

**EFFECT OF *Butea superba* AND 17- α -METHYLTESTOSTERONE (MT) ON
SEX REVERSAL AND SOME GROWTH PARAMETERS IN
THREE STRAINS (RED, GHANA AND CHITRALADA) OF
NILE TILAPIA (*Oreochromis niloticus*, L.)**

ROGELIO P. CARANDANG JR.

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE IN FISHERIES TECHNOLOGY**

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APPROVAL SHEET

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Title

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ABSTRACT

This study evaluated the potential of *B. superba* crude powder, liquid ethanol extract and freeze-dried extract powder for use in tilapia sex reversal and its effect in growth rate. The three-part experiment was done in earthen pond and aquaria. In the first part, *B. superba* crude root powder and two 17- α -Methyltestosterone (MT) dosages were given to three strains of Nile tilapia (Red, Ghana and Chitralada). Results revealed that MT treatments (40 and 60 mg kg⁻¹) had comparable effects in terms of male sex percentage, survival rate (SR), except in Red strain, feed conversion rate (FCR) and gain in weight (GW), except in Ghana strain. MT treatments were significantly different with the control group in terms of percent male sex (except in Chitralada strain), SR (except in Chitralada and T2 of Ghana strains) and GW (except in Red and T1 of Ghana strains). *B. superba* treatments (100, 200 and 300 g kg⁻¹) did not have significant difference with the control group and with the MT treatments in some cases. However, a 72.2 ± 25.5 % male sex ratio was obtained in the T4 (200 g kg⁻¹) of the Ghana strain. As to the effect of the treatments on the three strains, it was observed that they generally had comparable effects.

The second experiment utilized *B. superba* liquid ethanol extract and powder fed to Ghana strain Nile tilapia in earthen pond and aquaria. Male sex percentage in T1 (60 mg kg⁻¹ MT) in the earthen pond was highest (90%) and statistically significant among other treatments. The control, T2 (200 g kg⁻¹ *B. superba* crude powder), T5 (6 ml kg⁻¹), T4 (4 ml kg⁻¹) and T3 (2 ml kg⁻¹) had 45.2, 58.2, 56.7, 50.0 and 31.2 % male sex percentage, respectively. The lowest SR was in T0 (64.7%) which was significantly different among the treatments and the highest was in T1 (89.0%). In the aquaria, male sex percentage was highest in T1 (97.0%) and statistically significant among the treatments followed by T2 (68.1%). Lowest male sex ratio was observed in T5 (28.6%). SR was lowest in T0 (86.7%) and statistically significant with T1 (96.7%), and T4 (98.7%).

On the third part *B. superba* crude ethanol extract was freeze-dried into powder and fed to Nile tilapia in three levels (70, 80 and 90 g kg⁻¹). Highest male percentage observed in T1 (100.0%), 60 mg kg⁻¹ MT was statistically significant among all treatments (40.0-56.7%). No significant difference was observed in SR (84.4-98.9%). Final weight of T3 (70 g kg⁻¹) and T4 (80 g kg⁻¹) were statistically significant (122.7 ± 37.3 and 125.3 ± 27.6 grams, respectively) to T0 (56.0 ± 2.5 grams), T1 (72.3 ± 3.8 grams) and T2 (102.7 ± 32.7 grams). Feed conversion rate was statistically comparable between T3 (0.6 ± 0.1) and T4 (0.6 ± 0.1) but both were significantly different to T0 (1.4 ± 0.3) and T1 (1.0 ± 0.0).

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To all of you I humbly dedicate this piece of work.

Rogelio P. Carandang Jr., DVM

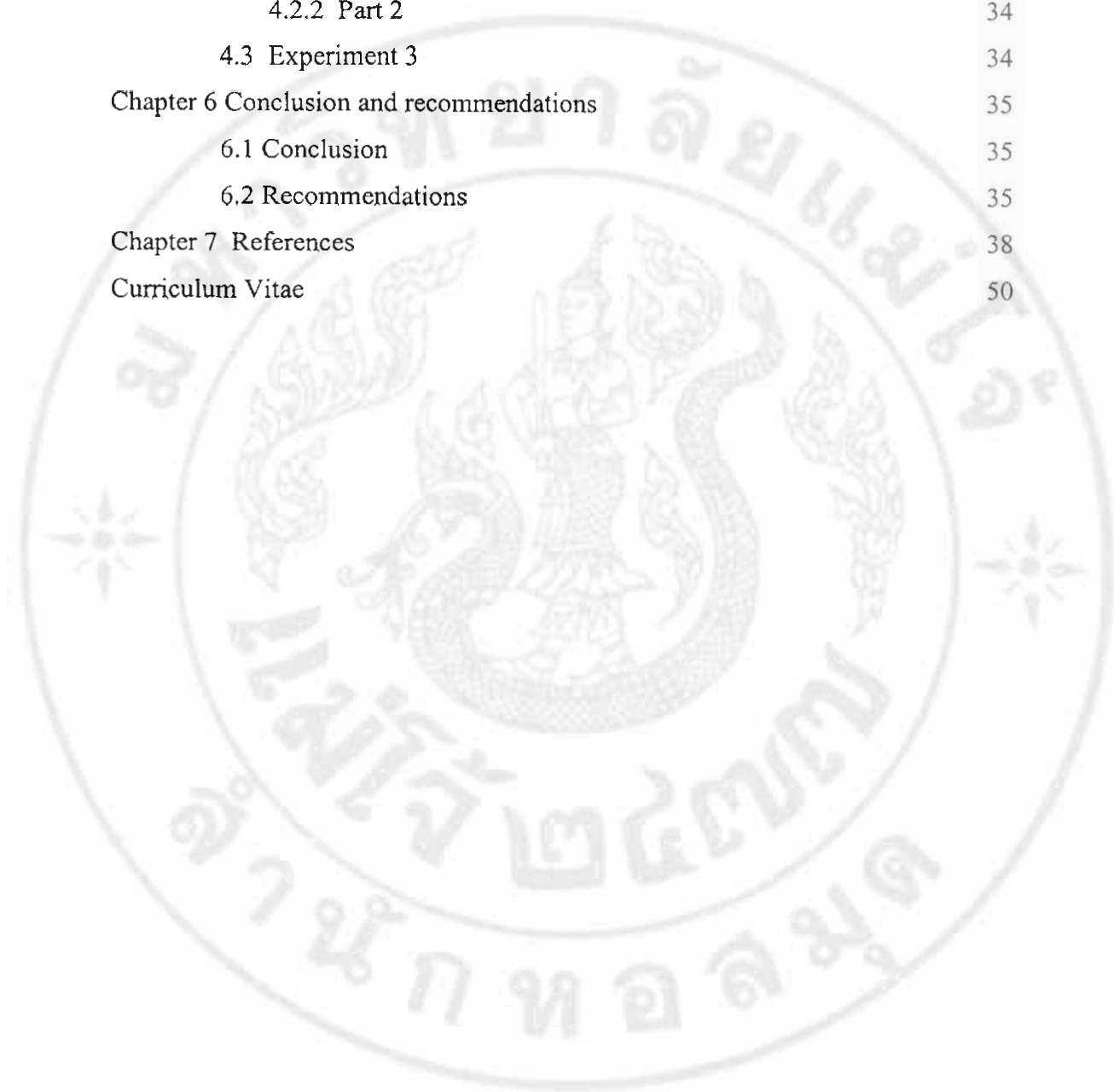
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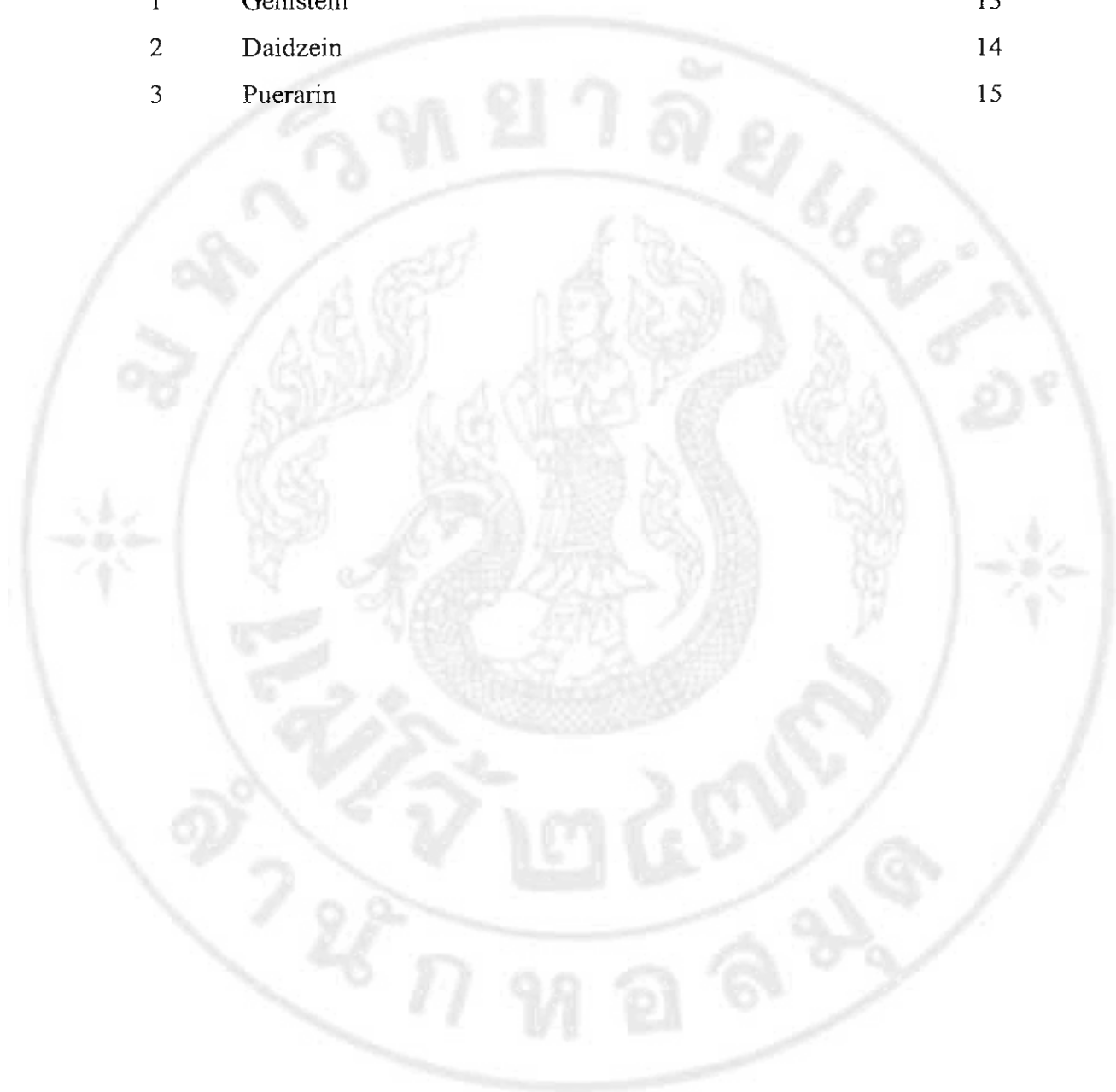


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Chapter 1

Introduction

Studies involving sex reversal of Nile tilapia species using 17- α -methyltestosterone is well documented. Numerous papers have reported that 60 mg kg⁻¹ was found to induce monosex male population (Nuanmanee *et. al.* 2004; Vorasayan and Petchrich, 2004; Phelps *et. al.*, 1995; Green and Teichert-Coddington, 1994; Killian and Kohler, 1991; Jo *et. al.*, 1988; Macintosh *et. al.*, 1988). Abucay and Mair (1997) observed incidental sex reversal of Nile tilapia when treated with 40 mg kg⁻¹ MT in a closed water system. They further suggested that the environmental and human health issues related to experimental and commercial sex reversal be reviewed. Years prior, Johnston *et. al.* (1983) found that MT was rapidly depleted to less than one percent of the initial dosage which makes the MT-treated tilapia safe for human consumption based on the acceptable level of hormones. However, they also recommended researches concerning the fate of MT in the environment to quell negative public perception regarding hormone-treated fish. This claim was supported by Green and Teichert-Coddington (2000); they found that MT treatment had no adverse or negative effect on human food safety and in the environment. The issues however cannot be rested fully due to consumer health concerns especially during these times where there are increasing cases of diseases involving drug and hormone residues. This eventuality had increased the popularity of the organic way of food production. Herbal preparations are so popular in the market today.

Researchers in response had varying success in inducing male sex reversal without the use of MT i.e. shock, heat and irradiation treatments, transgenic fish, aromatase inhibition, genetically male tilapia (GMT) etc. of which the GMT technology otherwise known as YY-supermale technology was the most successful. The YY-supermale technology is a good alternative for MT treatment but may not be applicable on all strains of tilapia based on initial study in the Chitralada strain (Tuan *et. al.*, 1998), so that hormone treatment is still the most practical and convenient way of sex reversal. The success of sex reversal using this hormone however depends on many factors (dose, timing and duration of treatment and mode of administration).

Moreover, it was observed that high dosage and prolonged treatment induces gonadal intersexuality and paradoxical feminization (<http://pdacrsp.oregonstate.edu>).

Red kwao kreua (*B. superba*) contains substance with cAMP phosphodiesterase inhibitors and inhibitory activity on acetylcholinesterase which provides alternative treatment for erectile dysfunction (ED) (Cherdshewasart and Nimsakul, 2003). Moreover, Cherdshewasart *et. al.* (2004) disclosed that this herbal plant could have an anti-estrogen or a cytotoxic property because the test subject-MCF-7, an estrogen receptor positive (ER⁺) human mammary adenocarcinoma did not proliferate when treated with *B. superba* ethanol extract. They further suggested that there could be phytoandrogens in the plant which is yet to be identified. One possibility for the anti-estrogenic property are isoflavonoids i.e. genistein and daidzein which were also reported to have weak estrogenic property (www.genistein.com). Isoflavones are thought to be aromatase inhibitors- drugs that reduce the level of estrogen in the body (www.dadamo.com). Manosroi and Manosroi (2005) found that six-year old red kwao kreua plant had a significant amount of genistein and daidzein contents (4.5 mg kg⁻¹ and 37.2 mg kg⁻¹ respectively). In another study, feeding rats with *B. superba* crude powder from roots resulted in an increased tendency of testicular weight and sperm count (Manosroi *et. al.*, 2006). Instead of the usual MT treatment, studies are now focused on alternative methods in generating all-male tilapia due to the rising health concern issues.

Plant sources with potential sex-reversing mechanism and growth-promoting effects could be worth studying since they are not subjected to scrutiny and licensing for use in food animals. Hence, this study.

1.1 Objectives of the Study

The general objective of the study was to investigate the effect of *B. superba* and MT on the percentage of male sex and on some growth parameters of Nile tilapia.

Specifically, it aimed to:

1. Determine and compare the percent male sex, survival rate (SR), feed conversion rate (FCR) and gain in weight (GW) between three strains (Red, Ghana, Chitralada) of Nile tilapia treated with *B. superba* crude powder and MT.
2. Determine and compare the percent male sex, SR, FCR and FW of Ghana strain Nile tilapia treated with *B. superba* crude powder and liquid ethanol extract and MT in earthen pond and aquaria.
3. Determine and compare the percent male sex, SR, FCR and FW of Nile tilapia treated with *B. superba* freeze-dried ethanol extract and MT in the aquaria.
4. Determine the potential use of *B. superba* in Nile tilapia production.

Chapter 2

Review of Related Literature

2.1 Nile Tilapia (*Oreochromis niloticus*, Linnaeus, 1758).

2.1.1 General characteristics

Genus: *Oreochromis*; Euryhaline; oviparous; omnivorous; gregarious, pelagic. The proper genus name for Common Tilapia (*Tilapia*, *Sarotherodon* or *Oreochromis*) has now been more or less resolved. The males are territorial and are hostile towards other males. Females tend to be more drab, and seem to travel in large schools. A breeding male will clean out a large depression on the substrate in which he will "invite" potential females to come and mate. The fish are mouth brooders, and it is usually the females who take care of the brood.

Family: Cichlidae (Cichlids); subfamily: Pseudocrenilabrinae

Order: Perciformes (perch-like)

Class: Actinopterygii (ray-finned fishes)

Common name: Nile tilapia

Maximum size: 60.0 cm SL (male/unsexed)

Maximum published weight: 4,324 g

Maximum reported age: 9 years

Environment: Benthopelagic; potamodromous; freshwater; brackish; depth range is 5 meters.

Climate: Tropical; 8 - 42°C; 32°N - 10°N

Distribution: Africa; coastal rivers of Israel; Nile from below Albert Nile to the delta; Jebel Marra; Lake Chad basin and the rivers Niger, Benue, Volta, Gambia and Senegal. Widely introduced for aquaculture with many existing strains. Several countries report adverse ecological impact after introduction.

Diagnosis: Dorsal spines (total): 16-18; Dorsal soft rays (total): 12-13; Anal spines: 3-3; Anal soft rays: 9-11; Vertebrae: 30-32. Genital papilla of breeding male is not tassellated. Jaw of mature male is not greatly enlarged (length of lower jaw 29-37 % of head length). The most distinguishing characteristic of the species is

the presence of regular vertical stripes throughout the depth of caudal fin. Margin of dorsal fin is grey or black. There are 7-12 vertical bars in caudal fin.

Biology: Occurs in a wide variety of freshwater habitats like rivers, lakes, sewage canals and irrigation channels. They are mainly diurnal and feed mainly on phytoplankton or benthic algae. They are marketed fresh and frozen. (www.fishbase.org).

2.1.2 Strains

2.1.2.1 Red strain

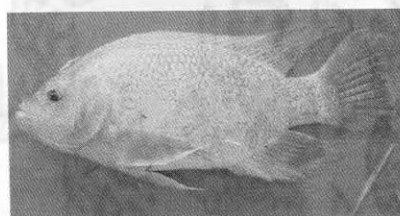


Plate 1 Red strain *O. niloticus*

The original Red tilapias were genetic mutants. The first Red tilapia, produced in Taiwan in the late 1960s, was a cross between a mutant reddish-orange female Mozambique tilapia and a normal male Nile tilapia. It was called the Taiwanese Red tilapia. Another Red strain of tilapia was developed in Florida in the 1970s by crossing a normal colored female Zanzibar tilapia with a red-gold Mozambique tilapia. A third strain of Red tilapia was developed in Israel from a mutant pink Nile tilapia crossed with wild Blue tilapia. The confusing and rapidly changing genetic composition of Red tilapia, as well as the lack of “head-to-head” growth comparisons between the different lines makes it difficult for a producer to identify a “best” red strain. (Popma and Masser, 1999 as cited by <http://aquanics.org>). This hybrid tilapia can grow well in various favorable environments. In Thailand, Thai Red tilapia is one of the most popular Nile tilapia because of its economic values. The organoleptic test compared between the Thai Red tilapia and Nile tilapia indicated about 80% of the testers preferred the Thai Red tilapia to the Nile tilapia for its nice-looking color, softness and fatty test of the flesh. Thus, commercial culture of tilapias has focused on Thai Red tilapia and Nile tilapia. Although a hybrid, Thai Red tilapia showed high growth rate in favorable environments. Under less optimal

conditions, the hybrid fish tends to be susceptible to stress and has a relatively low survival rate with only average fecundity (Macaranas *et al.*, 1997 as cited by Manosroi *et al.*, 2003).

2.1.2.2 Ghana strain

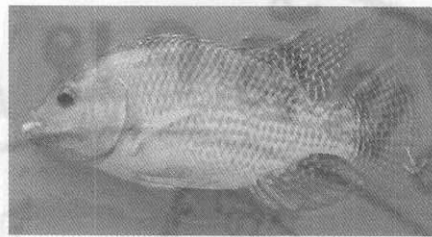


Plate 2 Ghana strain *O. niloticus*

Mires (1977) and Hulata (1988) as cited by Jeremy *et al.* (2000) first described the Ghana strain of *O. niloticus*. It was introduced in 1982 into Alabama (Hangroves, 2000 as cited by <http://etd.lsu.edu>). Smitherman *et al.* (1988) as cited by www.blackwell-synergy.com found that Ghana strain produced more seed and spawned more frequently than the ivory strain. The high variability in sex ratios also occurred in the Ghana line (<http://pdacrsp.oregonstate.edu>).

2.1.2.3 Chitralada strain

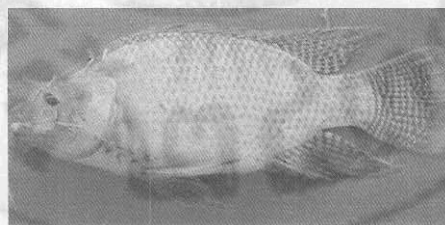


Plate 3 Chitralada strain *O. niloticus*

The Thai-Chitralada strain is derived from a strain held at the Royal Palace in Japan, which originates from Egypt. A stock of 50 fish was introduced to Thailand in 1965 as a gift of the Emperor of Japan to His Majesty, the King of Thailand. Since this time the fish have been kept in the Chitralada palace pond, well isolated from other tilapia species. The number of fish, which survived and bred to produce succeeding generations, is not known (Pullin and Capili, 1988; Pongsri, 1994; Capili, 1995 as cited by Tuan *et al.*, 1998). This introduction formed the base population for

the large majority of tilapia cultured in Thailand today. In one study, it was found out that this strain has better survival rate but lower fecundity, lower incidence of cannibalism than the GIFT strain but the growth were comparable (Nandlal, 2001). Borges *et. al.* (2005) found a significant temperature effect on sex ratio which indicates the thermosensitivity of the Chitralada strain.

2.1.3 Introduction to Tilapia sex-determination and sex-reversal

One of the basic factors in tilapia aquaculture is that male fish grows bigger and faster than the female. Also, in order to avoid unwanted spawning in a production unit, an all-male population is preferred. There are several methods used to skew sex ratios and increase the percentage of males in a population.

The first method developed was to simply cull through a population, discard the females and keep the males. This system is obviously wasteful and inefficient. In the 60s and 70s, Israeli scientists discovered that certain hybrid crosses resulted in skewed sex ratios favoring males. There are several theories regarding the genetic factors involving the number and location of sex genes on particular chromosomes. The drawback to this method is that two separate broodlines must be maintained. The crossing must be done very carefully and meticulous records should be kept to insure that the parent species are kept pure. Also, usually only one sex from each species is used for any particular cross because the reciprocal cross (using the other sex from each species) is not as successful. Another problem is that the number of young produced is rarely as high as a single species spawn. Therefore, to maintain a commercial scale hatchery will usually require significant resources and staff.

Evidence of the effect of environment on sex ratio has been observed in a number of fish species. Abucay *et. al.* (1999) found out that not all of the fries tested were affected by increase in temperature and that different genotypes have different sensitivity to environmental effects on sex ratio. They further suggested that high temperature can influence sex ratio not only in the direction to male but also to female. Baras *et. al.* (2001) as cited by Stickney (2002) was able to produce 90 % males when he exposed *O. niloticus* to 37.8 °C for 28 days.

The more common method of generating mostly male populations is through the use of steroids fed to sexually undifferentiated fry. Newly hatched tilapias are still

developing their gonads. Even though they are determined genotypically their phenotype or morphological characteristics can still be altered. By exposing the fish to forms of testosterone or estrogen, the gonad can switch. Typically the desire is to produce all males, so methyltestosterone is included in the diet for several weeks when the fish starts eating. Utilizing MT at 60 mg kg⁻¹ has consistently produced $\geq$ 95% male sex ratio (Vorazayan and Petchrich, 2004; Nuanmanee *et. al.*, 2004). Phelps *et. al.* (1995) proved that monosex population can be achieved using 60 mg kg⁻¹ MT in earthen ponds but a lower density would result to lower percent sex reversal. Abucay and Mair (1997) proved the phenomenon of incidental sex reversal when they observed male sex reversal in their Control group caged in the same tank with the treatment group given 40 mg kg⁻¹ MT. This research was originally intended to determine the importance of feeding duration and quality in the optimization of masculinization and feminization treatments of *O. niloticus*. The studies of Green and Teichert-Coddington (1994) proved that anabolic response from 60 mg kg⁻¹ MT treatment is not consistently observed and may be masked by the environment. Macintosh *et. al.* (1988) found that administration of 40 mg kg⁻¹ MT had a higher male sex reversal than 60 mg kg⁻¹ when they conducted the experiment in clear water tanks. They further observed the depressant effects (abnormally enlarged ovaries in females and testicular degeneration in some males which lowered the GSI) of MT when used in a higher dosage (60 mg kg⁻¹).

Sex-reversal can also be achieved by immersion in a solution (Cagauan *et. al.*, 2004). The hormones cause the gonads to develop as testes instead of ovaries and the fish will also take on male morphological characteristics. The hormone is only needed during the first month and after that the fish are feed normally for the rest of their lives. Using this technique farms can produce populations of greater than 90% male fish. These populations grow faster than equivalent populations of mixed sex fish and have significantly less reproduction in the growout systems.

One of the recent technologies developed is the Genetically Male Tilapia (GMT) otherwise known as YY-male technology. The YY-male technology was conceptualized as a method of generating monosex tilapia providing alternatives to hormonal sex reversal and hybridization which both have their disadvantages. The YY-male technology is a combination of sex reversal and progeny testing combined

in a breeding program, which is essentially selecting for sex. The initial stage induces the sex inversion of sexually undifferentiated tilapia fry into delta females (i.e. XY genetic males which are phenotypically female), which are then identified by progeny testing. Identified XY delta females are then crossed with normal XY males, with one third of the male progeny being YY males, which again are identified by progeny testing. However, Tuan *et. al.* (1998) found a high variability of sex ratio when they applied the technology to the Thai-Chitralada strain of *O. niloticus* thus, it is not apparent that the YY-male technology has universal applicability to all strains of *O. niloticus* as earlier work with other strains have also demonstrated greater variability of sex ratio (Shelton *et. al.*, 1983; Wohlfarth and Wedekind, 1991 as cited by Tuan *et. al.*, 1998). Other methods of producing all male or female population i.e. triploidy, quadriploidy, gynogenesis etc. have also their disadvantages. This remains clear that the most practical and convenient way of obtaining a one sex population is through direct hormonal application. An extensive survey of U.S. surface waters identified the presence of many pharmaceutical agents including natural and synthetic hormones (Kolpin *et al.* 2002 as cited by Detailed Review on Fish Screening Assays for the Detection of Endocrine Active Substances, 2004). This report follows decades of increasing reports of reproductive disturbances in fish and other wildlife that was attributed to exposure specific chemical agents or waste water effluents. Perhaps the most well known example is that of the feminizing effects of pesticide DDT in wildlife (Bitman *et al.* 1969 as cited by Detailed Review on Fish Screening Assays for the Detection of Endocrine Active Substances, 2004).

In fish, as with other vertebrates, reproduction requires the coordination of a variety of physiological processes culminating in release of viable gametes and successful fertilization. In many fish species a seasonal reproductive cycle exists whereby the gonads undergo a period of recrudescence or rapid growth prior to spawning. Fish are also similar to other vertebrate groups in that most species are gonochoristic, with separate male and female phenotypes. However, there is great diversity in reproductive strategies among fishes, such as internal or external fertilization, oviparity (fertilized eggs mature outside of the fish) or ovoviviparity/viviparity (internal development) and synchronous (annual spawning) or asynchronous spawning (repeated spawns during a spawning season).

In a broad sense, communication between cells and tissues can occur via the central nervous system and/or through release of chemical messengers or signals. Chemical signaling can be further divided into autocrine and paracrine actions to differentiate between effects on similar or different cell types. The endocrine system can be defined as any tissue or cells that release a chemical messenger (hormone) directly into the blood that signals or induces a physiological response in some target tissue.

The fish ovary has been described as essentially a hollow organ containing many lamellae that resemble the pages of a book (Scott 1987 as cited by Draft Detailed review paper on a Fish Two-Generation Toxicity Test, 2002). Oogenesis, which is the process of egg development, occurs within the lamellae and can be divided into several discrete phases.

In many fishes, the testes has a characteristic whitish appearance and elongated lobular shape within which exist tubules that are surrounded by a basement membrane that divides the space into intra- and inter-tubular compartments (Nagahama 1983; Scott 1987 as cited by Draft Detailed review paper on a Fish Two-Generation Toxicity Test, 2002). In teleosts, two distinctive forms of testes structure have been described and are termed tubular or lobular (Grier, 1981 as cited by Draft Detailed review paper on a Fish Two-Generation Toxicity Test, 2002), although technically speaking, tubules are present in both types.

2.2 Red Kwao Kreua (*Butea superba*)

2.2.1 General characteristics



Plate 4 *B. superba* tuber root

B. superba is an herb in the family Papilionaceae and has the characteristics of being a crawling vine that wraps itself around large trees. One branch has three leaves. The flowers are of a yellowish orange color and the plant grows in the open. The long roots of the plant are buried under the ground, similar to the roots of a yam. It reproduces through seeds and the separation of its roots. It exists only in the forests of Northern and Eastern regions and along Kanchanaburi, Province of Thailand. This plant species can be found in the same habitat as White kwao Kreua (*Pueraria mirifica*). The long-shape tubers are annually enlarged and accumulated at least 15 chemicals in the group of direct chain organic acid, sterol, sterol glycoside, flavonoid and flavonoid glycoside (www.stherb.com).

, A study on the growth of female Nile tilapia supplemented with white kwao kreua (*P. mirifica*) during winter season revealed high growth rate (Saetaphan and Sobin, 2006). In the study of Jintasaporn *et. al.* (2000), the growth rate of Snake skin gourami (*Trichogaster pectoralis*) when fed with white kwao kreua was not significant during the first 30 days of feeding but it significantly increased after 60 days of feeding which suggested that prolonged feeding of this herbal plant promoted growth. Similar studies using this herbal plant in chickens also reports significant growth rates and efficient feed conversion (Kongbuntad and Saytong, 2001; Kongbuntad and Nonsila, 2001). In a crude nutrient analysis of the *B. superba* freeze-dried tuber-root powder, it was revealed that the carbohydrate, protein, fat, fiber, moisture and ash content was 53.2 (261.5 kcal g⁻¹), 4.3, 3.5, 19.3, 10.6 and 9.1 % respectively.

The roots and stem of the plant are medicines used for strength and power. In addition, the roots and stem of the plant are considered to help increase male sexual performance. Thus, this plant has come to be known as a miracle herb.

Species

Due to the dispersal habitat of the plant, many variations had been found. At least cultivars have been studied and selected for commercialized plantation and were named as Butea I and II.

Properties

This herb exhibits some chemicals closely related to that of *P. mirifica* but other chemicals are far more different. (www.stherb.com).

Manosroi and Manosroi (2005) evaluated the bioactive compounds in the roots of different ages of *P. mirifica* and *B. superba* from various locations in Thailand. The extracted bioactive compounds from the roots by organic solvents were determined from HPLC fingerprints and compared with standard isoflavonoids (puerarin, daidzein and genistein).

B. superba from Chiang Mai had the highest amount of the active compounds. Puerarin contents in *B. superba* were lower whereas daidzein and genistein were greater than those found in *P. mirifica*. The contents of puerarin, daidzein and genistein in *B. superba* from Chiang Mai were 1.9, 37.2 and 4.5 mg kg⁻¹ of the dried root respectively.

For miroestrol contents (identified as phytoestrogen), the highest amount of 45.0 mg/kg of the dried root was found in *P. mirifica* collected from Chiang Mai province at the age of 5.5 years old. No miroestrol could be determined from roots of *B. superba*.

Also, Cherdshewasart *et. al.* (2004) investigated the differential anti-proliferation effect of white (*P. mirifica*), red (*B. superba*), and black (*Mucuna collettii*) kwao kreu plants on the growth of MCF-7 cells. They found out that *B. superba* led to no proliferation and an anti-proliferation effect on the growth of MCF-7 cells at 10, 100 and 1000 μ mL⁻¹ with an ED50 value of 370.91 μ L mL⁻¹. They concluded that *B. superba* exhibited only anti-proliferation effects on the growth of MCF-7 cells in relation with a possible anti-estrogen mechanism or a potent cytotoxic effect. They further theorized that the plant might contain phytoandrogen which is yet to be identified.

2.2.2 Flavonoid content

2.2.2.1 Genistein

Genistein belongs to the isoflavone class of flavonoids. It is also classified as a phytoestrogen. Phytoestrogens are plant-derived nonsteroidal

(www.pdrhealth.com). Genistein is a potent inhibitor of protein-tyrosine kinase and topoisomerase II, enzymes which are crucial to cellular proliferation. Genistein is also an inhibitor of angiogenesis and several steroid metabolizing enzymes, such as aromatase and 5- α -reductase (www.chinagreatvistachemicals.com).

2.2.2.2 Daidzein

Daidzein belongs to the isoflavone class of flavonoids. It is also classified as a phytoestrogen since it is a plant-derived non-steroidal compound that possesses estrogen-like biological activity. Daidzein has been found to have both weak estrogenic and weak anti-estrogenic effects.

Daidzein is the aglycone (aglucon) of daidzin. The isoflavone is found naturally as the glycoside daidzin and as the glycosides 6"-O-malonylgenistin and 6"-O-acetyldaidzin. Daidzein and its glycosides are mainly found in legumes, such as soybeans and chickpeas. Soybeans and soy foods are the major dietary sources of these substances. Daidzein glycosides are the second most abundant isoflavones in soybeans and soy foods; genistein glycosides are the most abundant. Non-fermented soy foods, such as tofu, contain daidzein, principally in its glycoside forms. Fermented soy foods, such as tempeh and miso, contain significant levels of the aglycone.

Daidzein is a solid substance that is virtually insoluble in water. Its molecular formula is $C_{15}H_{10}O_4$, and its molecular weight is 254.24 daltons. Daidzein is also known as 7-hydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one and 4', 7-dihydroxyisoflavone. Daidzin, which has greater water solubility than daidzein, is the 7-beta glucoside of daidzein. The structural formula of daidzein is:

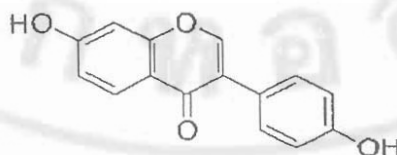


Illustration 2 Daidzein

Actions

Daidzein has estrogenic activity. Daidzein may have antioxidant activity. It may also have anti-dipsotropic, anti-carcinogenic, anti-atherogenic and anti-osteoporotic activity.

Mechanism of Action

Daidzein has weak estrogenic activity as measured *in vivo* and *in vitro* assays. *In vivo*, its estrogenic activity is the lowest of the soy isoflavones.

The mechanism of daidzein's possible anti-carcinogenic activity, as well as its possible anti-atherogenic activity, is unclear. Daidzein's weak estrogenic effect may play some role in the possible activity of soy isoflavones against prostate cancer. Likewise, daidzein's weak estrogenic effect, as well as its possible antioxidant activity, may contribute to its possible anti-atherogenic activity.

Daidzein's weak estrogenic effect may also be involved in its possible anti-osteoporotic activity. Daidzein has been found to have an anabolic effect in an osteoblastic cell line in culture, suggesting that it may be able to stimulate osteoblastic bone formation. It has recently been demonstrated that daidzein can promote bone formation and mineralization *in vitro*. Its activity in this regard is similar to that of genistein, another soy isoflavone (www.pdrhealth.com). The anti-estrogenic effect of daidzein may explain its anti-carcinogenic, anti-atherogenic and anti-osteoporotic activity. Epidemiological studies have long shown that people who consume a lot of soy have reduced incidence of prostate cancers. This benefit of soy could be explained by the anti-cancer and antioxidant activity of daidzein. (www.phytochemicals.com).

2.2.2.3 Puerarin

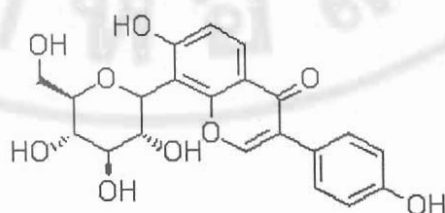


Illustration 3 Puerarin

compounds that possess estrogen-like biological activity. Lamartiniere (2000) mentioned that genistein, found in soy, have weak estrogenic and antiestrogenic properties. Genistein is the aglycone (aglucon) of genistin. The isoflavone is found naturally as the glycoside genistin and as the glycosides 6"-O-malonylgenistin and 6"-O-acetylgenistin. Genistein and its glycosides are mainly found in legumes, such as soybeans and chickpeas. Soybeans and soy foods are the major dietary sources of these substances. Non-fermented soy foods, such as tofu, contain higher levels of the genistein glycosides, while fermented soy foods, such as tempeh and miso, contain higher levels of the aglycone. Genistein is a solid substance that is practically insoluble in water. Its molecular formula is $C_{15}H_{10}O_5$, and its molecular weight is 270.24 daltons. Genistein is also known as 5, 7-dihydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one, and 4', 5, 7-trihydroxyisoflavone. Genistin, which is the 7-beta glucoside of genistein, has greater water solubility than genistein. Genistein has the following structural formula

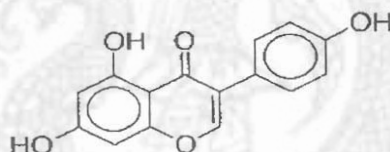


Illustration 1 Genistein

Actions

Genistein has estrogenic and antioxidant activities. It may also have anticarcinogenic, anti-atherogenic and anti-osteoporotic activities.

Mechanism of Action

Genistein has weak estrogenic activity as measured *in vivo* and *in vitro* assays. *In vivo*, its estrogenic activity is one-third that of glycitein and four times greater than that of daidzein. Genistein's anti-estrogenic action may be another possible mechanism to explain its putative anti-breast cancer activity. In the final analysis, the mechanism of genistein's putative anticarcinogenic activity is unclear.

Puerarin (daidzein-8-C-glucoside) is the major bioactive isoflavone of kudzu root (the root of *Pueraria lobata*).

Rasain *et. al.* (2004) suggested that puerarin is hydrolyzed to daidzein by bacterial enzymes in the large intestine and subsequently reduced to dihydrodaidzein and equol. The urine samples collected from rats during 43 hours after puerarin administration contained, in addition to puerarin, the deconjugated and reductive metabolites daidzein, dihydrodaidzein, and equol.

Detection of a large amount of unmetabolized puerarin and only small amounts of its oxidative metabolites, mono- and dihydroxylated puerarin, in the urine in the 0-4 hours period indicated that puerarin is absorbed without hydrolysis and may undergo limited conversion to phase I oxidative reaction products. These metabolites could be of the catechol or pyrogallol types. It is of considerable interest now to investigate which cytochrome P450 isozymes are involved in the formation of these metabolites and to study their biological properties. As catechol formation is considered to be a major pathway in the metabolism of endogenous estrogens (E2), puerarin may interfere with E2 metabolism if they share the same P450 forms.

2.3 Diets and feeding routines for tilapia fry

During early development, the metabolic activity and growth of fry is very high. Therefore, to sustain optimal growth, it should be ensured that the food available to fry contains sufficient quantities of protein for tissue construction and is nutritionally well-balanced.

2.3.1 Natural food

If ponds and outdoor tanks are used to rear fry, a wide selection of natural foods may be available to the fish. Their diets consist largely of a mixture of algae, bacteria and detritus. By adding organic and inorganic fertilizers to the water, the quantity and range of food items can be increased. Addition of organic manures introduces particulate matter which is rapidly broken down by bacteria, protozoans and small invertebrates and encourages the growth of algae, phytoplankton and zooplankton all of which form an excellent source of protein and add to the balance of

the diet. Some natural food is available to the fry at all times, providing essential vitamins and minerals which may be deficient in the supplementary diets but as the stocking density of fry is increased, the quantity of natural food available to each fry will decrease and therefore the quantity and quality of supplementary or formulated diets will become more important. Correctly formulated diets containing required amounts of proteins, lipids, amino acids, fatty acids, minerals and vitamins are crucial if healthy and strong fry are to be produced in the absence of natural foods. Below is the nutrient requirement of different sizes of Nile tilapia.

Nutrient (%)	Size range of fish			
	up to 0.5 g	0.5–10 g	10–35 g	> 35 g
Crude protein	50	35–40	30–35	25–30
Crude lipid	10	10	6–10	6–8
Digestible carbohydrate	25	25	25	25
Fiber	8	8	8–10	8–10

2.3.2 Feeding routine

Under natural conditions, tilapias are omnivorous and choose to browse on detritus, phyto- and zooplanktons, algae and small invertebrates of appropriate size. They are equipped with simple stomachless guts and as such feed continuously during daylight hours. Under hatchery conditions where formulated diets are fed, the growth and survival of tilapia fry will be influenced by factors such as particle size, feeding frequency/ration, and feeding method.

2.3.3 Particle size of food

The particle size acceptable to first-feeders depends on the size of the fry.

	Fish size		Particle size (mm)
	Weight (g)	Standard length (cm)	
Nile Tilapia <i>spp.</i> early fry	0.003–0.005	0.3–0.4	0.1–0.2
Mouth-brooders early fry	0.006–0.015	0.5–0.9	0.2–0.3
	0.05–0.25	1–2	0.25–0.5
	0.25–0.5	2–3	0.5–0.75
Fry of all species	0.5–1	3–4	0.75–1
	1–3	4–5	1–2
	3–30	5–9	2–3

2.4 Feeding frequency and ration

First-feeders grow best when they are fed continuously. They are equipped with a simple gut that is designed to cope with small quantities of food. Under hatchery conditions feeding frequency will be determined by the management of the rearing tanks. If green water systems are used, a feeding frequency of 3 to 4 times a day is adequate. If flow-through systems, high stocking density or clear water systems are used, first-feeders should be fed 6 to 8 times a day. Feeding at the correct frequency and ration is important for both survival and growth. Under low rations and feeding frequencies, size variations of individual fish will increase considerably. Under these conditions, cannibalism of small fry by larger individuals may be as high as 30 to 35%. Underfed tilapia fry become very aggressive.

The feeding frequency and ration may also be related to water temperature and oxygen stress. As the oxygen level and water temperature decrease or fish become stressed by disease, transportation, etc., food consumption will decrease. Under these conditions feeding ration will need to be reduced. Conversely, at higher temperatures, feeding should be increased. Feed may be delivered to tilapias by hand, or by use of demand or automatic feeders. (www.fao.org).

2.5 Physical and Chemical Water Characteristics

2.5.1 Water temperature

In summary, tilapias are best described as thermophilic. Although they can tolerate temperatures of 6° to 10°C for short periods, mortalities due to cold can occur below 12°C. Growth and reproduction are increasingly impaired at temperatures below 20°C and feeding ceases below 11 to 15°C. For most species, growth occurs between 20 to 35°C while above 37°C, increasing mortalities are likely to occur.

Since the aim of aquaculture is to achieve maximum production at minimum cost, it is of prime importance to know at which temperatures maximum growth occurs. Although information on tilapias lag behind than on other cultured species such as salmonids, nevertheless studies to date show that for most of the commercially-important species maximum growth occurs between 28° and 30°C. Above 32°C, growth rate and food intake decrease exponentially with increasing temperature. As a rule, tilapias do not grow well below 16°C, and under conditions several degrees above lethal will often develop fungal and bacterial infections resulting in high mortalities.

2.5.2 Dissolved oxygen

In intensively fertilized ponds where high levels of primary production can occur, it is not unusual for dissolved oxygen concentrations (DO) to fall to near zero at dawn. Oxygen fluctuations can also be observed in intensively-managed raceway or tank systems as a result of high BOD, caused by accumulations of uneaten food and feces. Filamentous algae growing on the sides of these systems can also influence DO levels. Periods of prolonged anoxia follow the collapse of an algal bloom. In practice, tilapias are highly tolerant of low DO concentrations and survival for short periods of time in less than 0.5 mg L⁻¹ have been widely reported. But it has been shown that growth of tilapia is adversely affected if DO saturation falls below 25% for prolonged periods. Exposure to DO levels of less than 20% saturation for 2 or 3 days can lead to mortalities. Tilapias are thus well adapted to survive and grow in most aquaculture situations although they will not, of course, survive complete anoxia. During low

oxygen conditions, fish should not be fed and handling should also be avoided as both cause dramatic increases in the respiration rate.

2.5.3 Total Ammonia Nitrogen (TAN)

TAN consists of two fractions, un-ionized ammonia (NH_3) and ionized ammonia (NH_4^+) and is the by-product of protein metabolism. TAN is excreted from the gills of fish as they assimilate feed and is produced when bacteria decompose organic waste solids within the culture system. The un-ionized form of ammonia-nitrogen is extremely toxic to fish. The fraction of TAN in the un-ionized form is dependent upon the pH and temperature of the water. At a pH of 7.0, most of the TAN is in the ionized form, while at a pH of 8.0 the majority is in the un-ionized form, while the lethal concentration of ammonia-nitrogen for many species has been established, the sublethal effects of ammonia-nitrogen have not been well defined. Reduction in growth rates may be the most important sublethal effect. In general, the concentration of unionized ammonia-nitrogen should not exceed 0.05 mg L^{-1} . (info@fishfarming.com).

2.5.4 pH

As evident from the Henderson-Hasselbach equation, pH is the change in concentration of H^+ concentration which decides the level of pH of water/blood. The pH, as well known, is the negative logarithm of hydrogen ion concentration within the pH range of 0 to 14. Fish grow between 6.5 and 9, and would prefer slightly alkaline water, close to neutral pH (Fig 2). Very low (acid) and very high (alkaline) pH are recorded in natural waters. A point in question close to us is the acid waters of the mangrove swamp; which goes very acid and acid waters develops pH down to 2% per occasion, alkaline pH is more a problem under drier conditions, when dissolved solids, and hardness of waters increase.

Both these conditions can be rectified by proper management for example by liming low pH soil/waters and adding organic matter (manures) to alkaline soils. The acid pH of mangrove swamps can also be corrected by other management methods such as by repeated drying and flushing with tide water (see “acid-sulfate

Wet samples can be frozen by placing them in a vacuum. The more energetic molecules escape, and the temperature of the sample falls by evaporative cooling. Eventually it freezes. About 15% of the water in the wet material is lost.

The simplest form of lyophilizer would consist of a vacuum chamber into which wet sample material could be placed, together with a means of removing water vapor so as to freeze the sample by evaporative cooling and freezing and then maintain the water-vapor pressure below the triple-point pressure. The temperature of the sample would then continue to fall below the freezing point and sublimation would slow down until the rate of heat gain in the sample by conduction, convection, and radiation was equal to the rate of heat loss as the more energetic molecules sublimed away were removed.

This simple approach creates numerous difficulties. When a material is frozen by evaporative cooling it froths as it boils. This frothing can be suppressed by low-speed centrifugation. Centrifugation also helps to dry faster by reducing material thickness and exposing a greater surface area.

An alternative is to freeze the material before it is placed under vacuum. This is commonly done with small laboratory lyophilizers where material is frozen inside a flask. The flask is then attached to a manifold connected to the ice condenser. To speed the process the material can be shell-frozen by rotating the flask in a low-temperature bath, giving a large surface area and small thickness of material.

For larger-scale equipment it is usual to place the material on product-support shelves inside the drying chamber, which can be cooled so that the material is frozen at atmospheric pressure before the vacuum is created. Without a controlled heat input to the sample its temperature would fall until drying was virtually at a standstill. For this reason it is usual to arrange a heat supply to the product-support shelves so that, after their initial use for freezing the product, they can be used to provide heat to replace the energy lost with the subliming water vapor and maintain the product at a constant low temperature (Snowman, 1988.).

soils” in this section).

Effects of pH on biological systems are significant. In the case of fish acid pH causes more or less the same effect on blood-oxygen capacity as the increase in $p\text{CO}_2$ (Bohr effect).

2.6 Rotary Evaporator (Rotavap)



Plate 5 Rotavapor apparatus

Rotary evaporators are used to remove solvents from reaction mixtures. A typical rotary evaporator has a heatable water bath to keep the solvent from cooling or even freezing during the evaporation process. The solvent is removed under vacuum, is trapped by a condenser and is collected for easy reuse or disposal. Most labs use a simple water aspirator vacuum on their rotavap, so a rotavap cannot be used for air and water-sensitive materials unless special precautions are taken (www.SafetyEmporium.com).

2.7 Principle of Lyophilization



Plate 6 Freeze-Dryer apparatus

Chapter 3

Materials and Methodology

The study was divided into three experiments.

3.1 Experiment 1: Comparison of male sex percentage, SR, FCR and GW on three strains (Red, Ghana and Chitralada) of Nile tilapia using MT and *B. superba* crude powder.

3.1.1 Experimental Design and Fish Strains

Randomized Complete Block Design (RCBD) was applied in this part. First-feeding recently hatched Nile tilapia fries were randomly distributed per strain (Red, Ghana and Chitralada) into a total of 54 (1x1x1 m) hapas divided into six Treatments: T0 (Control) pure fishmeal (FM); T1 (40 mg kg⁻¹ MT); T2 (60 mg kg⁻¹ MT); T3 (100 g kg⁻¹ *B. superba*) T4 (200 g kg⁻¹ *B. superba*) and T5 (300 g kg⁻¹ *B. superba*). Each Treatment was replicated three times with 100 fries per replication. The experimental treatment was conducted for 28 days in an approximately 30x20 m² earthen pond and water was maintained at 0.6 meter depth. At the end of 28 days, survival rate (SR) and growth performance in terms of feed conversion rate (FCR) and gain in weight (GW) were evaluated. After 60 days the percentage of male sex was obtained. The strain with the best percent male sex was used in the second experiment.

3.1.2 Feed preparation

The 40 and 60 mg MT were dissolved into separate 200 ml ethanol (95%), sprayed onto two separate one kilogram fishmeal then mixed to produce a ratio of 40 and 60 mg kg⁻¹ and air dried for 24 hours before feeding. Finely powdered *B. superba* tuber roots were thoroughly mixed with fishmeal to produce 100, 200 and 300 g kg⁻¹ *B. superba* and fishmeal ratio. The feed for the negative control group was pure fishmeal.

3.2 Experiment 2: Comparison of male sex percentage, SR, FCR and FW in Nile tilapia Ghana strain) using MT and *B. superba* ethanol crude liquid extract.

3.2.1 Experimental Design and Fish Strain

Based on the result of the previous study the strain with the highest percentage of male sex from *B. superba* Treatments was utilized. The experiment utilized Complete Randomized Design (CRD). It involved simultaneous experiments in aquaria and earthen pond for a period of 28 days (experimental treatment). Treatment 0 (negative Control) was purely fishmeal (FM). Treatment 1 contained MT at a ratio of 60 mg kg⁻¹ fishmeal. Treatment 2 had *B. superba* at 200 g kg⁻¹ fishmeal ratio. Treatments 3, 4 and 5 were *B. superba* ethanol extract at ratios 2, 4 and 6 ml kg⁻¹ fishmeal respectively.

The Part 1 first-feeding recently hatched Ghana strain Nile tilapia fries were randomly distributed into 18 hapas (1x1x1 m) set in an approximately 30x20 m² earthen pond and water was maintained at 0.6 meter depth. Treatments were divided into six replicated three times with 100 fries per hapa.

For Part 2, first-feeding recently hatched Ghana strain Nile tilapia fries were randomly distributed into eighteen aquaria (24 x 12 x 18 inches) divided into six Treatments replicated three times with fifty fries per aquarium. Each aquarium was provided with air stone and water volume was set at 60 liters.

At the end of 70 days feeding trial, male sex percentage, SR and growth performance in terms of FCR and final weight (FW) were evaluated.

3.2.2 *B. superba* extract preparation

An approximately one kilogram of *B. superba* root powder was soaked in a 95% ethyl alcohol for 24 hours then the liquid extract was filtered with sieving cloth. The 40 to 50 ml crude plant extract obtained in liquid form was run through rotary evaporator (R-114, BUCHI, Switzerland) to separate the solvent from the crude extract.

3.2.3 Feed preparation

The 60 mg kg⁻¹ MT preparation was prepared by dissolving MT into 200 ml (95 %) ethanol, sprayed onto the one kilogram fishmeal (FM), mixed and air dried for 24 hours before feeding. *B. superba* extract was thoroughly mixed with fishmeal (FM) to produce 2, 4 and 6 ml kg⁻¹ *B. superba* liquid extract and fishmeal ratio. The negative Control was purely fishmeal.

3.3 Experiment 3: Comparison of male sex percentage, SR, FCR and FW in Nile tilapia using MT and *B. superba* extract freeze-dried powder form.

3.3.1 Experimental Design and Fish Strains

Complete Randomized Design (CRD) was utilized. First-feeding recently hatched Nile tilapia fries were randomly distributed into a total of 15 (60 liters) aquariums divided into five Treatments: The treatment regimen was composed of T0 (pure fishmeal), T1 (60 mg kg⁻¹ MT and fishmeal ratio) and red kwao krea freeze-dried powder (T2, 70 mg kg⁻¹; T3, 80 mg kg⁻¹ and T4, 90 mg kg⁻¹ freeze-dried extract and fishmeal ratio). Each Treatment was replicated three times with 30 fries per replication. The experimental treatment was conducted for 28 days. At the end of 70 days feeding trial, male sex percentage, SR and growth performance in terms of FCR and FW were evaluated.

3.3.2 Extraction of *B. superba* compound

One kilogram *B. superba* root powder was soaked in 1.2 liters of ethanol (95%) for 24 hours then it was filtered using sieving cloth to separate the filtrate from the liquid component. The filtrate was again soaked in one liter of ethanol for 24 hours then sieved to separate the liquid component. This process was done for the third time using 800 ml ethanol. A total of 2.1 liters of ethanol solution was obtained. After every filtration the solution was run through rotary evaporator (Rotavapor114, BUCHI, Switzerland) and a total of 120 ml extract component was recovered. The

extract was freeze-dried (Freezone 6, LABCONCO, USA) to produce 50 grams crude powder.

3.3.3 Feed preparation

The 60 mg kg⁻¹ MT preparation was prepared by dissolving MT into 200 ml (95 %) ethanol, sprayed onto the one kilogram fishmeal (FM), mixed and air dried for 24 hours before feeding. *B. superba* free-dried extract powder was thoroughly mixed with fishmeal (FM) to produce 70, 80 and 90 gm kg⁻¹ *B. superba* freeze-dried extract powder and fishmeal ratio. The negative Control was purely fishmeal.

3.4 Feeding regimen

The amount of feed given (grams) was fixed based on the assumed average bodyweight per fry (grams) per week: week 1 (0.01), 2 (0.06), 3 (0.20), 4 (0.30). During the first, second, third and fourth week fries were given feed equal to 30, 20, 15 and 10 % of their assumed bodyweight with a feeding rate of five, four, three and two times per day respectively. This regimen was applied on all experiments.

3.5 Water Quality Parameters

Daily water temperature was recorded while dissolved oxygen (DO) (YSI MODEL 57, YSI Inc., USA), total ammonia-nitrogen (TAN) (DR 2000, HACH Co., USA) and pH (HI 9812, HANNA Instruments, Thailand) were obtained twice during the experiments.

3.6 Gonad examination

A total of ten percent fish samples in experiment 1, 10 and 30 % in earthen pond and aquaria in experiment 2 and 30 % in experiment 3 were randomly obtained in every replication from all the treatments after 60 days (experiment 1) and 70 days (experiments 2 and 3) for gonad examination. The gonads were surgically removed and placed on the slide then stained with a drop of Giemsa stain then a cover slide was put on top and pressed lightly and the specimen was examined microscopically (40x) (Nuanmanee, 2004). The male Nile tilapia gonad was identified by the presence of cyst-like structures containing spermatogonia and spermatocytes while the presence of oocytes at different stages of development was identified as female

gonad. The gonad of intersex had oocytes scattered among testicular tissue (Wasserman and Afonso, 2002).

3.7 SR, FCR, GW and FW

Shown below are the growth parameters obtained in the experiment (survival rate, feed conversion ratio (experiments 1, 2, and 3) and gain in weight (experiment 1) and final weight (experiment 2 and 3)).

$$\text{SR (\%)} = \frac{\text{\# of live fish}}{\text{total \# of loaded fish}} \times 100$$

$$\text{FCR} = \frac{\text{total feeds consumed}}{\text{net weight of fish}}$$

$$\text{GW} = \text{total final weight} - \text{initial weight}$$

$$\text{FW} = \text{initial weight} + \text{gain in weight}$$

3.8 Statistical analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) of the Statistical Package for Social Studies (SPSS 14.0).

Chapter 4

Results

Experiment 1

Presented in Table 1 is the percent male of Treatments from the three strains. Treatments 1 and 2 (MT treatments) were statistically comparable. Furthermore, MT Treatments were significantly higher compared to the Control except in the Chitralada strain. It was also evident however that the target $\geq 95\%$ male sex percentage was not observed. The percentage of intersex was consistently observed and was high in T1 (29.9%), T2 (26.7%) and T4 (13.0%). T3, T4 and T5 (*B. superba* Treatments) were also statistically comparable to T1 and T2 except between T2 and T3 of Ghana and Chitralada strains respectively and T2 and T5 of Chitralada strain. The range of percent male sex from the *B. superba* Treatments was 48.2 ± 9.9 (Red) to 72.2 ± 25.5 % (Ghana). The dosage did not affect statistically the percent male sex between the *B. superba* treatments although highest sex reversal was observed in T4 ($72.2 \pm 25.5\%$) of Ghana strain.

Comparison between strains indicated no significant difference whichever of the three strains was utilized similar result would be obtained.

Table 1. Comparison of mean male sex (%) $\pm$ SD between Treatments of three strains of *O. niloticus*.

Treatments	Red (%)	Ghana (%)	Chitralada (%)
T0 (100% fishmeal, FM)	29.5 ± 26.4^{Aa}	47.2 ± 21.0^{Aa}	69.2 ± 8.1^{Ab}
T1 (40 mg MT kg ⁻¹ FM)	69.3 ± 31.2^{Ab}	83.3 ± 15.3^{Abc}	70.1 ± 7.6^{Ab}
T2 (60 mg MT kg ⁻¹ FM)	73.3 ± 23.0^{Ab}	93.3 ± 5.8^{Ac}	94.4 ± 9.6^{Ab}
T3 (100 g <i>B. superba</i> kg ⁻¹ FM)	57.9 ± 11.8^{Aab}	62.2 ± 3.8^{Aab}	60.2 ± 13.1^{Aa}
T4 (200 g <i>B. superba</i> kg ⁻¹ FM)	48.2 ± 9.9^{Aab}	72.2 ± 25.5^{Aabc}	70.9 ± 16.3^{Aab}
T5 (300 g <i>B. superba</i> kg ⁻¹ FM)	52.4 ± 4.1^{Aab}	68.1 ± 6.4^{Aabc}	52.2 ± 32.7^{Aa}

* Means that do not share the same letter superscript in the same row (capital letters) and column (small letters) are statistically significant ($P < 0.05$).

Shown in Tables 2, 3 and 4 are the SR, FCR and GW between species respectively. In Table 2 the SR of Red strain was statistically significant to other strains in T2 while a significant difference in T4 was observed between the three

species. The unusual low SR of Red Nile tilapia species in T2 could have been brought about by the escape of the some fishes through a hole from the damaged cage net observed after the treatment proper.

Comparison among Treatments in the Red strain showed that significantly low and high SR was observed in T2 and T5 respectively as compared to the Control. In Ghana strain, only T1 was statistically better with the Control. No significant difference was observed among the Treatments in the Chitralada strain.

Table 2. Comparison of mean SR (%) $\pm$ SD between Treatments of three strains of *O. niloticus*.

Treatments	Red (%)	Ghana (%)	Chitralada (%)
T0 (100% fishmeal, FM)	77.3 $\pm$ 6.4 ^{Abc}	81.3 $\pm$ 13.4 ^{Aa}	78.0 $\pm$ 6.0 ^{Aa}
T1 (40 mg MT kg ⁻¹ FM)	67.7 $\pm$ 21.0 ^{Ab}	98.0 $\pm$ 3.5 ^{Ab}	85.7 $\pm$ 24.8 ^{Aa}
T2 (60 mg MT kg ⁻¹ FM)	33.3 $\pm$ 0.2 ^{Aa}	92.3 $\pm$ 13.3 ^{Bab}	72.0 $\pm$ 38.6 ^{ABa}
T3 (100 g <i>B. superba</i> kg ⁻¹ FM)	86.7 $\pm$ 6.0 ^{Abc}	92.3 $\pm$ 2.5 ^{Aab}	88.7 $\pm$ 2.0 ^{Aa}
T4 (200 g <i>B. superba</i> kg ⁻¹ FM)	85.0 $\pm$ 4.4 ^{Bbc}	92.3 $\pm$ 2.5 ^{Cab}	76.3 $\pm$ 3.2 ^{Aa}
T5 (300 g <i>B. superba</i> kg ⁻¹ FM)	89.3 $\pm$ 2.3 ^{Ac}	93.3 $\pm$ 1.5 ^{Aab}	90.0 $\pm$ 8.7 ^{Aa}

* Means that do not share the same letter superscript in the same column (small letters) and row (capital letters) are statistically significant (P<0.05).

As shown in Table 3 the FCR of Chitralada strain was significantly better and poorer between the other two species in T4 (1.0) and T5 (1.4) while the Ghana strain was significantly poorer from the other species in T3 (1.4). As can be noted in *B. superba* Treatments, poor FCR was observed in Ghana and Chitralada strains in T3 (1.4) and T5 (1.4) respectively. Between the MT treatments no statistical significance was observed between species.

Comparison between Treatments in the Red strain revealed no significant difference. In Ghana strain, only T3 (1.4) was statistically different among the Treatments while T1 (0.8) and T2 (0.8) were significantly different among all Treatments in the Chitralada strain.

Table 3. Comparison of mean FCR $\pm$ SD between Treatments of three strains of *O. niloticus*.

Treatments	Red	Ghana	Chitralada
T0 (100% fishmeal, FM)	0.9 $\pm$ 0.1 ^{Aa}	1.0 $\pm$ 0.3 ^{Aa}	1.3 $\pm$ 0.2 ^{Ab}
T1 (40 mg MT kg ⁻¹ FM)	1.3 $\pm$ 0.5 ^{Aa}	0.9 $\pm$ 0.0 ^{Aa}	0.8 $\pm$ 0.3 ^{Aa}
T2 (60 mg MT kg ⁻¹ FM)	1.4 $\pm$ 0.6 ^{Aa}	0.8 $\pm$ 0.2 ^{Aa}	0.8 $\pm$ 0.3 ^{Aa}
T3 (100 g <i>B. superba</i> kg ⁻¹ FM)	1.2 $\pm$ 0.2 ^{Aa}	1.4 $\pm$ 0.0 ^{Bb}	1.0 $\pm$ 0.1 ^{Aab}
T4 (200 g <i>B. superba</i> kg ⁻¹ FM)	0.9 $\pm$ 0.0 ^{Aa}	0.9 $\pm$ 0.0 ^{Aa}	1.0 $\pm$ 0.0 ^{Bab}
T5 (300 g <i>B. superba</i> kg ⁻¹ FM)	1.1 $\pm$ 0.2 ^{Aa}	0.9 $\pm$ 0.0 ^{Aa}	1.4 $\pm$ 0.2 ^{Bb}

* Means that do not share the same letter superscript in the same column (small letters) and row (capital letters) are statistically significant (P<0.05).

In Table 4, the GW of Ghana and Chitralada strains in T3 (36.0 $\pm$ 1.0 grams) and T5 (38.3 $\pm$ 6.1 grams) were significantly lowest. Ghana strain had a better GW in T5 (58.2 $\pm$ 6.3 grams) while Chitralada had a higher GW in T3 (50.5 $\pm$ 7.0). Chitralada strain had a better GW in T3 while Ghana was better in T5. Highest GW was noted in Ghana strain of T2 (76.3 $\pm$ 14.3).

In terms of GW, no significant difference was observed among the Treatments in the Red strain while T2 was significantly different among all Treatments in the Ghana strain. MT treatments were found to be statistically best among the Treatments in Chitralada strain

Table 4. Comparison of mean GW (grams) $\pm$ SD between Treatments in three strains of *O. niloticus*.

Treatments	Red (grams)	Ghana (grams)	Chitralada (grams)
T0 (100% fishmeal)	56.0 $\pm$ 7.5 ^{Aa}	53.9 $\pm$ 13.0 ^{Ab}	39.9 $\pm$ 6.2 ^{Aa}
T1 (40 mg MT kg ⁻¹ FM)	42.9 $\pm$ 16.9 ^{Aa}	57.8 $\pm$ 2.0 ^{Ab}	74.3 $\pm$ 27.3 ^{Ab}
T2 (60 mg MT kg ⁻¹ FM)	43.7 $\pm$ 24.4 ^{Aa}	76.3 $\pm$ 14.3 ^{Ac}	68.6 $\pm$ 20.9 ^{Ab}
T3 (100 g <i>B. superba</i> kg ⁻¹ FM)	45.6 $\pm$ 8.1 ^{ABa}	36.0 $\pm$ 1.0 ^{Aa}	50.5 $\pm$ 7.0 ^{Ba}
T4 (200 g <i>B. superba</i> kg ⁻¹ FM)	58.4 $\pm$ 6.4 ^{Aa}	57.5 $\pm$ 5.3 ^{Ab}	47.5 $\pm$ 4.0 ^{Aa}
T5 (300 g <i>B. superba</i> kg ⁻¹ FM)	48.5 $\pm$ 8.4 ^{ABa}	58.2 $\pm$ 6.3 ^{Bb}	38.3 $\pm$ 6.1 ^{Aa}

* Means that do not share the same letter superscript in the same column (small letters) and row capital letters) are statistically significant (P<0.05)

Experiment 2

As shown in Table 5, the male sex percentage in T1 (90%) was statistically significant with other Treatments. Highest SR was observed in T1 (89%) which was

comparable with T2 (82%), T3 (74.3%) and T4 (84.7%). The lowest SR was in T0 (64.7%) and statistically comparable with T5 (66.5%). Statistically comparable effects were observed in FCR and FW.

Table 5. Comparison of mean male sex (%), SR (%), FCR and FW (grams) $\pm$ SD in Ghana strain *O. niloticus* raised in earthen pond.

Treatments	Male sex (%)	SR (%)	FCR	FW (grams)
T0 (100% fishmeal, FM)	45.2 $\pm$ 5.0 ^a	64.7 $\pm$ 5.0 ^a	0.90 $\pm$ 0.01 ^a	167.25 $\pm$ 15.0 ^{0a}
T1 (60 mg MT kg ⁻¹ FM)	90.0 $\pm$ 10.0 ^a	89.0 $\pm$ 10.1 ^c	0.86 $\pm$ 0.09 ^a	174.25 $\pm$ 41.2 ^a
T2 (200 g <i>B. superba</i> kg ⁻¹ FM)	58.2 $\pm$ 10.5 ^b	82.0 $\pm$ 12.1 ^{abc}	0.86 $\pm$ 0.03 ^a	176.75 $\pm$ 37.8 ^a
T3 (2 ml <i>B. superba</i> kg ⁻¹ FM)	31.2 $\pm$ 28.5 ^a	74.3 $\pm$ 8.3 ^{abc}	0.85 $\pm$ 0.01 ^a	185.75 $\pm$ 24.8 ^a
T4 (4 ml <i>B. superba</i> kg ⁻¹ FM)	50.0 $\pm$ 26.5 ^a	84.7 $\pm$ 7.4 ^{bc}	0.85 $\pm$ 0.01 ^a	180.00 $\pm$ 16.5 ^a
T5 (6 ml <i>B. superba</i> kg ⁻¹ FM)	56.7 $\pm$ 5.8 ^a	66.7 $\pm$ 11.9 ^{ab}	0.86 $\pm$ 0.01 ^a	177.50 $\pm$ 17.5 ^a

* Means that do not share the same letter superscript in the same column are statistically significant (P<0.05).

Shown in Table 6 are the male sex percentage, SR, FCR and FW of Ghana strain Nile tilapia treated with *B. superba* liquid ethanol-extract. Highest male sex ratio was observed in T1 (97%) which was statistically significant among all Treatments. This was followed by T2 (68.1%); T4 (58.9 %); T0 (54.8%) and T3 (50.0%) which were statistically comparable. The lowest was observed in T5 (28.6%) but was statistically comparable with the Control. In terms of SR, T4 (98.7%) was highest followed by T1 (93.1%); T5 (94.0%) and T3 (93.3%) which were all statistically comparable. T2 (87.3%) and T0 (86.7%) were statistically lower than T4 and T1. No significant difference was observed among the Treatments for FCR. With regards FW, the highest was observed in T4 (59.7 grams) followed by T3 (57.1 grams) and T5 (54.4 grams) which were all statistically comparable. T2 had the lowest FW (46.7 grams) but was statistically comparable with T0 (51.0 $\pm$ 1.7 grams) and T1 (51.0 $\pm$ 2.6 grams).

Table 6. Comparison of mean male sex (%), SR (%), FCR and FW (grams) ($\pm$ SD) of Ghana strain *O. niloticus* raised in aquaria.

Treatments	Male	SR	FCR	FW (grams)
T0 (100% fishmeal, FM)	54.8 $\pm$ 21.9 ^{ab}	86.7 $\pm$ 4.2 ^a	1.3 $\pm$ 0.1 ^a	51.0 $\pm$ 1.7 ^{ab}
T1 (60 mg MT kg ⁻¹ FM)	97.0 $\pm$ 5.2 ^c	96.7 $\pm$ 5.8 ^b	1.3 $\pm$ 0.2 ^a	51.0 $\pm$ 2.6 ^{ab}
T2 (200 g <i>B. superba</i> kg ⁻¹ FM)	68.1 $\pm$ 18.8 ^b	87.3 $\pm$ 5.0 ^a	1.5 $\pm$ 0.6 ^a	46.3 $\pm$ 5.7 ^a
T3 (2 ml <i>B. superba</i> kg ⁻¹ FM)	50.0 $\pm$ 17.3 ^{ab}	93.3 $\pm$ 4.6 ^{ab}	1.2 $\pm$ 0.1 ^a	57.1 $\pm$ 2.1 ^c
T4 (4 ml <i>B. superba</i> kg ⁻¹ FM)	58.9 $\pm$ 8.3 ^b	98.7 $\pm$ 2.3 ^b	1.2 $\pm$ 0.0 ^a	59.7 $\pm$ 1.5 ^c
T5 (6 ml <i>B. superba</i> kg ⁻¹ FM)	28.6 $\pm$ 14.3 ^a	94.0 $\pm$ 5.3 ^{ab}	1.3 $\pm$ 0.0 ^a	54.4 $\pm$ 0.7 ^{bc}

* Means that do not share the same letter superscripts in the same column are statistically significant ($P < 0.05$).

Experiment 3

Shown in Table 7 are the male sex percentage, SR, FCR and FW of Nile tilapia after 70 days feeding. T1 (100.0%) was statistically the best among all Treatments in terms of male sex percentage while the rest of the Treatments were statistically comparable (40.0-56.7%). SR was statistically comparable among all Treatments. FCR was best in T3 (0.6) and T4 (0.6) but least efficient in T0 (1.4). The FW of T3 and T4 were highest (122.7 and 125.3 grams respectively) and statistically significant with T0 (56 grams) and T1 (72.3 grams).

Table 7. Comparison of mean male sex (%), SR (%), FCR and FW (grams) $\pm$ SD of *O. niloticus* raised in aquaria.

Treatment	Male sex (%)	SR (%)	FCR	FW (grams)
T0 (100% fishmeal, FM)	50.0 $\pm$ 0.0 ^a	98.9 $\pm$ 1.9 ^a	1.4 $\pm$ 0.3 ^c	56.0 $\pm$ 12.5 ^a
T1 (60 mg MT kg ⁻¹ FM)	100.0 $\pm$ 0.0 ^b	100.0 $\pm$ 0.0 ^a	1.0 $\pm$ 0.0 ^b	72.3 $\pm$ 3.8 ^a
T2 (70 g <i>B. Superba</i> kg ⁻¹ FM)	40.0 $\pm$ 10.0 ^a	98.9 $\pm$ 1.9 ^a	0.7 $\pm$ 0.2 ^{ab}	102.7 $\pm$ 32.7 ^{ab}
T3 (80 g <i>B. Superba</i> kg ⁻¹ FM)	56.7 $\pm$ 5.8 ^a	100.0 $\pm$ 0.0 ^a	0.6 $\pm$ 0.1 ^a	122.7 $\pm$ 37.3 ^b
T4 (90 g <i>B. Superba</i> kg ⁻¹ FM)	56.7 $\pm$ 15.3 ^a	99.3 $\pm$ 1.4 ^a	0.6 $\pm$ 0.1 ^a	125.3 $\pm$ 27.6 ^b

* Means that do not share the same letter superscript in the same column are significantly different ($P < 0.05$).

Chapter 5

Discussion

Discussion

Experiment 1

The percent male sex reversal of *B. superba* Treatments in some instances statistically did not differ between MT Treatments however, it is still clear that the latter had better and efficient male sex reversal. The use of 40 mg kg⁻¹ MT dosage yielded male sex percentage statistically comparable to that of 60 mg kg⁻¹ dosage hence, using both dosages could yield comparable effects in the earthen pond environment. Cagauan *et.al.* (2004) mentioned that male sex reversal using MT has less control of reversal efficiency when done in the natural environment where food is present. This result plus the low stocking density (Phelps *et. al.*, 1995) suggest that the earthen pond provides a source of food aside from the artificial feed being offered thus less treated feed may be taken in. Macintosh *et. al.* (1986) and Abucay and Mair (1997) were consistently successful in sex reversal of tilapia species using 40 mg kg⁻¹ MT dosage under closed water system. On the other hand, Pongtana *et. al.* (2004) utilized 60 mg kg⁻¹ MT to achieve 97.9 ±1.5% male sex tilapia for a period of 21 days. This practice is currently used in commercial mass production of sex reversed Nile tilapia fry. The incidence of intersex was also consistently observed especially in the MT treatments, which further suggests that the presence of readily available food in an earthen pond affected the efficiency of sex reversal using MT. It is also important to note that MT is an aromatizable androgen which means it can be converted into estrogen by an enzyme produced in the liver called aromatase (Alfonso *et. al.*, 2001 as cited by Alfonso and Lebouté, 2003). There were also varying results as to the effect of *B. superba* and MT treatments on FCR and GW. MT treatment was not a significant factor in terms of FCR and GW when treatment was done in natural environment unlike when it was done in closed or recirculating water systems (Jo *et. al.*, 1988). *B. superba*'s effect for male sex reversal needs to be evaluated by using other forms than crude powder but to produce an all male population is so far have not been observed. Intesex gonad was also consistently observed during this experiment. Ghana strain in T0 had 3.7 % intersex while Red, Ghana and Chitralada strains in T1

had 19.6, 10.0 and 29.9 % respectively. Red and Ghana strains in T2 had 26.7 and 3.3 % intersex respectively. Only Ghana strain in T3 (3.3 %) and T5 (7.9) had intersex while Red (8.9 %) and Ghana (13.0 %) strains had intersex in T4.

Experiment 2

Part 1

The application of MT in the earthen pond consistently produced slightly lower male sex ratio as compared to the optimum requirement but is considerably appreciable as was observed in our previous study (Meng-Umphun *et.al.*, 2005, on press). In addition, was the observation that FCR and FW were not affected by the inclusion of either MT or *B. superba*. Anabolism as suggested in other studies which utilized MT could be variable or is offset depending on the amount of available food in the earthen pond. The 200 grams per kilogram dosage had higher male sex percentage although statistically comparable with the Control and *B. superba* liquid extract Treatments. Better SR was observed among the Treatments as compared with the Control which had the lowest SR which suggests no harmful components of the *B. superba* to the fish.

Part 2

Treatment of MT to achieve a monosex population ($\geq 95\%$) is observed in confined systems. In this part of the study treatment was done in aquaria and as expected it resulted to high male sex percentage. As with the experiment in the earthen pond, addition of 200 g per kilogram crude powder had higher male percent as compared with the Control and liquid extracts but not to the required percentage to produce a monosex population. Another interesting result is the very low percentage of male Nile tilapia in T5 (28.6%) where 6 ml *B. superba* extract was used. This plant is believed to have phytoandrogen but other studies also suggest it has both antiestrogenic and estrogenic effects owing to the presence of isoflavone genistein and daidzein. Better survival rate was also consistent in MT and *B. superba* treatments which were above 90%. The FCR was comparable as was in the earthen pond but *B. superba* liquid extract treatments had better FW in T3 and T4. The MT treatment did not effect to improve FW and FCR which suggests anabolism as

theorized with the use of MT was not a factor. The high fiber content of T2 (200 g kg⁻¹ *B. superba*) may have reduced the nutritional value of the fishmeal so that it had the highest FCR and FW. This was in contrary when done in the earthen pond because of the presence of natural food compensated for the lack in the feed mixture.

Experiment 3

The study suggests that *B. superba* freeze-dried extract did not effect sex change however, the FW was better compared to both Controls. Based on the results of the study the plant in general possesses traits that promote growth in Nile tilapia aside from its use as a rejuvenation and treatment of impotence in male (Chershewashart, 2003). Most papers relating to the use of *B. superba* are concentrated mainly on the benefits to the reproductive system of male, anti-viral and anti mutagenic effects. A study on the growth of female Nile tilapia supplemented with *P. mirifica*, an herbal plant belonging to the same family with *B. superba*, during winter season revealed higher growth rate (Saetaphan and Sobin, 2006). In the study of Jintasaporn *et. al.* (2000), the growth rate of Snake skin gourami (*Trichogaster pectoralis*) when fed with *P. mirifica* was not significant during the first 30 days of feeding but it significantly increased after 60 days of feeding which suggested that prolonged feeding of this herbal plant promoted growth. Based on the crude nutrient analysis done in our experiment, unusually high energy content was observed in the *B. superba* freeze-dried extract powder (261.5 kcal g⁻¹) which could have directly contributed to the high growth rate of the experimental fish.

Chapter 6

Conclusion and Recommendations

6.1 Conclusion

Based on the results obtained, the following were concluded:

1. *B. superba* did not effect all-male population in all the experimental procedures applied in the study.
2. The use of either 40 or 60 mg kg⁻¹ MT produced statistically comparable male sex percentage when done in earthen pond.
3. In general, the SR in all the experiments was within the satisfactory range of Nile tilapia production.
4. The FCR, GW and/or FW of *B. superba*-treated Nile tilapia were more often comparatively better than those in the Control and MT.
5. Interestingly, Nile tilapia fed with fishmeal fortified with *B. superba* freeze-dried root extract was able to enhance growth in Nile tilapia.
6. Based on the first experiment, similar result was obtained in terms of male sex percentage between the three strains. Moreover, the SR among the strains was within satisfactory range of Nile tilapia production. Generally, similar FCR and GW were also noted.

6.2 Recommendations

Based on the experiments conducted the following are recommended for future studies.

1. Evaluate the growth performance of the Nile tilapia until growing and or breeding stage.
 2. Evaluate the sperm morphology, volume and density of male tilapia breeder.
 3. Evaluate physiologically, morphologically and histologically the ovaries and testicles of *B. superba*-treated breeder fish.
 4. Evaluate the sex reversal effect and growth performance of Nile tilapia given with a combination of *B. superba* and MT at different rates.
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Chapter 7

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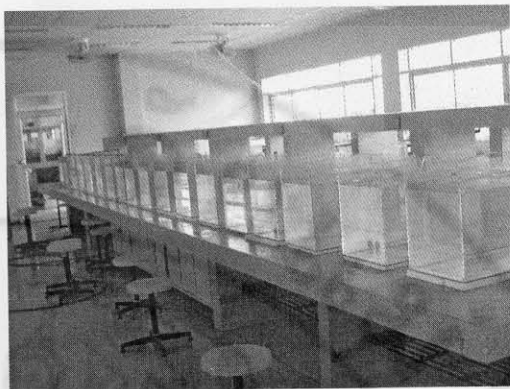
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LIST OF ADDITIONAL PLATES

Plate 7 Experimental areas



Earthen pond

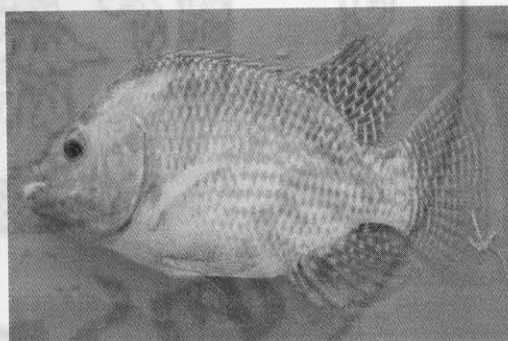


Aquaria

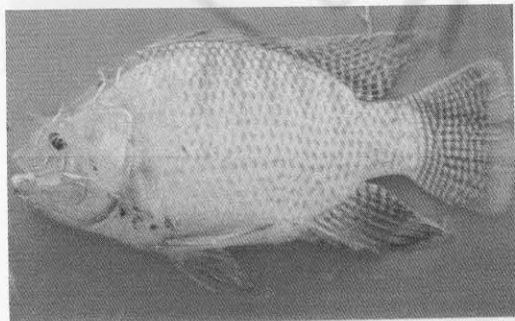
Plate 8 Experimental Fish



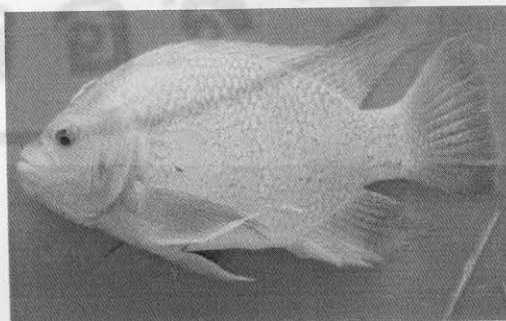
Nile tilapia fries



Nile tilapia (ghana strain)



Nile tilapia (chitralada strain)



Nile tilapia (red strain)

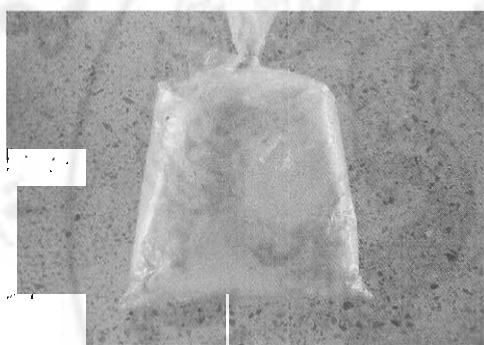
Plate 9 Experimental compounds



17- α -Methyltestosterone (MT)



Butea superba extract

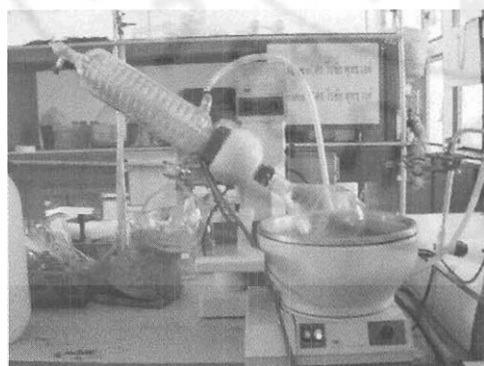


Butea superba powdered root



Butea superba powdered crude extract

Plate 10 Equipment for extraction and freeze drying

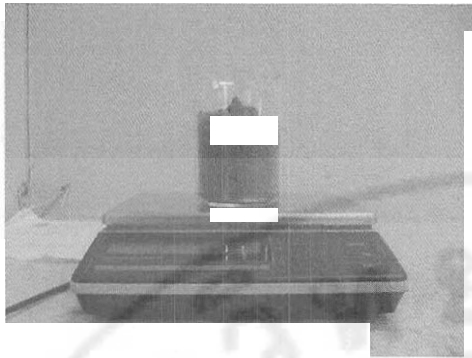


Rotary evaporator



Freeze-drying machine

Plate 11 Feeds and Feeding

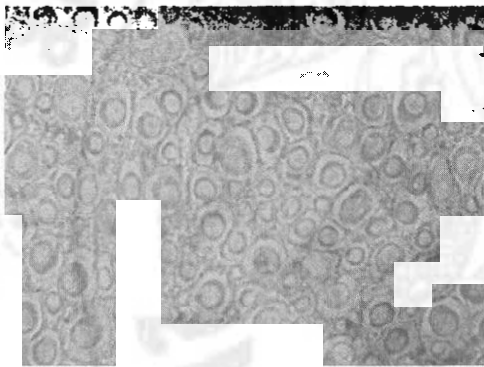


Fishmeal

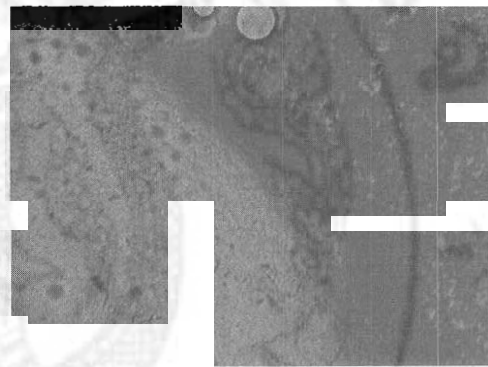


Feeding

Plate 12 Male, Female and intersex gonads of Nile tilapia.



Nile tilapia ovary under the (LPO)



Nile tilapia testis under the microscope (LPO)



Nile tilapia intersex under the microscope (LPO)

LIST OF APPENDICES

Appendix 1. Water temperature, Dissolved Oxygen, pH and Total Ammonia Nitrogen in the earthen fish pond of Experiment 1.

Water temp. ($^{\circ}\text{C}$)	DO (mg/l)	pH	TAN (mg/l)
26.5	3.0	8.7	0.7

Appendix 2. Water temperature, Dissolved Oxygen, pH and Total Ammonia Nitrogen in the aquaria of Experiment 2.

Treatments	Water temp. ($^{\circ}\text{C}$)		DO (mg/l)		pH		TAN (mg/l)	
	Week 2	Week 4	Week 2	Week 4	Week 2	Week 4	Week 2	Week 4
T0	26.5	27.0	8.1	7.0	7.3	6.8	0.79	0.24
T1	26.5	27.0	8.1	6.8	7.3	6.6	0.77	0.21
T2	26.8	27.0	8.0	6.7	7.3	6.8	0.73	0.32
T3	26.2	27.0	8.2	7.0	7.6	6.8	0.82	0.24
T4	26.1	27.0	8.3	6.8	7.8	6.7	0.79	0.20
T5	26.9	27.0	7.9	7.0	7.3	6.7	0.70	0.15

Appendix 3. Water temperature, Dissolved Oxygen, pH and Total Ammonia Nitrogen in the earthen fish pond of Experiment 2.

Week	Water temp. ($^{\circ}\text{C}$)	DO (mg/l)	pH	TAN (mg/l)
2	26.9	3.1	6.8	0.67
4	31.0	9.0	8.38	0.14

Appendix 4. Water temperature, Dissolved Oxygen, pH and Total Ammonia Nitrogen in the earthen fish pond of Experiment 3.

Treatments	Water temp. ($^{\circ}\text{C}$)		DO (mg/l)		pH		TAN (mg/l)	
	Week 2	Week 4	Week 2	Week 4	Week 2	Week 4	Week 2	Week 4
T0	23.0	26.0	4.2	7.9	7.6	7.7	2.2	1.0
T1	23.0	26.0	4.0	7.6	7.6	7.5	2.0	0.6
T2	23.0	26.0	4.1	7.4	7.5	7.4	2.3	0.9
T3	23.0	26.0	4.0	7.2	7.5	7.4	2.0	0.5
T4	23.0	26.0	4.0	7.0	7.5	7.4	2.2	0.5

Appendix 5. Abstract of Published paper on Asian Fisheries Science Volume 19 no. 4
(on press).

The potential of red kwao kreua (*Butea superba*) in inducing sex reversal on
three strains (red, ghana, chitralada) of Nile tilapia (*Oreochromis niloticus* L.)
and the effect of 17- α -Methyltestosterone (MT)

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Keywords: *Butea superba*, 17- α -Methyltestosterone, sex reversal, *Oreochromis niloticus*

Abstract:

The present study aimed to investigate the potential of red kwao kreua and compare the effect between two MT dosage regimens in terms of inducing sex reversal, survivability rate, feed conversion ratio and gain in weight on red, ghana and chitralada strains of Nile tilapia. A 100 g kg⁻¹, 200 g kg⁻¹ and 300 g kg⁻¹ red kwao kreua dried and pounded roots and 40 and 60 mg kg⁻¹ MT were prepared and mixed per one kilogram fishmeal. Results revealed that MT treatments had a comparable effect in terms of male sex ratio, SR, FCR and GW and are statistically significant as compared to the control. Red kwao kreua treatments did not have significant difference with the control and with the MT treatments in some cases. However, a 72.2 $\pm$ 25.5 % male sex ratio was obtained in the treatment 4 of the ghana strain. As to the effect of the treatments on the three strains it was observed they generally have comparable effects.

Appendix 6. Paper presented to the 2nd International Symposium on Cage Aquaculture in Asia (CAA2).

THE EFFECT OF RED KWAO KREUA (*Butea superba*) AND 17- α -METHYLTESTOSTERONE (MT) ON SOME GROWTH PARAMETERS AND IN INDUCING SEX REVERSAL ON GHANA STRAIN NILE TILAPIA (*Oreochromis niloticus* L.) RAISED IN HAPAS

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Abstract:

The study aimed to investigate the effect of red kwao kreua and two MT dosage regimens in terms of inducing sex reversal, survival rate (SR), feed conversion ratio (FCR) and gain in weight (GW) on ghana strain of Nile tilapia. A 100 g·kg⁻¹(T3), 200 g·kg⁻¹(T4) and 300 g·kg⁻¹(T5) red kwao kreua pounded roots, 40 (T1) and 60 mg·kg⁻¹(T2) MT and 100% fishmeal (T0) were prepared and mixed per one kilogram fishmeal and fed to first feeding fry for a period of 28 days caged in hapas in an earthen pond. Results revealed that male sex ratio in T0 (47.2 $\pm$ 21.0%) was statistically significant with T1 (83.3 $\pm$ 15.3%) and T2 (93.3 $\pm$ 5.8%) and was not significantly different with T3 (62.2 $\pm$ 3.8%), T4 (72.2 $\pm$ 25.5%) and T5 (68.1 $\pm$ 6.4%). Comparison between red kwao kreua treatments revealed no significant difference. T4 and T5 were also statistically comparable with MT Treatments. Moreover, dosage did not affect statistically the male sex ratio between MT Treatments in the earthen pond and the target $\geq$ 95% male sex ratio was not achieved. However, the incidence of intersex was common. Survival rate in the Control (81.3 $\pm$ 13.4%) was significantly different with T1 (98.0 $\pm$ 3.5%) but not statistically significant with T2 (92.3 $\pm$ 13.3%), T3 (92.3 $\pm$ 2.5%), T4 (92.3 $\pm$ 2.5%) and T5 (93.3 $\pm$ 1.5%). The FCR in the Control (1.0 $\pm$ 0.3) was statistically significant with T3 (1.4 $\pm$ 0.0) but not with T1 (0.9 $\pm$ 0.0), T2 (0.8 $\pm$ 0.2), T4 (0.9 $\pm$ 0.0) and T5 (0.9 $\pm$ 0.0). Regardless of the dosage, MT was not observed to provide better FCR in the earthen pond. In terms of GW (grams), the Control (53.9 $\pm$ 13.0) was statistically different with T2 (76.3 $\pm$ 14.3) and T3 (36.0 $\pm$ 1.0) and comparable with T1 (57.8 $\pm$ 2.0), T4 (57.5 $\pm$ 5.3) and T5 (58.2 $\pm$ 6.3).

Appendix 7. Paper presented to the 7th Maejo Conference.

The potential of red kwao kreua (*Butea superba*) in inducing sex reversal on three strains (red, ghana, chitralada) of Nile tilapia (*Oreochromis niloticus* L.) and the effect of 17- α -Methyltestosterone (MT)

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Abstract

The present study aimed to investigate the potential of red kwao kreua and compare the effect between two MT dosage regimens in terms of inducing sex reversal, survivability rate (SR), feed conversion ratio (FCR) and gain in weight (GW) on red, ghana and chitralada strains of Nile tilapia. A 40 (T1) and 60 mg kg⁻¹ (T2) MT and 100 g kg⁻¹ (T3), 200 g kg⁻¹ (T4) and 300 g kg⁻¹ (T5) red kwao kreua dried and pounded roots were prepared and mixed per one kilogram fishmeal. The highest male sex reversal was observed in T2 at 73.3% $\pm$ 23.0, 93.3% $\pm$ 5.8 and 94.4% $\pm$ 9.6 in red, ghana and chitralada strains respectively but statistically comparable with T1. Red kwao kreua treatments in most cases had higher percent male sex reversal compared with the control (T0) but not statistically significant. A 72.2 $\pm$ 25.5 % male sex ratio in the T3 of the chitralada strain was obtained highest. Moreover, MT treatments had comparable effect in terms of SR, FCR and GW and were statistically significant as compared to T0. As to the effect of the treatments on the three strains it was observed they generally had comparable effects.

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