UNIVERSITI PUTRA MALAYSIA

EFFECTS OF SELECTED HERBAL EXTRACTS ON SEXUAL MATURITY, GROWTH PERFORMANCE AND IMMUNE RESPONSE OF NILE TILAPIA (Oreochromis niloticus Linnaeus)

ZANA HAMA GHARIB KAREEM

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By

ZANA HAMA GHARIB KAREEM

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

April 2015

DEDICATION

To my father and my son; my father's dream was seeing me going abroad for education and my dream is seeing my son doing the same.

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

EFFECTS OF SELECTED HERBAL EXTRACTS ON SEXUAL MATURITY, GROWTH PERFORMANCE, IMMUNE RESPONSE OF NILE TILAPIA (*Oreochromis niloticus* Linnaeus)

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Chairman: Associate Professor Yasser Mohamed Abdelhadi, PhD
Faculty: Agriculture

Precocious puberty is a serious problem, which prevents fish from reaching its full aquaculture potential and is regarded as a limitation to the Nile tilapia farming. Delaying sexual maturity of Nile tilapia is crucial in order to control the number of unwanted fish in the pond leading to solving the overcrowding, food shortage, diseases and growth problems. The aim of this study was to investigate the effect of four screened herbs extracts (Neem leaves (*Azadirachta indica*), Asthma weed (*Euphorbia hirta*), Papaya seed (*Carica. papaya*) and Camphor bark (*Cinnamomum camphora*)) on the sexual maturity, growth performance and immune response of Nile tilapia.

Thus a 90 days feeding trial (experiment I) was conducted on gonado/somatic index (GSI), histopathological examination of the gonads (as an indication of gonadal development), growth performance, feeding efficacy, body indices (hepato/ somatic index (HSI), spleeno/somatic index (SSI), blood parameters as an indication of immune response of Nile tilapia. In this experiment the fish fed *ad libitum* on diets supplemented with different crude plant extracts from *C. camphora*, *E. hirta*, *A. indica*, and *C. papaya* at 2 g /kg diet and a control diet. This experiment was followed by a 14 days challenge test (experiment II) with a virulent Gram+ve bacterial strain of *Streptococcus agalactiae*. All treatments and control group were triplicated and each treatment consisted of 30 fish (Total body weight of 90-110 g and Total Length of 18-20 cm). The most effective herbal extract according to results of experiments I and II, which was *C. papaya*, was selected and applied in experiment III in a different doses (0.5, 1, 2 g/kg diet) and two different periods of feeding (3 and 20 weeks) on 420 sac-larvae (3 days old) of Nile tilapia with initial weight of 0.015 g and total length of 9.43 mm. The sac-larvae were equally and randomly distributed into 21 glass tanks (20 sac-larvae/ aquarium). Results of this study showed that *C. papaya* extract 2 g/kg diet was the most effective herb on delaying gonadal development and sexual maturity in both males and females Nile tilapia. *C. papaya* extracts as well as significantly increased the growth performance

it did not affect the immune response of Nile tilapia. Similarly, dietary *A. indica* was significantly reduced the GSI of the fish, but it had no effect on growth performance and it had negative effect on immune response, as evidenced by significantly lower and higher HSI and plasma alanine aminotransferase ALT, respectively.

The diet supplemented with *C. camphora* and *E. hirta*, extracts also significantly improved the growth performance of Nile tilapia. While, the extracts of *C. camphora* and *E. hirta* were not significantly differed the sexual maturity of fish when compared with control group. Further, crude body lipid was lower in fish fed on the diet supplemented with *C. camphora*, *E. hirta and C. papaya* treatments, but was only significantly lower for the *E. hirta* treatment compared to the control. The results of experiment II showed that after 14 days of bacterial challenge, Nile tilapia fed on *C. camphora* extract supplementation had significantly higher survival rate and relative percent survival (RPS), compared to the control group, while the other treatments were not significantly different when compared to control group. Results indicated that dietary *C. camphora* extract was the most effective prophylactic to *S. agalactiae* and can be a cost-effective and eco-friendly alternative to antibiotics.

According to the results of experiment III, the diet supplemented with *C. papaya* fed for a period of 20 weeks at a dose of 2 g/kg was significantly ($P \leq 0.05$) reduced the GSI of males and females of Nile tilapia, while diet supplemented with 0.5 and 1 g/kg were not affected significantly ($P \geq 0.05$) the GSI of fish compared to control group. However, the diets supplemented with *C. papaya* 1 and 2 g/kg fed for 20 weeks improved significantly ($P \leq 0.01$) the growth performance of Nile tilapia.

In addition, the results revealed that, the diet supplemented with different doses of *C. papaya* extracts (0.5, 1 and 2 g/kg) fed for 3 weeks were not affected significantly ($P \geq 0.05$) the sexual maturity and growth performance of the Nile tilapia.

Overall, it could be concluded that *C. papaya* extracts at 2 g/kg dose is the best among the screened used herbal extracts and most effective on delaying sexual maturity and enhancing growth performance of Nile tilapia. It can be recommended as a natural reproductive inhibitor and feed additive for Nile tilapia. While dietary supplemented with *C. camphora* extract, was the most effective prophylactic herbal extract against *S. agalactiae*.

Abstrakt tesis yang dikemukakan kepada Senat Universiti Putra Malaysia Sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN EKSTRAK HERBA TERPILIH KE ATAS KEMATANGAN SEKSUAL, PRESTASI PERTUMBUHAN DAN BALAS IMUN TILAPIA Nil (*Oreochromis niloticus* Linnaeus)

Oleh

ZANA HAMA GHARIB KAREEM

April 2015

Pengerusi: Profesor Madya Yasser Mohamed abdelhadi, PhD
Fakulti: Pertanian

Baligh cepat matang adalah masalah yang serius, yang menghalang ikan daripada mencapai potensi akuakultur yang penuh dan dianggap sebagai had kepada pertanian tilapia Nil. Melambatkan kematangan seksual Nile tilapia adalah penting untuk mengawal jumlah ikan yang tidak dikehendaki di dalam kolam yang membawa kepada masalah kesesakan, kekurangan makanan, penyakit dan masalah pertumbuhan. Tujuan kajian ini adalah untuk mengkaji kesan empat herba ekstrak ditayangkan (neem daun (*Azadirachta indica*), Asma rumpai (*Euphorbia hirta*), biji betik (*Carica papaya* pada) dan kapur barus kulit (*Cinnamomum camphora*)) pada kematangan seksual, prestasi pertumbuhan dan tindak balas imun tilapia Nil.

Oleh itu, tempoh pemakanan selama 90 hari (eksperimen I) telah dijalankan untuk menilai prestasi pertumbuhan, keberkesanan makanan, gonad / somatik indeks (GSI) sebagai tanda perkembangan gonad, indeks badan, parameter darah, dan pemeriksaan histopatologi terhadap gonad ikan tilapia Nil. Dalam ujikaji ini, ikan diberi makan tumbuhan mentah yang berbeza terdiri daripada ekstrak kulit kayu *C. camphora*, *E. hirta*, *A. indica*, *C. papaya* 2 g / kg diet dan diet kawalan pada kadar "*ad libitum"*. Ini diikuti oleh 14 hari eujikaji (eksperimen II) dengan Gram virulen + ve strain bakteria *Streptococcus agalactiae*. Setiap kumpulan rawatan dijalankan dalam rangkap tiga dan setiap kumpulan terdiri daripada 30 ikan (Jumlah berat badan 90-110 g dan jumlah panjang 18-20 cm). Ekstrak herba yang paling berkesan mengikut keputusan eksperimen I dan II (ekstrak biji betik (PSE)) telah dipilih dan digunakan dalam dos yang berbeza (0.5, 1, 2 g / kg diet) untuk dua tempoh pemberian makanan yang berbeza (3 dan 20 minggu masing-masing) pada 420 kantung-larva- tilapia Nil berusia 3 hari dengan berat permulaan sebanyak 0.015 g dan jumlah panjang daripada 9.43 mm (percubaan III). kantung-Larva- telah di letak kan secara rata dan rawak ke dalam tangki kaca 21 (20 kantung-larva / akuarium). Hasil kajian ini menunjukkan bahawa ekstrak biji betik 2 g / kg diet merupakan herba yang paling berkesan untuk melambatkan perkembangan gonad dan kematangan seksual dalam kedua-dua jantan dan betina ikan tilapia Nil, dan prestasi pertumbuhan ikan yang

dirawat juga meningkat secara ketara berbanding ikan dalam kumpulan kawalan tetapi tidak mempunyai kesan terhadap sistem imun ikan. Sama seperti kumpulan yang diberi makan *A. indica* yang mengurangkan GSI ikan, tetapi ia tidak mempunyai kesan ke atas prestasi pertumbuhan dan menunjukkan kesan negatif ke atas tindak balas imun, apabila aras HSI dan plasma alanina aminotransferase ALT yang ditunjukkan adalah tinggi.

Kumpulan yang di beri rawatan dengan kulit kayu kapur barus dan rumpai asma ekstrak juga menunjukkan peningkatan dari segi prestasi pertumbuhan. Walaubagaimanapun, tiada perbezaan yang ketara di antrara ekstrak kulit kayu kamper, rumpai Asma, daun Neem berbanding dengan kumpulan kawalan. Walau bagaimanapun, tiada perbezaan yang ketara dari segi prestasi pertumbuhan di antara ekstrak neem dan kumpulan kawalan. Sementara itu, semua parameter di dalam darah tidak ada perubahan yang ketara. Selepas 14 hari cabaran bakteria (Eksperimen II), tilapia Nil yang diberi makan dengan ektrak kulit kayu kamper mempunyai kadar kelangsungan hidup yang lebih baik dan relatif peratus survival (RPS) yang lebih tinggi, berbanding dengan kumpulan kawalan, manakala kumpulan rawatan yang lain tidak mempunyai perbezaan yang ketara berbanding kumpulan kawalan. Hasil ujikaji juga menunjukkan bahawa pemakanan ekstrak biji betik boleh menggalakkan pertumbuhan dan melambatkan kematangan gonad untuk kedua-dua tilapia jantan dan betina dengan ketara.. Sebaliknya, ekstrak kulit kayu kamper adalah pencegahan yang paling berkesan dari jangkitan *S. agalactiae* dan boleh menjadi alternatif yang kos efektif dan mesra alam menggantikan penggunaan antibiotik.

Menurut keputusan eksperimen III, gonad ikan yang di beri makan 2 g / kg ektrak biji betik untuk tempoh 20 minggu menunjukkan kadar pengurangan min GSI, manakala kumpulan yang di beri makan 0.5 dan 1 g / kg ektrak biji betik tidak mempunyai perbezaan yang ketara bagi pengurangan min GSI di ($P \geq 0.05$) berbanding dengan kumpulan kawalan. Walau bagaimanapun, ikan yang diberi makan diet eksstrak biji betik pada kadar 1 dan 2 g / kg dan selama 20 minggu tidak mempunyai kesan yang ketara ($P \leq 0.01$) dari segi prestasi pertumbuhan dan kematangan sexualnya. Tambahan lagi, keputusan tempoh kedua untuk ikan yang di beri makan extrak biji betik untuk tempoh 3 minggu mendedahkan bahawa tidak terdapat perbezaan yang ketara ($P \geq 0.05$) antara min GSI dalam ikan walaupun di beri makan extrak biji betik pada dos yang berbeza (0.5, 1 dan 2 g / kg).

Secara keseluruhan, ia boleh disimpulkan bahawa ekstrak biji betik pada kadar 2 g / kg dos adalah yang terbaik di antara ekstrak herba yang lain dan paling berkesan untuk melambatkan kematangan seksual dan berupaya meningkatkan prestasi pertumbuhan tilapia Nil dan juga boleh disyorkan sebagai perencat semulajadi pembiakan dan makanan tambahan dalam industi ikan tilapia Nil. Manakala diet ditambah dengan *C. camphora* ekstrak, adalah merupakan ekstrak herba profilaktik yang paling berkesan terhadap *S. agalactiae*.

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I certify that a Thesis Examination Committee has met on 28 April 2015 to conduct the final examination of Zana Hama Gharib Kareem on her thesis entitled "Effects of Selected Herbal Extracts on Sexual Maturity, Growth Performance and Immune Response of Nile Tilapia (*Oreochromis niloticus* Linnaeus)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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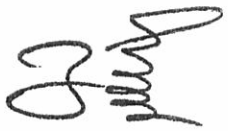

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ALT	Alanine aminotransferase
AST	Aspartate transaminase
AWE	Asthma weed extract
CBE	Camphor bark extract
CF	condition factor
CFU	Colony Forming Unit
DO	Dissolved oxygen
FCR	Feed conversion ratio
FW	Finale weight
GSI	Gonado somatic index
HIS	Hipato somatic index
I/P	Intraperitoneal injection
MCHC	mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
NLE	Neem leaf extract
PCV	Packed cell volume
PSE	Papaya seed extract
RBC	Red blood cells
RPS	Relative percent of survival
SAS	Statistical Analysis System
SGR	Specific growth rate (SGR
SSI	Spleen somatic index
TBW	Total body weight
TL	Total length
TSA	Tryptic Soya agar
TSB	Tryptic Soya Broth
SR	Survival rate
WG	weight gain
WBC	White blood cells

CHAPTER 1

INTRODUCTION

1.1 Introduction

Food production is undoubtedly, is one of the most important issues which looms large in every aspects of human life. The three top sources of food production are agriculture, fishery and livestock industry. With gradual increase in the world population, these food production activities, specifically fishery have increased over the years. Nowadays aquaculture is an effective alternative to wild fishery and it has mitigated the intensifying pressure on wild fishery around the globe. Nowadays there is a considerably growing demand for the products of aquaculture fishery all over the world (Rosenlund & Skretting, 2006). With the importance of fishery for human life, it is essential to use techniques and strategies to make aquaculture more efficient industry. This industry has important economical values, low costs and high commercial demand.

Nile tilapia is considered as an important fish food source which has been transplanted to various regions all over the world. This fish has a number of salient characteristics such as fast breeding, strong resistance, hardiness, rapid growth, flexible and multivariate eating habits, and nice taste. These characteristics enable Nile tilapia to be cultured in different systems of aquaculture and to live under a broad range of environmental conditions. Therefore, Nile tilapia is the most common type of tilapia cultured in different parts of the world. Furthermore, it can live in low oxygen conditions, overcrowding, endure difficult ecological systems and serve as a an important source of fish food in many regions around the globe (Khalil et al., 2014).

There is a considerable body of research in the area of aquaculture which has focused on different aspects of tilapia species including tilapia diseases (Abd El-Rhman et al., 2009), reproductive performance (Charo-Karisa et al., 2007; Ng & Wang, 2011), growth performance (Zahran et al., 2014; Zychowski et al., 2013), genetic improvement (Bentsen et al., 2012; Ponzoni et al., 2007; Rutten et al., 2005) and immunology (Barros et al., 2014; Guimarães et al., 2014; Park & Choi, 2012).

There are many types of feed additives used to enhance the growth performance of different fish species. The feed additives encompass a wide range of chemical and no-chemical materials. Herbs and plants comprise a significant portion of feed additives in the literature of aquaculture research. One of the reasons for the enormous use of plants and herbs as feed additive is to decrease the use of chemicals because chemicals have side effects on animals. In addition, the use of natural materials such as herbs and plants as an effective strategy against diseases has a long history in old civilizations such Egypt, China and India (Zaki et al., 2012). There have been significant research attempts directed towards the use of plants and herbs to improve productive performance and nutrition of the animals (Mohamed et al.,2003).

Recent research in the area of fish nutrition shows that the nutrition potential of a variety of natural herbs and spices including black seeds, peppermint, caraway seed have been examined in fish nutrition.(El-Dakar & Hassanien, 2008); Zaki et al., 2012).

Another line of inquiry in the field of aquaculture addresses the effects of herbs and plants on the growth performance, resistance enhancement and immunity Improvement for fish species including Nile tilapia (Ahmad & Abdel-Tawwab, 2011; Dügenci et al., 2003; Reverter et al., 2014).

Fish, as an important source food, plays a pivotal role in human life, as it is rich in a variety of vitamins, minerals, proteins and essential micronutrients including omega 3 which are all important for good health. The importance of fish in human diet has been accentuated in many reports. For example, according to FAO (2012) in 2006, fish made up 17% of the whole sources of animal protein and 6.5% of the whole sources protein including plant protein. The past five decades has experienced a dramatic increase in the fish supply with an annual consumption rate of 3% all over the world in a forty-year-period between 1961 and 2010. It is worth mentioning that aquaculture including fish is the most rapidly growing sector among all of the animal-food producing industries which rise 6% annually in a period from 1990 to 2010 (FAO, 2012; Reverter et al., 2014).

1.2 Problem Statement

According to Department of Fisheries Malaysia (2010) freshwater fish culture in Malaysia contributes 155,398.6 ton valued at RM 760.3 million, representing 26.7% of the total production and constituted 27.2% of the overall aquaculture subsector (Department of Fisheries Malaysia, 2010). In Malaysia, freshwater fish is cultured using pond culture, ex-mining pool, freshwater cage, cement tank, canvas tank, and freshwater pen culture systems. The highest total freshwater fish production (59.7%) has been reared in the pond culture system and the types of fish cultured in this system are freshwater catfish (64.9%) and tilapia (18.2%). The rate (18.2%) of culturing tilapia is low if compared it to the other type of freshwater fish.

Unsolicited multiplication and overpopulation due to early sexual development, acknowledged being the chief constraint in the cultivation of most species of tilapia, especially; *O. niloticus* and *O. mossambicus*. Most teleosts, particularly females avert energy which could be exploited for somatic development, into germ cell creation and behavioral relations. Furthermore, rivalry with newcomers in restrained conditions promotes suppression of stocked fish from growing, and may cause in containing large amounts of 30-50% of collected biomass unmarketable newcomers (Vera Cruz & Mair, 1994).

Sexual maturity has long been an important area of research which has received ample attention in aquaculture, and many studies have addresses sexual maturity among some fish species particularly the Nile tilapia. Sexual maturation important factors affecting the growth and performance of the fish. There is a considerable number of studies which have addressed the effect of chemicals on timing of maturation (Singh & Tripathi 2012), changing the sex of the fish (Afonso, et al.,

2001;Vera Cruz & Mair, 1994). The most noticeable method used for sexual alteration in the literature of aquaculture is hormonal approach. Another technique used for single sex production is gynogenesis. However, this method is not economical as it causes high rate of fish mortality but is an effective method for determining sex differentiation (Devlin & Nagahama, 2002).

Although there are many studies which have focused on sexual maturity of fish, most of these studies have investigated the effects of chemicals and hormones on sexual maturity, and little research has examined the effects of herbs and plants on sexual maturity and the timing of maturation. Furthermore, there are only a few studies have investigated the effects of extracted herbs on the sexual maturity of fish species, and even fewer studies have focused on assessing the effects extracted herbs on the sexual maturity of Nile tilapia.

In addition, one of the problems in fish producing industry is precocious maturity among some fish species. Precocious maturity in Nile tilapia is one of issues causing uncontrolled reproduction and overcrowding, and consequently leads to food shortages, diseases, and growth problems (Toguyeni et al., 2002). Precocious maturity is a problem which hinders the fish to reach its full aquaculture potential and is considered as a limitation in Nile tilapia farming. Therefore, to control unwanted breeding and enhancing the growth performance of the fish, this study seeks to delaying the maturity of Nile tilapia by examine the effect some herbal extracts on the sexual maturity of the, fish in addition to investigate of their effect on growth performance and immune response of fish.

1.3 Significance of the Study

Nile tilapia in tropical countries, tilapia farming and industry has been influenced by a variety of infectious diseases and bacteria, causing affliction and economic damages and posing threats to the development of this industry. The dietary supplementation techniques and strategies employed for other sea animals and aquaculture species may be effective and applicable to dietary research on Nile tilapia and to the nutrition investigations for that fish species. The use of specific types of feed and dietary supplementation is a strategic method not only to enhance the immune response of the animals but also they may be used as an effective and useful alternative to antibiotics for treating and curing fish diseases (Barros et al., 2014; Oliva-Teles, 2012).

Today, herbal medicine is an a rapidly expanding area as an effective and alternative medicine or treatment for humans and numerous types of drugs derived from herbs and plants have been widely used by human being for different medical purposes. The plan-made drugs are very important in treatments and cures as they are highly rich in various nutrients and these natural drugs may be taken and used as feed additives (Chang, 2000). The medicinal herbs as a feed additive for fish seem to be more effective and useful than chemicals, and consequently they may have an accumulative effect and fewer side effects on human health. Medical plants may be used as feed additive or as an attractant to enhance the level of feed intake, increase feed utilization, improve weight, balance nutrition and improve growth performance (Goda, 2008; Ahmad & Abdel-Tawwab 2011; Liu, et al., 2011).

Thus, finding the alternatives to the chemical production by using the herbs that available in tropical countries especially Malaysia, as a natural reproductive inhibitor and least economic costs as a feed additive is very importance in tilapia pond culture

1.4 Objectives of the study

1. To investigate the effect of four screened herbs extracts (Neem leaves, Asthma weed, Papaya seed and Camphor bark) on the sexual maturity of Nile tilapia.

2. To determine the effect of methanolloic extracts of these herbs on the growth performance of Nile tilapia.

3. To examine the effect of these herbal extracts on the immune response of Nile tilapia.

4. To study the effect of such herbal extracts on the whole body composition of Nile tilapia.

5. To determine the dose and time related effect of the most effective herbal extract on the sexual maturity, growth performance and survival rate of Nile tilapia.

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