



UNIVERSITI PUTRA MALAYSIA

**SOME BIOGENIC AMINES IN THE PRAWN HAEMOLYMPH DURING
OVARIAN GROWTH AND THEIR EFFECTS ON VITELLIN
BIOSYNTHESIS IN THE PENAEID PRAWN,
PENAEUS MERGUEINSIS, DEMAN**

CHONG HUEY BING

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By

CHONG HUEY BING

**Thesis Submitted in Fulfilment of the Requirements for the
Degree of Master of Science in Faculty of Medicine and Health Sciences
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June 2000



Specially dedicated to

MY PARENTS



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

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A study on the biogenic amines in the haemolymph of penaeid prawn, *Penaeus merguensis*, at different stages of ovarian growth was conducted. The gonadosomatic index (GSI) was used to show the stages of the ovarian growth. The qualitative and quantitative determination of the biogenic amines were accomplished by using the high performance liquid chromatography with electrochemical detection (HPLC-ECD) method, which provides a high sensitivity and selectivity in determining the biogenic amines. The biogenic amines can be detected at the picogram levels using this method.

The haemolymph of *P. merguensis* at the immature stage (GSI 0.1-4.0) contained the highest level of 5-hydroxyindole acetic acid (5-HIAA) (250 pg/mg). It decreased



at the maturing stage (GSI 4.1-8.0) and increased again at the mature stage (GSI 8.1-12.0). Both norepinephrine (NE) and dopamine (DA) were not detected in the haemolymph at the immature stage. The level of adrenaline (AD) was found to be in increasing order from immature (10 pg/mg) to maturing (20 pg/mg) and mature (140 pg/mg) stages. The concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) increased from immature (60 pg/mg) to maturing (120 pg/mg) stages, but decreased at the mature stage (100 pg/mg). Neither 5-hydroxytryptamine (5-HT) nor 5-methoxytryptamine (5-MT) were detected in the haemolymph samples of *P. merguensis* in the present study.

An unknown compound was detected in the haemolymph of *P. merguensis* at different ovarian growth stages. The levels of this unknown compound decreased from immature to maturing and mature stages, implying that it might be needed for the ovarian growth processes.

The effectiveness of some selected biogenic amines, 5-HT, 5-HIAA and melatonin in stimulating *in vitro* vitellin biosynthesis in the ovarian tissues of *P. merguensis* were studied. Combinations of 5-HT with brain (BR) and 5-HT with thoracic ganglion (TG) treatments significantly ($P < 0.001$) induced vitellin biosynthesis. Ovarian tissues incubated with 5-HT in the absence of brain and thoracic ganglion did not significantly ($P > 0.05$) stimulate the vitellin biosynthesis in the ovarian tissues of *P. merguensis*. The treatments with 5-HIAA or melatonin itself significantly ($P < 0.05$) induce vitellin biosynthesis as compared to the control.

However, the results of these treatments were insignificant ($P > 0.05$) when compared with the BR and TG treatments.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains.

**BEBERAPA BIOAMINA DALAM HEMOLIMF UDANG SEMASA
PERKEMBANGAN OVARI DAN KESANNYA TERHADAP BIOSINTESIS
VITELIN PADA UDANG LAUT, *PENAEUS MERGUIENSIS*, DE MAN**

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Satu kajian terhadap bioamina dalam sampel hemolimf dari udang laut, *Penaeus merguensis* pada peringkat perkembangan ovari yang berlainan telah dijalankan. Indeks gonadosomatik (gonadosomatic index, GSI) menunjukkan peringkat perkembangan ovari udang laut. Kehadiran bioamina-bioamina ditentukan melalui teknik “high performance liquid chromatography with electrochemical detection” (HPLC-ECD). Teknik ini dapat memberi kepekaan dan kuasa pemilihan yang tinggi terhadap bioamina yang diselidiki. Bioamina-bioamina tersebut dapat dikesan sehingga ke takat pikogram.

Keputusan kajian menunjukkan bahawa sampel hemolimf *P. merguensis* pada peringkat tidak matang (immature) (GSI 0.1-4.0) mengandungi kepekatan



5-hydroxyindole acetic acid (5-HIAA) yang paling tinggi (250 pg/mg). Ia menurun pada peringkat mencapai kematangan (maturing) (GSI 4.1-8.0) dan meningkat semula pada peringkat matang (mature) (GSI 8.1-12.0). Kedua-dua jenis bioamina iaitu norepinephrine (NE) dan dopamine (DA) tidak dapat dikesan pada peringkat tidak matang. Kepekatan adrenaline (AD) meningkat sepanjang peringkat perkembangan ovari manakala kepekatan 3,4-dihydroxyphenylacetic acid (DOPAC) meningkat dari peringkat tidak matang (60 pg/mg) ke peringkat mencapai kematangan (120 pg/mg), tetapi menurun pada peringkat matang (100 pg/mg). Keputusan kajian ini juga menunjukkan bahawa kesemua sampel hemolimf tidak mengandungi sebarang 5-hydroxytryptamine (5-HT) dan 5-methoxytryptamine (5-MT).

Suatu bahan yang tidak dapat dikenalpasti telah dijumpai dalam hemolimf *P. merguensis* pada peringkat kematangan ovari yang berlainan. Kepekatan bahan ini menurun sepanjang peringkat kematangan ovari. Ini menunjukkan bahawa bahan ini mungkin diperlukan dalam proses kematangan ovari udang.

Beberapa bioamina seperti 5-HT, 5-HIAA dan melatonin telah dipilih untuk mengkaji keberkesanannya dalam merangsang biosintesis vitelin *in vitro* pada tisu ovari *P. merguensis*. Kesan yang ketara ($P < 0.001$) dapat dilihat pada rawatan 5-HT bersamaan tisu otak dan 5-HT bersamaan torasik ganglion berbanding dengan kawalan. Rawatan dengan 5-HT tanpa tisu otak dan torasik ganglion tidak menunjukkan kesan yang ketara ($P > 0.05$) dalam peningkatan biosintesis vitelin.

Tisu ovari yang menerima rawatan 5-HIAA atau melatonin sahaja memberi kesan yang ketara ($P < 0.05$) berbanding dengan kawalan. Tetapi, kesan kedua-dua rawatan tersebut adalah tidak ketara ($P > 0.05$) jika dibanding dengan rawatan tisu otak dan torasik ganglion.

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TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL SHEETS	xi
DECLARATION FORM	xiii
LIST OF TABLES	xvii
LIST OF FIGURES	xviii
LIST OF PLATES	xx
LIST OF ABBREVIATIONS	xxi
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE RIVIEW	5
2.1 Life Cycle of Penaeid Prawn	6
2.2 Factors Affecting Ovarian Growth of Penaeid Shrimps in Captivity	7
2.2.1 Environmental and Behavioural Factors	7
2.2.2 Nutritional Factors	11
2.3 Endocrinological Aspects in Controlling Reproduction in Crustaceans	13
2.3.1 Inhibiting and Stimulating Factors	14
2.3.2 Vitellogenin Stimulating Hormones	16
2.4 Identification, Localization and Distribution of Biogenic Amines in Crustaceans	17
2.4.1 Dopamine (DA)	18
2.4.2 Norepinephrine (NE)	19
2.4.3 Adrenaline (AD)	20
2.4.4 Histamine (HA)	21
2.4.5 5-Hydroxytryptamine (5-HT, Serotonin)	21
2.4.6 Melatonin	23
2.4.7 Tryptamine	25
2.4.8 Octopamine (OA)	25



2.4.9	Tyramine	26
2.5	The Role of Biogenic Amines in Crustacean Reproduction	26
2.6	Analysis of Biogenic Amines	27
3	HPLC-ECD DETERMINATION OF BIOGENIC AMINES IN THE HAEMOLYMPH OF <i>PENAEUS MERGUIENSIS</i> AT DIFFERENT STAGES OF OVARIAN GROWTH	30
3.1	Introduction	30
3.2	Materials and Methods	31
3.2.1	HPLC-ECD System and Optimization of the Chromatographic Conditions	31
3.2.2	Preparation and Analysis of Biogenic Amine Standards	33
3.2.3	Sampling and Freeze-drying of the Prawn Haemolymph	33
3.2.4	Extraction and Analysis of Biogenic Amines from Prawn Haemolymph	34
3.3	Results	35
3.3.1	Optimization of Chromatographic Conditions	35
3.3.2	Analysis of Biogenic Amine Standards	42
3.3.3	Analysis of Biogenic Amines in Prawn Haemolymph	45
3.4	Discussion	52
4	THE EFFECTS OF BIOGENIC AMINES ON THE <i>IN VITRO</i> VITELLIN BIOSYNTHESIS IN THE OVARIES OF <i>PENAEUS MERGUIENSIS</i>	57
4.1	Introduction	57
4.2	Materials and Methods	58
4.2.1	Sampling of Prawns	58
4.2.2	Purification of Vitellins	59
4.2.3	Immunization of Rabbits	60
4.2.4	Ouchterlony Test	61
4.2.5	Experimental Treatments	62
4.2.6	Chemical Analysis	65
4.2.7	Computation of Data and Statistical Analysis	67
4.3	Results	69
4.3.1	Ouchterlony Test	69
4.3.2	Experimental Treatments	70
4.4	Discussion	72
5	GENERAL DISCUSSION AND CONCLUSIONS	76
	BIBLIOGRAPHY	82



APPENDICES		96
Appendix A	Equations for the Determination of the Gonadosomatic Index (GSI) and Gonadal Index (GI)	97
Appendix B	Solutions and Mobile Phases for Biogenic Amine Analyses	98
Appendix C	Buffers and Solutions for the Extraction of Vitellin	100
Appendix D	Solutions for the Ouchterlony Test	101
Appendix E	Media and Other Tissue Culture Solutions	102
Appendix F	Chemicals Used in the Analysis of Vitellins Synthesized	104
Appendix G	The Gonadosomatic Index (GSI) and Gonadal Index (GI) of Immature Female Prawns, <i>P. merguensis</i> Used in the Tissue Culture	107
Appendix H	The Total Percentage of Vitellin Synthesized <i>in vitro</i> in the Ovarian Tissues of <i>P. merguensis</i>	108
Appendix I	The Arcsine Transformed Data of the Total Percent Vitellin Synthesized	109
Appendix J	Statistical Analysis on Arcsine Transformed Data	110
VITA		113

LIST OF TABLES

Table		Page
1.	Retention times (RT) of biogenic amine standards and the internal standard, DHBA (chromatographic conditions: 3.9 x 300 mm C-18 column; mobile phase containing 7% methanol, pH 4.3; oxidation potential of 0.70 V)	42
2.	Retention times (RT) of 5-HT, 5-MT and the internal standard, DHBA (chromatographic conditions: 3.9 x 150 mm C-18 column; mobile phase containing 20% methanol, pH 4.3; oxidation potential of 0.70 V)	43
3.	Treatments of the ovarian tissues of <i>P. merguensis</i>	63



LIST OF FIGURES

Figure		Page
1	A generalized life cycle of a penaeid prawn (Motoh, 1981)	6
2	Biosynthesis of catecholamines. The dashed lines indicate inhibition of tyrosine hydroxylase by norepinephrine and dopamine (Ganong, 1995)	20
3	Synthesis of melatonin (Ebadi, 1993)	24
4.1	Chromatograms of biogenic amine standards (DOPAC, NE, 5-HIAA, AD, DHBA, and DA) with mobile phase at pH 4.1, 4.3 and 4.5	35
4.2	Chromatograms of biogenic amine standards (DOPAC, NE, 5-HIAA, AD, DHBA, and DA) with mobile phase at pH 4.7 and 4.9	36
5.1	Chromatograms of biogenic amine standards (DOPAC, NE, 5-HIAA, AD, DHBA, and DA) at different concentrations (5%, 7% and 9%) of methanol in the mobile phase	37
5.2	Chromatograms of biogenic amine standards (DOPAC, NE, 5-HIAA, AD, DHBA, and DA) at different concentrations (11% and 13%) of methanol in the mobile phase	38
6	Relative peak heights of DOPAC, NE, 5-HIAA, AD, DHBA and DA as a function of the oxidation potential	39
7.1	Chromatograms of biogenic amine standards (DOPAC, NE, 5-HIAA, AD, DHBA, and DA) at oxidation potentials of 0.65 V, 0.70 V and 0.75 V	40
7.2	Chromatograms of biogenic amine standards (DOPAC, NE, 5-HIAA, AD, DHBA, and DA) at oxidation potentials of 0.80 V and 0.85 V	41

8	Chromatogram of biogenic amine standards (DOPAC, NE, AD, 5-HIAA, DHBA and DA)	43
9	Chromatogram of 5-HT and DHBA standards	44
10	Chromatogram of 5-MT and DHBA standards	44
11	Chromatogram of haemolymph extract of <i>P. merguensis</i> (GSI = 3.26)	46
12	Chromatogram of haemolymph extract of <i>P. merguensis</i> (GSI = 5.75)	46
13	Chromatogram of haemolymph extract of <i>P. merguensis</i> (GSI = 6.84)	47
14	Chromatogram of haemolymph extract of <i>P. merguensis</i> (GSI = 8.30)	47
15	Chromatogram of haemolymph extract of <i>P. merguensis</i> (GSI = 11.27)	48
16	Chromatogram of haemolymph extract of <i>P. merguensis</i> (GSI = 13.41)	48
17	The concentration of biogenic amine (pg/mg) of freeze-dried haemolymph of <i>P. merguensis</i> at each stage of ovarian growth	49
18	Chromatogram of the unknown compound (chromatographic conditions: 3.9 x 150 mm C-18 column; mobile phase containing 20% methanol, pH 4.3; oxidation potential of 0.70 V)	50
19	The average peak area of the unknown compound at each stage of ovarian growth	51
20	Total percent vitellin synthesized <i>in vitro</i> following different treatments with brain (BR), thoracic ganglion (TG), 5-HT and BR, 5-HT and TG, 5-HT, 5-HIAA and melatonin	71

LIST OF PLATE

Plate		Page
1.	Immunoprecipitation lines from Ouchterlony test	69



LIST OF ABBREVIATIONS

AD	Adrenaline
ANOVA	Analysis of Variance
BL	Body length
BR	Brain
BCS	Biodegradable Counting Scintillant
CL	Carapace length
DA	Dopamine
DHBA	3, 4-Dihydroxybenzylamine
DOC	Sodium deoxycholate
DOPA	Dihydroxyphenylalanine
DOPAC	3,4-Dihydroxyphenylacetic acid
ECD	Electrochemical detector
EDTA	Ethylene Diamine Tetra-Acetate
GCMS	Gas chromatography-mass spectrometry
GI	Gonadal index
GIH	Gonad inhibiting hormone
GSH	Gonad stimulating hormone
GSI	Gonadosomatic index
HA	Histamine
HCG	Human chorionic gonadotrophin



HClO ₄	Perchloric acid
5-HIAA	5-Hydroxyindole acetic acid
HPLC	High performance liquid chromatography
HPLC-ECD	High performance liquid chromatography with electrochemical detection
5-HT	5-Hydroxytryptamine
JH	Juvenile hormone
MF	Methyl farnesoate
5-MT	5-Methoxytryptamine
NAT	N-acetyltransferase
NE	Norepinephrine
OA	Octopamine
PAG	Polyacrylamide Gel
PFP	Pentafluoropropionic anhydride
PMSF	Phenylmethylsulfonylfluoride
RIA	Radioimmunoassay
RT	Retention times
SDS	Sodium dodecyl sulphate
TCA	Trichloroacetic acid
TG	Thoracic ganglion
TGE	Thoracic ganglion extract



CHAPTER 1

INTRODUCTION

The shrimp culture industry is one of the most lucrative industries in many countries with aquacultural development, including Malaysia. The large scale production of penaeid shrimps is a recent development, stimulated by a common pattern of increasing demand and on the other hand, by a decline of natural supplies caused by over-fishing, aquatic pollution and diseases.

The spawner (broodstock) is essential for the production of penaeid shrimp postlarvae in penaeid shrimp culture. However, at present in some countries, marine prawn seed supply is still dependent on postlarvae collected from coastal waters. The availability of the spawners has also been mainly dependent on the capture of wild female spawners from the sea. This is because the ovaries of captive females of several penaeid species, for example, *Penaeus monodon* Fabricius, *Penaeus vannamei*, *Penaeus semisulcatus*, and *Penaeus japonicus* do not easily attain sexual maturity without external manipulation. Therefore, development of methods to induce sexual maturation of female shrimps in captivity should be given high priority to assure a stable supply of spawners and seed availability.

Various attempts have been made to stimulate ovarian growth, such as manipulation of diet (Middleditch *et al.*, 1979; Nascimento *et al.*, 1991), manipulation of environmental conditions (Hillier, 1984; Luis and Ponte, 1993), and hormonal regulation such as eyestalk ablation (Adiyodi and Adiyodi, 1970; Fingerman, 1970; Kleinholz and Keller, 1979; and Charniaux-Cotton, 1985). Among these methods, eyestalk ablation is the most popular and commonly applied. Panouse (1943) first showed that eyestalk ablation induced ovarian maturation in the shrimp, *Leander serratus* (Pennant). This was subsequently confirmed by Primavera (1978) and Emmerson (1983), using other species including *P. monodon*.

Unfortunately, these methods are not totally successful and the results are inconsistent. For example, the unilateral eyestalk ablation method is completely at variance with the natural physiology of the animal, and this manipulation may have a negative effect on the quality of the eggs. Conte *et al.* (1977) found that female shrimps that have undergone unilateral eyestalk ablation suffered from high mortality when placed in net cages in the pond. Browdy and Samocha (1985a, b) showed that there is a decrease in fecundity (as measured by the number of eggs per spawn) for the ablated females.

In order to study the hormonal regulation of crustacean reproduction, it is important to know the specific events and the origin of yolk produced during sexual maturation. Ovarian maturation in crustaceans consists of two major processes: oogenesis and vitellogenesis. During oogenesis, oogonia accumulate glycoproteins to become primary oocytes. When the oocytes reach a diameter typical of the