IN VIVO ACTIVITY OF GONADOTROPIN- RELEASING HORMONE (cGnRH-II, sGnRHa and LHRHa) IN AFRICAN CATFISH, (Clarias gariepinus B.) AND JAVANESE BARB, (Barbodes gonionotus B.)

NORHIDAYAH BT MOHD TAUFEK

FP 2010 10
IN VIVO ACTIVITY OF GONADOTROPIN- RELEASING HORMONE (cGnRH-II, sGnRHa and LHRHa) IN AFRICAN CATFISH, (Clarias gariepinus B.) AND JAVANESE BARB, (Barbodes gonionotus B.)

By

NORHIDAYAH BT MOHD TAUFEEK

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Masters of Science

August 2010
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Masters of Science

IN VIVO ACTIVITY OF GONADOTROPIN-RELEASING HORMONE (cGnRH-II, sGnRHa and LHRHa) IN AFRICAN CATFISH, (Clarias gariepinus B.) AND JAVANESE BARB, (Barbodes gonionotus B.)

By

NORHIDAYAH BT MOHD TAUFEEK

August 2010

Chair: Assoc. Prof. Sharr Azni Bin Harmin, PhD

Faculty: Agriculture

Gonadotropin hormone releasing hormone (GnRH) is a peptide hormone that is responsible for stimulating the release of gonadotropins from pituitary and consequently influence the steroid hormone production level in the ovary. Six experiments have been conducted to determine the effectiveness of cGnRH-II as an inducing agent for maturation and ovulation in Clarias gariepinus and Barbodes gonionotus. The first experiment determined the effectiveness of native cGnRH-II when compared with analog LHRHa, and sGnRHa with saline used as control. The concentration of all the hormones used in this experiment was 20μg/kg. For the second experiment, three different concentrations were given; 2 μg/kg, 20 μg/kg and 200 μg/kg with saline as a control. Finally, the third experiment examined the pimozide (PIM) as dopamine inhibitor with the combination of cGnRH-II to accelerate the maturation and ovulation. The hormones involved were cGnRH-II 200 μg/kg; 5mg/kg PIM; cGnRH-II 200 μg/kg + 5mg/kg PIM and saline. All the hormones
were administered as a single injection. The experimental parameters observed were hormonal changes in the fishes and Germinal Vesicle (GV) movement during the experiment. Each group consisted of six sexually mature females ranging from 450-550 g Body Weight (BW). The GV was examined prior to injection at 0h, 6h, 12h and 24h post injection and ranked from 1 (GV at central location) to 4 (at ovulation stage). Blood were sampled at the same time as the GV examination. Plasma sample were analyzed for Testosterone (T) and 17β-Estradiol (E2) concentrations using Enzyme Link Immunosorbent assay (ELISA). Hatching and fertilization rates were determined and data for plasma steroid hormones and the GV were analyzed using analysis of variance (ANOVA).

For African catfish, sGnRHa showed the most outstanding effect compared to the two treatments (LHRHa and cGnRH-II) since it is more resistance to degradation when compared with native peptides. The 200\(\mu\)g/kg cGnRH–II proved to be effective in stimulating maturation and ovulation. Saline injected fish remained unchanged where the initial GV was at stage 1.00±0.00. The cGnRH-II alone was enough to induce maturation and ovulation in African catfish. All fish ovulated in both groups (cGnRH-II alone and cGnRH-II + PIM) while the GV showed significant migration throughout the experiment (P<0.05). Fertilization rate constituted 73% and 80% while hatching rates were 58% and 67% for cGnRH-II alone and cGnRH-II + PIM treatments, respectively.
For Javanese barb, sGnRHa was more potent compared to the cGnRH-II and LHRHa. In the graded dosage experiment, 200 μg/kg cGnRH-II seemed to induce for oocyte maturation but no ovulation occurred. However, addition of Pimozide increase the plasma steroid level compared to cGnRH-II alone. The plasma steroid hormone for the saline treated group in all the experiment remained the same throughout the study (P>0.05). Although no ovulation was observed, the plasma steroid hormones and GV of cGnRH-II treated group showed significant effect compared to saline group (P<0.05).

The cGnRH-II was proven to effectively induced maturation and ovulation in both the Javanese barb and African catfish although large number of dosage was required. The use of a potent cGnRH-II analog for the improvement of spawning induction therapies is strongly suggested to increase the performance of cGnRH-II.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

AKTIVITI HORMON PEREMBESAN GONADOTROPIN (cGnRH-II, sGNRHa DAN LHRHa) PADA KELI AFRIKA, (Clarias gariepinus B.) DAN LAMPAM JAWA, (Barbodes gonionotus B.)

Oleh

NORHIDAYAH BT MOHD TAUFEK

Ogos 2010

Pengerusi: Prof. Madya Dr. Sharr Azni Bin Harmin

Fakulti: Pertanian

Hormon perembesan Gonadotropin (GnRH) ialah sejenis hormone peptida yang bertanggungjawab untuk merangsang pengeluaran gonadotropin dari kelenjar pituitari dan seterusnya mempengaruhi pengeluaran hormon steroid dalam ovari. Enam eksperimen telah dijalankan untuk melihat keberkesanan cGnRH-II sebagai agen yang mempercepatkan kematangan dan peneluran Clarias gariepinus dan Barbodes gonionotus. Eksperimen pertama melihat potensi cGnRH-II native apabila dibandingkan dengan LHRHa dan sGnRHa analog dan salin sebagai kawalan. Semua hormon menggunakan kepekatan yang sama iaitu 20 μg/kg. Bagi eksperimen kedua pula, tiga kepekatan yang berbeza telah diberikan kepada tiga kumpulan (2 μg/kg, 20 μg/kg and 200 μg/kg) dan salin. Eksperimen ketiga pula melihat keberkesanan pimozid sebagai penghalang dopamin dengan kombinasi cGnRH-II untuk mempercepatkan kematangan dan peneluran. Hormon yang terbabit adalah cGnRH-II 200 μg/kg; 5mg/kg PIM; cGnRH-II 200 μg/kg + 5mg/kg PIM dan salin. Kesemua ikan hanya diberi sekali suntikan hormon sahaja. Parameter
bagi eksperimen di atas adalah perubahan hormon pada ikan dan pergerakan Germinal Vesicle (GV) semasa eksperimen. Setiap kumpulan mengandungi enam ekor ikan betina yang telah matang dengan berat badan 450-550 g setiap seekor. GV telah diperiksa sebelum suntikan iaitu jam 0, 6 jam, 12 jam dan 24 jam selepas suntikan. Germinal Vesicle telah diklasifikasikan mengikut tahap yang bermula pada 1 (GV berada di tengah oosit) sehingga tahap 4 (semasa peneluran). Darah diambil pada masa yang sama dengan pemerhatian GV. Plasma daripada sampel Testosterone (T) dan 17β-Estradiol (E2) dianalisis kepekatannya menggunakan teknik Enzyme Link Immunosorbent assay ‘ELISA’. Kadar penetasan dan persenyawaan dikira dan data bagi kadar plasma hormon steroid dan GV dianalisis menggunakan analysis of variance (ANOVA).

Bagi keli Afrika, sGnRHa menunjukkan kesan yang paling efektif berbanding LHRHa dan cGnRH-II kerana ia lebih resistan berbanding peptida yang native. 200μg/kg cGnRH–II telah terbukti efektif untuk merangsang kematangan dan peneluran. Ikan yang disuntik saline tidak menunjukkan perubahan dimana GV berada pada tahap 1.00±0.00 sepanjang eksperimen. Hormon cGnRH-II yang disuntik bersendirian sudah mencukupi untuk merangsang kematangan dan peneluran bagi keli afrika. Kedua-dua kumpulan (cGnRH-II sahaja dan cGnRH-II + PIM) menunjukkan 100% peneluran dan GV menunjukkan perubahan signifikan sepanjang eksperimen (P<0.05). Kadar persenyawaan adalah 73% (cGnRH-II sahaja) dan 80% (cGnRH-II + PIM) manakala kadar penetasan pula menunjukkan 58% (cGnRH-II sahaja) and 67% (cGnRH-II + PIM)
Bagi lampam Jawa, sGnRHa menunjukkan kesan lebih efektif berbanding LHRHa dan cGnRH-II. Bagi eksperimen untuk menentukan kepekatan optimum, 200 μg/kg cGnRH-II terbukti berkesan untuk merangsang kematangan oosit walaupun tiada peneluran berlaku. Namun begitu, penambahan pimozid dapat meningkatkan kadar hormon steroid berbanding hanya menggunakan cGnRH-II sahaja. Kadar plasma hormon steroid bagi kumpulan yang disuntik salin tidak mengalami perubahan signifikan sepanjang eksperimen (P>0.05). Walaupun tiada peneluran berlaku, kadar plasma hormon steroid dan GV bagi kumpulan yang disuntik dengan cGnRH-II menujukkan perubahan signifikan berbanding salin (P<0.05).

cGnRH-II telah menunjukkan kesan efektif untuk merangsang kematangan dan peneluran walaupun memerlukan dos yang tinggi. Penggunaan cGnRH-II analog adalah digalakkan untuk menghasilkan keputusan yang lebih baik untuk proses pembiakan dan meningkatkan keberkesanan cGnRH-II.
ACKNOWLEDGEMENT

I would like to start by thanking my supervisor, Assoc. Prof. Dr. Sharr Azni Bin Harmin, for the support, understanding and for believing in me. I would also like to express my gratitude to my co-supervisor, Dr. Annie Christianus for her guidance and patience throughout the studies.

I sincerely thank Masters student, Mr. Mohammad Fadhil Syukri Ismail, for teaching me the use of ELISA. I would like to extend my thanks to the staff at Aquaculture Research Station in Puchong, Selangor for their kindness in lending their hand during the experiments. Million of thanks to Mr Jasni Mohd Yusoff, Mr Azmi Yaacob, Mr Mohammad Syahrizan Shaharudin and Mr Roszainal Yusop for their help.

To all my colleagues in Aquaculture Department, it has been a great pleasure to work with all of you. Special thanks to Mr Mohammad Fahmi Adam, Ms Noraini Omar and Ms Norhana Mohammad for helping me out during the sampling period. I would also like to express my grateful to Department of Fisheries Bukit Tinggi, Pahang for their guidance in fish breeding and supplying the Javanese barb for free. Finally, thanks to my beloved family for their unconditional love and support over the years.

Financial support for these studies has been provided from a grant by Ministry of Science, Technology and Information, Malaysia (MOSTI). Grant number 5487718
I certify that an Examination Committee has met on date of viva voce to conduct final examination of Norhidayah Bt Mohd Taufek on her degree thesis entitled “In vivo activity of Gonadotropin Releasing Hormone (cGnRH-II, sGnRHα and LHRHα) in African catfish, *Clarias gariepinus* and Javanese barb, *Barbodes gonionotus*” in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the student be awarded the (Master of Science)

Members of the Examination Committee were as follows:

**Siti Shapor Siraj, PhD**
Professor.
Faculty of Agriculture,
Universiti Putra Malaysia
(Chairman)

**Aziz Arshad, PhD**
Associate Professor
Faculty of Agriculture,
Universiti Putra Malaysia
/Internal Examiner)

**Che Roos Saad, PhD**
Associate Professor
Faculty of Agriculture,
Universiti Putra Malaysia
(Internal Examiner)

**Siti Azizah Mohd Nor, PhD**
Associate Professor
Pusat Pengajian Sains Kajihayat
Universiti Sains Malaysia
Malaysia
(External Examiner)

**SHAMSUDDIN SULAIMAN, PhD**
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Masters of Science. The members of the Supervisory committee were as follows:

**Sharr Azni Bin Harmin, PhD**  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Annie Christianus, PhD**  
Lecturer  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

___________________________  
HASANAH MOHD GHAZALI, PhD  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: December 2010
DECLARATION FORM

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

________________________________________
NORHIDAYAH BT MOHD TAUFEEK

Date:
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>viii</td>
</tr>
<tr>
<td>APPROVAL Error! Bookmark not defined.</td>
<td>xi</td>
</tr>
<tr>
<td>DECLARATION FORM</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF TABLE</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATION</td>
<td>xvii</td>
</tr>
</tbody>
</table>

## CHAPTER

1. **INTRODUCTION**

2. **LITERATURE REVIEW**

   2.1 Aquaculture industry, biology of African catfish, *Clarias gariepinus* and Javanese barb, *Barbodes gonionotus*.  
   2.2 Female reproductive morphology and ovarian development  
   2.3 Synthesis of 17ß-estradiol and Testosterone  
   2.4 Reproductive dysfunction in cultured fish  
   2.5 Hormonal induction and spawning  
   2.6 Application of GnRH in cultured fish  
   2.7 Induction of cGnRH-II to promote maturation and ovulation  
   2.8 The use of dopamine antagonist with GnRH on oocyte maturation

3. **GENERAL METHODOLOGY**

   3.1 Experimental fish  
   3.2 Hormone preparation, injection and blood sampling  
   3.3 Ovulatory Response  
   3.4 Steroid Enzyme-linked Immunosorbent assay  
      3.4.1 Pre-assay preparation  
      3.4.2 Assay Protocol  
      3.4.3 Plate Set-Up  
      3.4.4 Analysis  
   3.5 Statistical analysis

4. **EFFECT OF CHICKEN GONADOTROPIN-RELEASING HORMONE (cGnRH-II) ON PLASMA STEROID HORMONE, MATURATION AND OVULATION IN AFRICAN CATFISH, *Clarias gariepinus* (BURCHELL)**

   4.1 Introduction