PRODUCTION OF TRIPLOIDY IN RED TILAPIA [Oreochromis mossambicus (Peters, 1852) X Oreochromis niloticus (Linnaeus , 1758]

PRADEEP P.J

DOCTOR OF PHILOSOPHY
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Dedicated to

all tilapia lovers.....

&

all well wishers.....

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in fulfillment of the requirement for the
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PRODUCTION OF TRIPLOIDY IN RED TILAPIA [*Oreochromis mossambicus* (Peters, 1852) X *Oreochromis niloticus* (Linnaeus, 1758)]

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FEBRUARY' 2011

Chairman : **Associate Professor Anuar bin Hassan, Ph.D.**
Member : **Dr. Anil Chatterji, Ph.D.**
Dr. Shahreza Mohammad Sheriff, Ph.D.

Institute : **Institute of Tropical Aquaculture**

Tilapias are widely distributed species in the tropical and subtropical regions of the world with high tolerance to different environmental conditions. This characteristic has made this species more attractive for culture. The red tilapia is a cross breed of *Oreochromis niloticus* (Linnaeus, 1758) and *O. mossambicus* (Peters, 1852) which is a fertile hybrid tilapia strain originated through continuous selective breeding. The reduction of growth in cultured tilapia due to uncontrolled reproduction under restricted environment is the most disadvantageous phenomenon. The production of sterile triploid tilapia to overcome the problems of tilapia aquaculture is very obvious and as such

the efficiency of this technique was evaluated with red tilapia as the model in the present study.

In the present study a proper synchronized breeding protocol for triploidy induction has been developed. Selected ready to spawn brooders on the same day along with a dose of 1500 IU/Kg HCG hormone was found effective in stimulating the spawning of red tilapia under simulated conditions. The synchronized breeding technique applied in the present study has always given predicted spawning. The efficient re-circulatory incubation system specifically designed has always yielded higher percentage of hatching in red tilapia. For distinguishing the accurate ploidy level, karyotyping method was used. For this earlier done method on karyotyping was modified to get excellent chromosome metaphase spreads from the embryonic tissues of red tilapia. The optimized modified technique for getting the best parameters for chromosome preparations in red tilapia were; colchicine concentration 0.01% for 4-6 hours, hypotonic treatment of 0.56% KCl for 40 minutes, carnoy's fixation 3:1 and 10% Giemsa stain prepared in 0.01 M phosphate buffer (pH 7) for 20 minutes. The efficacy of morphometric indices of the erythrocytes as a tool to differentiate diploids and triploids was confirmed using chromosome preparations from the same fish in the present study. It was proven that the erythrocyte measurements can be used successfully for distinguishing the ploidy and all the morphometric indices of the erythrocytes of diploid and triploid red tilapia showed significant results ($P<0.005$). The mean values of cellular major and

minor axis and cellular surface area were; 10.43±0.51 µm; 6.69±0.36 µm and 54.70±4.05 μm^2, respectively in diploids, whereas in triploid (percentage increments) were; 13.32±0.37 µm (27.7%); 7.46±0.44 µm (11.5%) and 77.70±6.28 (42.0%) μm^2, respectively. Similarly in diploid, the nuclear major and minor axis and surface area were; 4.47±0.34 µm; 2.50±0.25 µm and 9.08±1.45 μm^2, respectively as compared to 5.89±0.38 µm (31.7%), 2.93±0.34 µm (17.2%) and 13.66±2.29 μm^2 (50.4%), respectively, in triploid individuals. The cellular, nuclear and cytoplasmatic volumes in diploids were; 245.56±29.39 μm^3, 15.89±4.25 μm^3 and 229.68±26.95 μm^3, respectively whereas in triploids 390.67±51.69 μm^3 (59%); 26.78±6.53 μm^3 (68.5%) and 362.81±47.60 μm^3 (57.9%), respectively.

The main focus of the present study was to optimize the best treatment protocol for triploidy induction in red tilapia using cold- and heat-shocks. My study has successfully demonstrated that the best timing to prevent the release of second polar body was at 4 minutes after fertilization of the eggs of red tilapia. The best treatments for triploidy induction in red tilapia were optimized as 41° C temperature with 5 minute duration for 4 minute after fertilization using heat-shock which resulted in 91.8±0.4% triploidy with 68.7±2.4% survival. However, for cold-shock the shock duration of 30 minutes at temperature 9° C applied for 4 minutes after the fertilization was the best which yielded 98.7% triploidy and 82.1% triploidy yield. The comparison of the growth performance between the diploid (control) and triploids produced by the best treatment procedure optimized in the present

study, showed relatively better growth in triploid groups. At the end of 120 days of culture period, the maximum average weight (215.5±3.61 g) and percentage increment (39.63%) was observed in fish produced by heat-shock, followed by cold-shock (192.7±2.68 g, 37.05%) and control group (191.9±1.74 g, 34.13%) respectively. The von Bertalanffy's growth equation applied in the study described the growth of red tilapia adequately where the maximum attainable weights were; 650 g (heat-shocked triploids), 490 g (cold-shocked triploids) and 440 g in control group. The percentage of the males in triploid population was the highest (82.9%) in heat-shocked group, followed by cold-shock group (54.8%) and the lowest for control group (50%). Histological analyses of testis and ovarian tissues of all the treatment groups of red tilapia showed clear evidence of abnormal gametogenesis in triploid groups which further confirmed that the triploids were sterile.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGHASILAN TRIPLOID IKAN TILAPIA MERAH [*Oreochromis mossambicus* (Peters, 1852) X *Oreochromis niloticus* (Linnaeus, 1758)]

PRADEEP P.J.

FEBRUARY' 2011

Pengerusi : **Professor Madya Anuar bin Hassan, Ph.D.**
Ahli : **Dr. Anil Chatterji, Ph.D.**
Dr. Shahreza Mohammad Sheriff, Ph.D.

Institut : **Institut Akuakultur Tropika**

Tilapia ialah spesis ikan yang bertabur luas di kawasan tropika dan subtropika serta mempunyai ketahanan yang tinggi terhadap pelbagai keadaan persekitaran. Ciri-ciri ini membuatkan ia lebih diminati untuk diternak. Ikan merah tilapia [*Oreochromis niloticus* (Linnaeus, 1758) x O. mossambicus (Peters, 1852] adalah spesis hibrid subur yang dihasilkan melalui pembiakkan terpilih berterusan. Pengurangan dalam tumbesaran pada ikan tilapia disebabkan oleh pembiakan yang tidak terkawal di dalam persekitaran yang terhad adalah suatu fenomena yang bersifat merugikan. Penghasilan triploid yang mandul telah dilihat sebagai kaedah yang berkesan untuk mengatasi masalah pembenihan ikan dan tilapia merah digunakan dalam kajian ini.

Dalam kajian ini, protocol pembikan seragam yang betul untuk triploidy telah pun dihasilkan. Dos hormon HCG pada kadar 1500 IAU/kg didapati berkesan dalam merangsang pembiakan ikan merah tilapia di bawah keadaan terkawal. Kesediaan induk untuk membiak dinilai melalui pemerhatian perilaku mengawan. Bagi meningkatkan kadar penetasan telur tilapia, satu sistem pengeraman menggunakan kaedah sistem air pusingan yang cekap telah direka dan ia boleh menghasilkan peratus kemandirian dan penetasan yang tinggi bagi ikan merah tilapia. Kaedah karyotip digunakan bagi mengkaji ketepatan tahap ploidi. Teknik ini telah memberikan serakan kromosom metafasa yang baik bagi kromosom dari tisu embrio ikan merah tilapia. Rawatan menggunakan kepekatan colchicines 0.01% selama 4-6 jam dan kepekatan 0.56% KCl selama 40 minit dengan menggunakan larutan Carnoy pada nisbah 3:1 merupakan parameter terbaik bagi penyediaan kromosom. Bagi pewarnaan yang baik, pewarnaan Giemsa 10% yang disediakan dalam 0.01M penimbal fosfat (pH 7) selama 20 minit merupakan parameter yang paling berkesan. Keberkesanan perbezaan ciri morfometrik bagi eritrosit ikan merah tilapia triploid dan diploid dilakukan dan di dapati mempunyai perbezaan yang signifikan secara statistik ($P<0.005$). Bagi ikan diploid, nilai min bagi panjang paksi sel major, panjang paksi sel minor dan luas permukaan sel adalah 10.43±0.51 µm, 6.69±0.36 µm dan 54.70±4.05 μm^2 masing-masing manakala nilai min bagi panjang paksi sel major, panjang paksi sel minor dan permukaan sel bagi triploid (peratus peningkatan) ialah 13.32±0.37 µm (27.7%); 7.46±0.44 µm (11.5%) and 77.70±6.28 (42.0%) μm^2 masing-masing. Bagi diploid, nilai panjang paksi nukleus major, panjang paksi nukleus minor dan luas permukaan nukleus

ialah 4.47±0.34 µm; 2.50±0.25 µm dan 9.08±1.45 μm^2 masing-masing berbanding dengan 5.89±0.38 µm (31.7%), 2.93±0.34 µm (17.2%) dan 13.66±2.29 μm^2 (50.4%) masing-masing bagi individu triploid. Jumlah isipadu sel, nukleus dan sitoplasmik bagi diploid ialah 245.56±29.39 μm^3, 15.89±4.25 μm^3 dan 229.68±26.95 μm^3 masing-masing manakala bagi triploid, nilai tersebut ialah 390.67±51.69 μm^3 (59%); 26.78±6.53 μm^3 (68.5%) dan 362.81±47.60 μm^3 (57.9%) masing-masing.

Kejutan panas dan sejuk adalah fokus utama kajian bagi penyediaan protokol untuk ikan tilapia bagi induksi triploid.Keputusan kajian ini menunjukkan bahawa masa untuk menghalang pelepasan badan polar telur yang kedua ialah 4 minit selepas persenyawaan. Rawatan kejutan suhu pada 41°C menghasilkan 91.8±0.4% triploid dengan 68.7±2.4% kemandirian apabila rawatan kejutan dimulakan 4 minit selepas persenyawaan selama 5 jangkamasa minit pendedahan. Jangkamasa kejutan selama 30 minit pada suhu 9 °C yang dikenakan 4 minit selepas persenyawaan memberi keputusan yang terbaik menghasilkan 98.7% triploid dengan 82.1±1.6% triploid terhasil. Perbandingan tumbesaran antara diploid (kawalan) dan triploid menunjukkan bahawa triploid mempunyai tumbesaran yang bagus. Peratus peningkatan tumbesaran yang maksimum (39.63%) dilihat pada ikan triploid yang terhasil secara suhu panas berbanding dengan 192.7±2.68 g (37.05%) pada ikan triploid yang terhasil secara kejutan suhu sejuk dan 191.9±1.74 g (34.13%) pada ikan kawalan selepas tempoh ternakan selama 120 hari. Formula pertumbuhan von Bertalanffy's menghuraikan tumbesaran

ikan merah tilapia dengan terperinci di mana nilai berat maksimum yang boleh dicapai adalah 650g (triploid yang terhasil secara kejutan suhu panas), 490g (triploid terhasil secara kejutan suhu sejuk) dan 440 g pada ikan kawalan. Peratus ikan jantan di dalam populasi triploid adalah tertinggi (82.9%) pada ikan triploid yang terhasil secara kejutan suhu panas, 54.8% diperolehi pada ikan triploid yang terhasil secara kejutan suhu sejuk manakala kawalan mempunyai jumlah jantan yang paling kurang (50%). Kajian histology terhadap tisu testis dan ovari ikan tilapia bagi semua kumpulan rawatan menunjukkan bukti gametogenesis luar biasa bagi kumpulan triploid yang menunjukkan kumpulan triploid adalah mandul.