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GROWTH PERFORMANCE MORPHOMERISTIC AND GENETIC CHARACTERIZATION OF CROSSBREEDING BETWEEN GIFT AND UPM RED TILAPIA

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By

NWACHI OSTER FRANCIS

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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February 2018

Chairman : Yuzine B Esa PhD
Faculty : Agriculture

This study was carried out to evaluate the possibility of producing hybrids of different crossing between Genetically Improved Farm Tilapia (GIFT) and UPM red tilapia (*Oreochromis* sp.) aiming to produce reddish coloured version of new generations of a first filial (F_1) reciprocal (nF1), second filial (F_2), backcross (BcF_1) and its reciprocal ($nBcF_1$). The strains produced were assessed for possibility of inheriting the phenotypic traits of UPM red tilapia and qualitative traits of GIFT, their growth performance in aquarium and fish cages. To prevent misidentification for stock management, morphomeristic traits and mitochondria control region (mtDNA) analyses were carried out for classification and identification.

In experiment one, broodstock of UPM red tilapia (Pure 1) and GIFT (Pure 2) were selected based on standard method and paired into the ratio of female to male (3:1) to produce F_1 and reciprocal nF1. Offspring of F_1 were then selected and used as the parents stock to produce F_2 and backcross by mating (UPM red tilapia x F_1) to give BcF_1 and (GIFT x F_1) to give $nBcF_1$. The inheritance trait of body colour of different crossing produced four different phenotypic colourations; reddish colour, red with dark blotches, wild type (dark) and mixed red-wild type of F_1, nF_1, Pure 1 and Pure 2 with various percentages of reddish colour phenotype. The F_2, BcF_1 and $nBcF_1$ produced faded wild type, red with dark blotches, red with dark posterior patches and mixed wild type. The highest percentage (64.61%) of reddish colour type were produced from the F_1. The successful of the crosses were determined based on the time of fry were produced. Results showed nF_1 was produced at a mean time span of 21.93 days after four successful trials compared to 14.53, 14.72 and 14.2 days by the Pure 2, Pure 1 and F_1. Similarly the F_1 outperform the others by producing a mean total fry of 154.65 compared to Pure 1, Pure 2, and nF_1 which were 148.16, 149.37, and 137.77.

There is high level of possibility that the body colour of male influenced the female attracted to male based on shorter number of days for the successful of spawning.

In experiment two, the growth performance and feed conversion ratio (FCR) of Pure 1, Pure 2, F_1 and BcF_1 were assesses. The F_1 and BcF_1 were selected among the other strains that were produced due to ability of these strains producing higher percentage of reddish colour of fry. The experiment was conducted in two culture systems which were aquarium and fish cages. The F_1, BcF_1 and their base parent (Pure 1 and Pure 2) were stocked at a stocking density of $0.1L^{-1}$ with mean body weight and total length of 7.84 g and 7.77 cm in aquarium, 300 tails per cage (3.5m×1.2m×3.5 m) in cages with size of 8.19 g at 8.46 cm in triplicates. Growths were presented in a mean body weight and recorded 332.15 g for F_1 compared to 264.69 g, 271.42 g and 320.02 g with the values of FCR 1.1 for pure 1, pure 2 and BcF_1, respectively. In aquarium, weight gain of F_1 was 40.55 g compared to 33.46 g, 40.87 g and 39.15 g with FCR of 1.38, 1.29 and 1.01 for Pure 1, Pure 2 and BcF_1 respectively. The F_1 has the highest FCR in both aquariums (1.0) and cages (1.08). Besides, F_1 and BcF_1 in both systems showed higher growth rate compared to their base parents at a culture days of 52 in aquarium and 150 days in cages.

In experiment three, morphomeristic components of Pure 1, Pure 2, F_1 and BcF_1 were measured based on truss network protocol by measuring 20 morphometric and five meristic components to predict and classified each strain using discriminate analysis (DFA) and principal component analysis (PCA) using SPSS and Unscramble$^{@}$X statistical tool software. The discriminate coefficient score of upper lip length recorded the highest (.825) among the others, which is for predicting the group at which the strains belong. The pelvic length, lower lip length and cheek depth were at .690, .629, and. 525, while their meristic counts for dorsal fin and anal fin were .993 and .992. Eigen values of three Functions derived were .721, 3.202 and 150.406 in morphometric, and .313 and 1.408 in two Functions for meristic counts as the best predicting for discrimination for each cross. The morphometric predicting components loaded 52% at PC1, 11% for PC2, and 59% at PC1 and 29% for PC2 for the meristic component. The random prediction values for morphometric and meristic were 80, 72, 92 and 96% and 96, 72, 60 and 12% for Pure 1, Pure 2, F_1 and BcF_1 respectively. This shows that morphometric traits could be used in assigning all the strains to their base parents. However based on meristic traits, BcF_1 could not be assigned to their base parents. It can infer that 93% of the prediction correlate correctly classify to the strain using their morphometric trait, while prediction level resulted to correct placing at 60% of meristic traits.

In experiment four, identification of Pure 1, Pure 2 their F_1 and BcF_1 was carried out to evaluate the genetic variability and phylogeny of each strain using mtDNA. Genomic DNA was extracted from each strain (n=26) from 25 mg of muscles tissue using Promega USA test kit. The extracted DNAs were viewed in a gel electrophoresis under ultraviolet light using Gel doc XR system Pc and Mac from USA for the qualitative validation. The extracted DNAs were subjected to polymerise chain

reaction (PCR) at their mitochondria control region using ORMT-F 5'-CTAACTCCCAAAGCTAGGAATTCT-3'and ORMT-R 5'-CTTATGCAAGCGTCGATGAAA-3'primer at pre-denaturation step for 94^0c at 3 min in a cycle of 35, denaturation at 94^0c for 30 s, annealing at 54^0C for 40 s, extension at 72^0C for 40 s and a final extension step of 72^0C for 10 min.

The multiple sequences were aligned with clustalW in BioEdit software. Evolutionary analysis of each strain was conducted with MEGA software using neighbour-joining (NJ) tree, maximum parsimony, and maximum likelihood with control region sequence and out-groups from the same family; (*Oreochromis. niloticus* (Genbank accession number: KC 811379.1) and (*Tilapia zilli* (Genbank accession number: AF 3288531). This was constructed with the Kimura 2 parameter distance model. The branching order of the tree was tested by bootstrapping at 1050 replicates data. Pure 1, Pure 2, F_1 and BcF_1 were found varied based on the variation in the degree of polymorphism. Pure 2 has the highest nucleotide diversity (0.0302) with a total of 4 control regions while the other stains have one single region. Pure 2 has the highest haplotype diversity (0.2933) although, out of five of the haplotype that were observed in the samples of Pure 1, F_1 and BcF_1 shared one between them. It is of note that the phylogenetic analysis in this study verified monophyletic relationship based on the high value of consistency index (0.805556) and retention index (0.758621). The high level of relatedness with consistence overlap and clustering are linked to the low genetic distance (0.1). Despite the closeness of the strain to each other, their control region analysis reveal a uniqueness that was specific to individuals and could be used for identification

Overall F_1 and BcF_1 that are reddish in colour were able to produce with higher FCR recorded than their parents. Improvement of mean weight gain revealed heterosis of the F_1 and BcF_1 as a result of qualitative traits from Pure 2. Pure 1 and Pure 2 females showed more attracted to male of same colour or male of reddish colouration during mating. Lastly, every strain could be assigned to their base parents using their predicting components (morphomeristic), and the mtDNA control region was found useful in validated the identity of each strains.

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

PRESTASI PEMBESARAN, MORFOMETRIK DAN PERINCIAN GENETIK BAGI KACUKAN DI ANTARA GIFT DAN TILAPIA MERAH UPM

Oleh

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Februari 2018

Pengerusi : Yuzine B Esa PhD
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Kajian ini dijalankan untuk menghasikan kacukan yang berbeza diantara Genetically Improved Farm Tilapia (GIFT) dan tilapia merah UPM (*Oreochromis* sp.) bertujuan untuk menghasilkan generasi baru iaitu generasi filial pertama (F_1), silang kacuk (nF1), generasi kedua filial (F_2), timbal balas (BcF_1) and silang timbal balas ($nBcF_1$) yang berwarna merah. Generasi baru yang terhasil di nilai berdasarkan keupayaan mewariskan fenotipik baka daripada tilapia merah UPM dan kualiti baka daripada pada GIFT, prestasi pembesaran di dalam akuarium dan sangkar. Untuk mngelakkan kekeliruan bagi pengurusan stok, analisis baka morfometrik dan bahagian tetap mitokondria dijalankan untuk pengelasan dan pengenalpastian.

Bagi ujikaji kedua, induk tilapia merah UPM (induk asal 1) dan GIFT (induk asal 2) dipilih berdasarkan kaedah yang ditetapkan dan dipasangkan dengan nisbah betina kepada jantan (3:1) untuk menghasilkan kacukan balik nF1. Generasi pertama daripada F_1 dipilih semula sebagai induk untuk menghasilkan F_2 dan timbal balas dengan mengacukkan (tilapia merah UPM x F_1) untuk menghasilkan BcF_1 dan (GIFT x F_1) untuk menghasilkan $nBcF_1$. Baka bagi pewarisan warna badan bagi kacukan yang berbeza menghasilkan empat fenotipik warna yang berbeza; merah, merah dengan tompok gelap, warna liar (gelap), dan campuran warna merah-liar bagi F_1, nF_1, induk asal 1 and induk asal 2 dengan peratusan fenotip warna yang pelbagai.. Bagi F_2, BcF_1 dan $nBcF_1$ menghasilkan warna liar yang pudar, merah dengan tompok gelap, warna merah dengan warna gelap di bahagian hadapan dan campuran warna liar. Peratusan tertinggi (64.61%) bagi warna jenis merah dihasilkan daripada F_1. Kejayaan kacukan ditentukan berdasarkan masa anak ikan dihasilkan. Keputusan menunjukkan nF_1 dihasilkan pada purata tempoh masa 21.93 dalam tempoh empat kejayaan percubaan berbanding 14.53, 14.72 dan 14.2 bagi induk asal 1, 2 dan F_1. Begitu juga, F_1 lebih menonjol berbanding yang lain dengan menghasilkan purata jumlah anak ikan

154.65 berbanding induk asal 1, 2, dan nF_1 iaitu 148.16, 149.37 dan 137.77. Terdapat kemungkinan warna badan induk jantan mempengaruhi pemilihan bagi induk betina tertarik kepada induk jantan berdasarkan jumlah hari yang lebih pendek bagi kejayaan pengawanan.

Dalam kajian kedua, prestasi pembesaran dan kadar penukaran makanan (FCR) bagi induk asal 1, induk asal 2, F_1 dan BcF_1 dinilai. F_1 dan BcF_1 dipilih di antara kacukan yang lain kerana keupayaan menghasilkan peratusan anak ikan yang berwarna merah. Kajian di jalankan dalam dua sistem yang berbeza iaitu akuarium dan sangkar. Kacukan F_1, BcF_1, induk asal 1 dan 2 di kumpulkan pada kepadatan $0.1L^{-1}$ dengan purata berat dan panjang 7.84 g dan 7.77 sm di dalam akuarium, dan 300 ekor per sangkar (3.5m×1.2m×3.5 m) dengan saiz 8.19 g dan 8.46 sm dalam tiga replikat. Pembesaran diwakili oleh purata berat badan telah merekodkan 332.15 g bagi F_1 berbanding 264.69 g, 271.42 g dan 320.02 g dengan nilai FCR adalah 1.1 bagi induk asal 1, induk asal 2 dan BcF_1. Kenaikan berat bagi F_1 adalah 40.55 g berbanding 33.46 g, 40.87 g dan 39.15 g dengan nilai FCR adalah 1.38, 1.29 dan 1.01 bagi induk asal 1, induk asal 2 dan BcF_1. Kacukan F_1 memiliki nilai FCR tertinggi di kedua-dua system iaitu akuarium (1.0) dan sangkar (1.08). Di samping itu, kacukan di kedua-dua system menunjukkan kadar pembesaran yang lebih tinggi berbanding induk asal pada hari pengkulturan ke 52 di dalam akuarium dan 150 hari di dalam sangkar.

Dalam kajian ketiga, komponen morfometrik bagi induk asal 1, induk asal 2, F_1 dan BcF_1 di ukur berdasarkan kaedah jaringan truss dengan mengukur 20 morfometrik dan lima komponen metrik untuk meramal dan mengelaskan setiap kacukan menggunakan analisis diskriminan (DFA) and analisis prinsip komponen (PCA) menggunakan perisian statistik SPSS dan Unscramble$^{@}$X. Perbezaan skor koefisin bagi panjang bibir mulut atas merekodkan nilai yang paling tinggi (.825) di antara yang lain bagi meramalkan kumpulan mana kacukan tersebut berada. Panjang pelvik, bibir bawah dan pipi depan adalah .690, .629, dan .525, manakala bilangan meristik bagi sirip dorsal dan anal adalah .993 and .992. Nilai Eigen bagi tiga Fungsi yang dihasilkan, adalah 0.721, 3.202 dan 150.406 bagi morfometrik, dan .313 dan 1.408 bagi dua Fungsi untuk meristik sebagai jangkaan terbaik untuk membezakan setiap kacukan. Jangkaan komponen morfometrik berada 52% pada PC1, 11% pada PC2, dan 59% pada PC1 dan 29% pada PC2 bagi komponen meristik. Nilai jangkaan secara rawak bagi morfometrik dan meristik adalah 80%, 72%, 92% dan 96% , 96%, 72%, 60% and 12% bagi induk asal 1, induk asal 2, F_1 dan BcF_1. Ini menunjukkan morfometrik boleh digunakan untuk mengelaskan kesemua kacukan kepada induk asal. Walaubagaimanapun, berdasarkan meristik, BcF_1 tidak boleh dikelaskan kepada induk asal. Ini boleh disimpulkan bahawa 93% jangkaan yang berkaitan boleh mengelaskan kacukan secara tepat menggunakan pewarisan morfometrik, manakala tahap jangkaan menghasilkan 60% untuk meletakkan pewarisan meristik secara tepat.

Dalam kajian keempat, pengenalpastian induk asal 1, induk asal 2, F_1 dan BcF_1 dijalankan untuk menilai kepelbagaian genetik dan filogenetik setiap kacukan menggunakan mtDNA. Genom DNA yang diekstrak daripada setiap kacukan (*n*=26)

daripada 25 mg tisu otot menggunakan kit ujian Promega USA. DNA yang telah diekstrak di lihat menggunakan kaedah elektroforesis di bawah cahaya ultra unggu menggunakan Gel doc XR system Pc and Mac daripada USA untuk pengesahan kualiti. DNA yang telah diekstrak menjalani tindakan berantai polimerase (PCR) pada bahagian kawalan mitokondria menggunakan primer ORMT-F 5'-CTAACTCCCAAAGCTAGGAATTCT-3'dan ORMT-R 5'-CTTATGCAAGCGTCGATGAAA-3' pada langkah awal *denaturation* pada 94^0c selama 3 min pada kitaran 35, *denaturation* pada 94^0C selama 30 s, *annealing* pada 54^0C selama 40 s, pemanjangan pada 72^0C selama 40 s dan pemanjangan akhir pada 72^0C selama 10 min.

Jujukan berbagai di selaraskan dengan perisian clustalW in BioEdit. Analisis evolusi bagi kacukan dijalankan menggunakan perisian MEGA menggunakan pokok jiran yang bergabung (NJ), parsimony maksimum dan kemungkinan maksimum dengan bahagian jujukan kontrol dan kumpulan-luar daripada keluarga; (*Oreochromis. niloticus* (Nombor akses Genbank: KC 811379.1) dan (*Tilapia zilli* (Nombor akses genbank: AF 3288531). Ianya dibina menggunakan parameter Kimura 2 jarak model. Cabang Order bagi setiap pokok diuji dengan data bootstrapping pada 1050 replikat. Didapati induk asal 1, induk asal 2, F_1 dan BcF_1 berbagai berdasarkan kepelbagaian tahap polimorfisam. Induk asal 2 memiliki kepelbagaian nukleotida (0.0302) yang paling tinggi dengan jumlah empat kawasan kawalan manakala kacukan lain memiliki satu bahagian kawalan. Induk asal 2 memiliki kepelbagaian haplotip ynag paling tinggi walaupun daripada Lima haplotip yang diperhatikan di dalam sampel induk asal 1, F_1 dan BcF_1 berkongsi salah satu di antaranya. Di perhatikan analisis filogenetik di dalam kajian ini menjelaskan hubungan *monophyletic* berdasarkan nilai indek keseimbangna yang tinggi (0.805556) dan indek penahanan (0.758621). Tahap keberkaitan yang tinggi dengan seimbang bertindih dan kluster dihubungkan kepada jarak genetic (0.1) yang rendah. Walaupun terdapat kedekatan setiap kacukan di antara satu sama lain, analisis kawasan kawalan mendedahkan keunikan yang spesifik untuk setiap individu dan boleh digunakan untuk pengenalpastian.

Secara keseluruhanya, F_1 dan BcF_1 yang berwarna merah mampu dihasilkan dengan FCR yang tinggi direkodkan daripada induk asal mereka. Peningkatan purata berat mendedahkan heterosis bagi F_1 dan BcF_1 sebagai hasil baka kualitatif daripada induk asal 2. Induk asal 1 dan induk asal 2 menunjukkan tarikan terhadap induk jantan yang memilik warna yang sama atau warna merah semasa pengawanan. Akhirnya, setiap kacukan boleh di kelaskan kepada induk asal berdasarkan komponen jangkaan (morfometrik) dan mtDNA kawasan kawalan di dapati berguna dalam mengesahan identiti setiap kacukan.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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µl	Micro litre
bp	Base pair
cm	Centimetre
dH20	Double distilled water
dNTPs	Deoxynucleotide triphosphate
mg	Milligram
min	Minute
ml	Millilitre
mM	Millimolar
PCR	Polymerase Chain reaction
s	Second
F_1	First filial generation
nF_1	Reciprocal first filial generation
F_2	Second filial generation
BcF_1	Backcross
$nBcF_1$	Reciprocal backcross
nDNA	Nuclear DNA
mtDNA	Mitochondria DNA
DNA	Deoxyribonucleic acid
Hd	haplotype diversity
H	haplotype
Ω	nucleotide diversity
SPR	Subtree-Pruning-Regrafting

v	volt
NCBI	National Centre for Biotechnology Information
sq km	Square kilometre
Mt	Metric ton
FCR	Feed conversion ratio
BLAST	Basic Local Alignment Search Tool
SNP	Single nucleotide polymorphism
RAPD	Random Amplification of Polymorphic
AFLP	Amplified Fragment Length Polymorphism

CHAPTER 1

INTRODUCTION

1.1 Research Background

Aquaculture is the farming production of aquatic organisms such as fish, molluscs, crustaceans and aquatic plants in a water bearing receptacle which includes; earth ponds, concrete tanks and raceways. In this situation the farmer or culturist makes effort to replicate or create as much as possible the condition nearest to the natural environment (Overturf, 2009). Tilapia is a fresh water fish belonging to the family Cichlidae and the name 'Tilapia' was given by African Bush man meaning 'fish' (Trewavas, 1982). Tilapia is referred to as 'aquatic chicken' because of its flexibility to reproduce in captivity, they are source of animal protein that is needed in poorer countries and hence linked with a potential candidate for providing the needed food security especially in the developing countries who are struggling with the need to provide the recommended per caput intake of protein per day (Canonico et al., 2005).

Tilapia is also a common name that is used for fishes of genus tilapia. Trewavas (1982) reclassified several hundred of tilapia into three genera: *Oreochromis*, *Sarotherodon* and *Tilapia*. The *Oreochromis* are maternal mouth brooders (the female fish take up the fertilised egg in the mouth incubate the egg until hatching takes place they protect the swim up fry until they can fend for themselves). The *Sarotherodon* are paternal mouth brooders while the genus *Tilapia* are the substrates spawners, they lay their eggs on substrates until hatching. The most reared species is the *Oreochromis niloticus* while *Oreochromis mossambicus* was the first tilapia to be widely distributed; it is given the name Java tilapia because of its adoption as food fish in Indonesia (Russell et al., 2012). Tilapia is a native of Africa although introduced to the tropical and even the subtropical region of the world. With the aim of increasing food production which is important for food security, recreational purposes and ample opportunities for angler to hunt for a fish that avoid been caught, aquatic weed control and for research purposes (Canonico et al., 2005).

Strain enhancement correlated to selection of traits and donation of selected traits to a recurrent pair. With the hope that final outcome (hybrid) will have enough of the wanted traits by exploiting the variation of the desirable trait (Kumar et al., 2017; Springer and Schmitz, 2017). Strains could be improved by addiction of phenotypic traits of interest or quantitative characteristics. The process of selection and hybridisation actually served as a means of getting this done. Hybridisation could be between species (interspecific) or genera (intergeneric) with the main aim of modelling strain based on requirement. Growth improvement were the focus of most aquaculture species however, this comes with reduced culture time, reduced maintenance period and final feed conversion ratio that will invariably amount to higher production from the technical resources from the farm.

Hybridisation is an important tool for stock improvement, various additions could be made at pairing stock with different traits. Most stock improvement processes start with hybridisation coupled with selection. Mating of species with different traits create the modern day strains with special abilities in terms of colour, growth and fillet quality and quantity. The 'Genetically Improved Farmed (GIFT) is the carefully selected and hybridized stock from work carried out by the World Food institute with the aim of producing tilapia strain that would not fail the culturist during production however, in adopting this strain of genetically improved fish, the receiving countries were advised to study the ecology of their water body so that it will not affect the biodiversity in a negative way or develop local strain using the GIFT technology. The results of such research and studies by Haque et al. (2016); Khaw et al. (2016) and Hamzah et al. (2014) on the superior traits found in GIFT give rise to the emergence of New GIFT and GIFU in China the 'Akosombo tilapia' in Ghana, all these new developments point toward improving production of farmed fish that express the traits of interest.

Universiti Putra Malaysia through the process of selection and hybridisation also developed a relatively fast growing strain UPM red tilapia but with an inferior growth rate compared to GIFT. Production of red tilapia started in Taiwan in the 1960s (Kuo, 1989), success recorded in terms of increased acceptability rate brought about the use of different strain of *O. mossambicus* and *O. niloticus* (Romana-Eguia et al., 2004). The first emphasis on red tilapia were based on colour rather than size hence the production of Florida red tilapia by crossing *Oreochromis hornorum* with red gold *O. mossambicus*. Wohlfarth et al. (1990) infer that most red tilapia do not breed true hence, a need to understand the allele of parent based population.

The introduction of red tilapia act as a turning point in the way Malaysians see tilapia. The first tilapia introduced into Malaysia was *O. mossambicus (*black in colour) the colour and the fact that it quickly colonize any water body it entered made it unattractive. However, the introduction of red tilapia and the relatively fast growing *O. niloticus* makes its favoured by culturist and a commercial success in Malaysia (Department of Fisheries, 2015; Ang et al., 1989). This opens a need to produce a fish that will match the need of the people in terms of reddish colour and fast growing. Breeding program in aquaculture is related to the express phenotype and growth, increase in weight and length in short time indicate short production circle and culture advantage. Good feed conversion rate is an important tool used in determining the success of any new strain (Omasaki et al., 2017). In culture, feed takes up to 50-70% of production cost which made it an input with the largest recurrent expenditure (Chau et al., 2013); (Verdal et al., 2017).

Genetic improvement linked with growth is associated with the ability of the developed strain to convert feed to flesh. Weight gain at culture gives insight on the feed conversion ability of strain. Omasaki et al. (2017) and (Neely et al. (2008) reported the use of feed conversion ratio and weight gain as a tool in measuring success in selection and genetic improvement. The use of less feed to achieve same

goal increase the efficient use of feed, feed efficiency as opined by Martins et al. (2011) as the ratio of feed intake to the weight gain and the use of less feed to produce same output. Hence, there is a need to produce a fish that will have good feed conversion ratio and appreciable weight gain at culture

Stock improvement program end in the production of slightly different strain from the parent however, their effective management is important if effect of gene exchange and direction of gene flow is known because the knowledge of interaction of species within a population enhance plan for breeding program. Similarly, genetic basis for the manifestation of a particular phenotype could be identified by their morphomeristic traits; measured distances (morphometric) and count of their appendages (meristic). Measured component can be used to predict the family of the strain; cultured fish from wild stock and stock identification (Rawat et al., 2017; Geiger et al., 2016; Okomoda et al., 2016). A study by Turan et al. (2006) reveal that the simplest tool for classifying fish is through statistical method although, the use of molecular method like random amplification of polymorphic DNA (RAPD) and Mitochondria control region (mtDNA) was made to increase the accuracy of prediction. The first point of contact and identification is on the field hence there is a need for first contact identification with the use of their morphomeristic traits before further investigation for the purpose of confirmatory test using their molecular traits

1.2 Problem Statement

The rate at which tilapia is accepted is influenced by the expressed phenotypic traits as a result of the willingness by consumers who attached special interest on the reddish coloured tilapia that has the ability to reach good size at culture. Producing a fish with quantitative traits of GIFT strain but reddish in colure like the UPM red tilapia will not only result to the favoured reddish coloured but a fish that will reach market size during culture. A number of time fish species has been wrongly classified at first contact on the field, resulting in obtaining wrong data. Misidentify strain create problem for the scientist and culturist. Differentiation of strain by their morphomeristic and genetic variability enhance the chances of correct stock identification.

1.3 Justification of Study

Tilapia is an important fish of interest because of its ability to provide cheap protein at quickest possible time. The main constrain to acceptance is based on the demand for a specific phenotypic traits (reddish colour). Consumers placed value on the reddish coloured tilapia compared to the wild type (black) despite the fact that they have same basic need in terms of feed and management procedure at culture. This placed size and colour of interest as condition that is important for the fish to maintain its wide appeal to the populace and a need to produce fish that will reach market at the same time express reddish colour which is the phenotypic traits of interest at culture. Malaysia and some other countries are willing to pay premium price for reddish

coloured tilapia compared to their wild type. History of tilapia introduction to countries like Malaysia placed colour and body size as a factor that increases the change of attitude from regarding tilapia as a fish to be eliminated before stocking as folk's fish which is highly valued food fish.

1.4 Objectives

The general objective of this study is to develop a strain of tilapia that can reach market size at culture and express the phenotypic traits of interest (reddish)

The specific objectives are:

1) To produce and examine the proportion of reddish coloured hybrid (F_1, F_2), their reciprocals (nF_1) and backcross (BcF_1 and $nBcF_1$), the preference of female fish to conspecific and or novel coloured (reddish) male from the cross between GIFT and UPM red tilapia.
2) To evaluate the performance of the reddish coloured hybrid and backcross in two culture receptacles (aquarium and fish cage) based on food conversion ratio and weight gain.
3) To determine morphomeristic traits that could be used to positively identify the parents from the offspring in isolation.
4) To examine the phylogenetic and genetic variability between reddish coloured hybrid (F_1), backcross (BcF_1) to their parents (GIFT and UPM red tilapia).

1.5 Scope of Study and Limitations

The study exclusively involved production of hybrids and backcross from base parent stock of UPM red tilapia and GIFT and selection of filial generations that expressed the reddish colour phenotypic traits of interest.

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