, 3 (1992)

3. A.K. Biswas, T. Morita, G. Yoshizaki, M. Maita and T. Takeuchi. Aquaculture. **243**, 229-239(2005)

4. N.R. Bromage, M.J.R. Porter, and C.F. Randall. Aquaculture.**197**, 63–98 (2001)

5. V.M.Cassone. Chronobiology. International. **15**, 457-473 (1998)

6. J. Chyb, Mikolajczyk, B.J endocrinol.**63**, 87-97 (1999)

7. P. Ekstrom, and H. Meissl. Fisher. 7, 199–284 (1997)

8. M.R Flack, J. Froehlich, A.P Bennet. J biol chem. **269**,14–20 (1994)

9. J.E. Juell, and J. E. Fosseidengen. Aquaculture. **233**, 269–282 (2004)

10. P. Melamed, G. Gur, H. Rosenfeld, H. Elizur, A. Yaron. Biochem. **17**, 93–98 (1997)

11. F. Minniti, M. Maisano,A. Giannito,A. Mauceri, and F. Sasuolo. Italian journal of zoology. **76**, 269-278 (2007)

12. L.A. Miranda, C. A. Strussmann, and G. M. Somoza.Fish physiology biochemical. **35**, 101–108 (2008)

13. Prayogo.n.a, g.e. wijayanti, murwantoko, m. Kawaichi and p. Astuti. (2012) effect of photoperiods on melatonin levels, estradiols level and the expression of cgnrh-ii and sgnrh genes, in hard-lipped barb (*osteochilus hasselti* c.v.).*Journal of global veterinaria*, 8(6) : 591-597.

14. N.A. Prayogo, G.E. Wijayanti, I. Sulistyo,P. Sukardi. Biodiversitas.**17**, 523-530 (2016)

15. N.A. Prayogo, A. Siregar, P. Sukardi. Turkish journal fishery and aquatic science. **16**, 1003-1009 (2016)

16. T. Qingbo, M. Mazur, and P. L. Mellon. Molecular endocrinology.**19**, 2769–2779 (2005)

17. L. Rodriguez, M. Carrillo, L. Sorbera, Y. Zohar, and S. Zanuy. General and comparative endocrinol. 136: 37-48. (2004)

18. H. Rosenfeld, B. Levavi-sivan, P. Melamed, Z. Yaron, and A. Elizur.Biochem. **17**, 85– 92 (1997)

19. J.E. Skjæraasen, T. Nilsen, and O. S. Kjesbu. Fish aquatic science. **63**, 310–320 (2006)

20. I. Sulistyo, J. Rinchard, P. Fontaine, J.N. Gardeur, B. Capdeville, and P. Kestemont. Aquatic living resource. **11**, 101-110 (1998)

21. I. Sulistyo,P. Fontaine, J. Rinchard, J.N. Gardeur, H. Migaud, B. Capdeville, and P. Kestemont. Aquatic living resource. **11**, 101-110 (1998)

22. V.L. Trudeau, B.D. Sloley, and R.E. Peter. Possible role for taurine in regulating pituitary hormone release in fish *taurine minisymposium, fourth international congress on amino acids* (Vienna, Austria, 1995)

23. A.A. Ulloa, C. Timossi, M.P. Damian. **11**, 205–215 (1999)

24. T.N. Utoh,A. Horie, Y. Okamura, S. Yamada, N. Tanaka, A. Mikawa, H. Akazawa, and P. Oka. Fisheries science.**69**, 181–188 (2003)

25. V.L.A. Vieira, S.J.S. Johansen, R. Bickerdike, and I.A. Aquaculture.**246**, 197– 208 (2005)

26. H. Volkoff, and R.E. Peter. Gen comp endocrinol. **116**, 347–355 (1999)

27. Q. Wang, K.W. Sham, S. Ogawa, S. Li, I.S. Parhar, C.H. Cheng, X. Liu, and H. Lin. Cell. Endocrinol. (2013)

28. F. Xiong, and C.L. Hew. J. Zool. **69**, 2572–2578 (1991)

29. Z. Yaron. Aquaculture. 129, 49-73(1995)

Abbreviations

1. GnRH: Gonadotrophin Releasing Hormone
2. GtH : Gonadotrophin Hormone
3. mRNA: Messenger RiboNucleoAcid

4. DNA : Deoxy NucleoAcid
5. cDNA: complementary Deoxy Nucle Acid
6. GnRH: Gonadotrophin Releasing Hormone
7. TPA : tetradecanoyl phorbol-13-acetate