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Efficacy of Red Kwao Krua (*Butea Superb* Roxb.) Crude Extract for All Male Production of Nile Tilapia (*Oreochromis Niloticus*)

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Abstract Efficacy testing of Red Kwao Krua, (*Butea superb*a) crude plant extracts, to produce all-male Nile tilapia (*Oreochromis niloticus*) was conducted. Three day old post hatched Nile tilapia were fed with a diet containing six different concentrations of Red Kwao Krua crude extracts (0, 40, 80, 120, 160, and 200 mg. kg^{-1} fish feed) and a concentration of17 α-methyltestosterone (60 mg. kg^{-1} fish feed) for twenty one consecutive days. Thereafter the fish were fed with normal diet, containing 40% protein, until the age of 2 months. Experimental fish were randomly sampled for sex determination by Acetocarmine Squash Method. Results indicated that crude extracts from Red Kwao Krua are effective for manipulating the sex of Nile tilapia. Highest percent of all male (100%) was obtained from diets containing 200 mg. kg^{-1} of Red Kwao Krua crude extracts and 60 mg. kg^{-1} of 17α-methyltestosterone, in fish feed. They were significantly higher ($P<0.05$) than those diets containing 160(97.33 %), 120(96.33%)80 , (81.33%), and 40 (74.33%) mg kg^{-1} of Red Kwao Krua crude extracts in fish feed. The control group showed 58.50% male sex. There were no difference in growth and survival for all treatments and control groups. These findings suggested that Red Kwao Krua can be used as an effective replacement for synthetic hormone, currently used in tilapia sex reversal.

Keywords: Nile tilapia, *Oreochromis niloticus*, Red Kwao Krua, *Butea superb* Roxb.

Introduction

In aquaculture, raising fish for the food market is often more advantageous if the fish are all the same sex. For Thai fish farmers, mono-sex culture of male tilapia, is intrinsically desirable, as several predominant advantages can be achieved. For example, by having all-male tilapia, a higher average growth rate is achieved, since tilapia males exhibit marked superior growth. Energy expended for reproduction is eliminated; and harvest size variations are reduced (Beardmore *et al.*, 2001). These factors lead to higher final profitability. Of the various techniques developed to produce all male

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tilapia, hormonal treatments are often used, as being most effective for sex reversal of tilapia (Penman and McAndrew, 2000; Beardmore *et al.,* 2001). Hormone sex reversal has become the commercial procedure of choice to produce all male tilapia. This technique is efficient and simple for tilapia, because gonads of newly hatched tilapia are undeveloped. Exogenous sex hormone interferes at this early life stage to induce gonadal development and produce single-sex populations. Administration of 17α-methyltestosterone, a synthetic androgen hormone, is considered to be the most effective and economically practicable method producing all male tilapia (Guerrero and Guerrero, 1988).

In Thailand, high costs and importation difficulties of the synthetic hormone, 17α-methyltestosterone, currently limits its use for production of all male tilapia. Moreover, application of synthetic hormones in fish feed is prohibited in a number of countries, due to environmental and health concerns; and major wholesalers are requiring their products be hormone free. Thus, endocrine active plant derived substances, such as Red Kwao Krua (*Butea superb* Roxb.), that produce similar effects on tilapia; might possibly be promoted as a substitute for 17α-methyltestosterone.

Red Kwao Krua (*B. superba*) is an indigenous herb in Thailand, used in many Thai folk medicinal recipes, as a phytoandrogen, as it produces substances with similar effects as testosterone. (Manosroi and Manosroi, 2005). Using Red Kwao Krua would be an alternative to synthetic hormones. Attempts to use Red Kwao Krua for mono-sex production have been documented before (Mengumphan *et al.,* 2006). These earlier results were not satisfactory, when compared to the use of 17-α methyltestosterone; possibly due to using a different method to induced hormone sex reversal. Therefore, the present study applied the use of crude extract from Red Kwao Krua, under identical procedures to those of using 17α- methyltestosterone, in sex reversal and the effectiveness on Nile tilapia sex ratio was determined.

Materials and methods

Preparation of Red Kwao Krua crude extract

The Red Kwao Krua crude extract was modified from Chainarong *et al.,* (2005). The tuber roots of Red Kwao Krua were cleaned, sliced and minced into small pieces, dried at 45 °C and grinded to fine powdered particles. The powder was successively extracted using 75 % ethanol (1/4 w/v) in 250 ml Eelenmeyer flasks then agitated for 48 hr using mechanical shaker at 250 rpm. The total extracted volume was filtrated using filter cloth. The filtrate was kept in deep freezer for one day before it was crystallized by Freeze-Dryer until

laminated. The crystals of crude extracts then were kept in desiccators until evaluation.

Feed preparation

Seven treatment feeds were prepared using the same feed composition at 44% crude protein. A ratio of 75% fish meal, 25% rice band and 2% vitamin premix were the ingredients used to compose of the control diet. One treatment feed served as control, one was formulated from control diet to contain17α-methyltestosterone (MT, Sigma chemicals Co, USA) at a dose of 60 mg kg^{-1} feed. The 5 others were formulated from control diet to contain Red Kwao Krua crude extract at a concentration of 40, 80, 120, 160, and 200 mg kg^{-1} feed. MT treated diet was prepared by dissolving them into 120 ml alcohol (95%), sprayed onto the one kilogram diet and air dried under shading for 24 hours. A treated diet containing Red Kwao Krua crude extract was prepared by dissolving them into separate 100 ml distilled water, sprayed onto the one kilogram control and air dried under shading for 24 hours. All treated diets were stored in the refrigeration at 4^{o}C before trial.

Experimental procedure

Nile tilapia fry (3 days old) were stocked into 21 (1x1x1 m) hapas suspended in an approximately 10x20 m^2 earthen pond, at a density of 500 fry per hapa. Water depth in the pond was maintained at 0.8 m. The feed amount was fixed based on the assumed average biomass per fry per week. First week the fry were fed 30% of their biomass. On the second week they were fed 20% of their biomass. On the third week they were fed 15% of their biomass and on the forth week they were given 10% of their biomass. The daily ration was divided into four meals, from 07.00 am, to 5.30 pm with 3.5 hours interval (Sanches and Hayashi, 2001) and adjusted weekly based on results of weekly population samples. After four weeks, fry in all treatment was fed 10% of their biomass with commercial pellet feed (30% crude protein). Scrubbing of hapas was done every 3 days to prevent clogging of the hapas by pond sediments.

After 60 days, all surviving fish in each hapa were counted to calculate survival using the formulae: Survival rate (%) = (Number at harvest x 100) / number at stock. A total of twenty percent fish samples were taken randomly in each hapa from all treatments for growth determination using weight and length measurements. Percentage of male sex was also computed using the formulae: obtained male (%) = (obtained male x 100) / sampled fish.

Gonad examination

The gonads of sampled fish were removed (Figure 1) and placed on a clean glass slide. A few drops of aceto-carmine stain were added and the gonad squashed with a cover slip (Guerrero and Shelton, 1974) then the slides were examined under microscope 10 x powers to differentiate their gonad. Tissues gonad were recorded as male, female or intersex when testicular, ovarian tissue or both testicular and ovarian tissue were observed, respectively (Figure 2).

Fig. 1. Finding and removing the gonad from a tilapia fingering

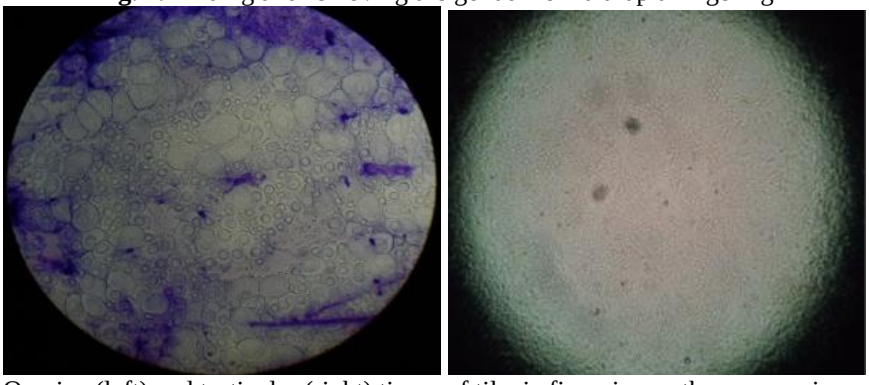

Fig. 2. Ovarian (left) and testicular (right) tissue of tilapia fingering as they appear in a gonadal squash

Statistical analysis

This experiment was conducted following a completely randomized design. The data were first tested for normality (Shapiro-Wilk test) and homogeneity of variance (Lavene test). When necessary, data were normalized by arcsine transformation, but only non-transformed means are presented in the tables and figures. When the data did not have normal distribution and homogeneous variance, the Kruskal-Wallis (KW) test was conducted to test the overall difference for all the treatments. One-Way Analysis of variance (ANOVA) using the statistical package (SPSS) 11.5 for Windows was used to analyze the differences among the treatments. Paired comparisons between treatment means were performed with Duncan's new multiple Range Test. Significant levels were considered at $P < 0.05$.

Results

When Red Kwao Krua crude extract was used as a source of exogenous sex hormone, it was effective in producing a tilapia single sex male population. Tilapia test populations fed rations containing 200 mg / kg^{-1} feed with Red Kwao Krua crude extract did not differ from MT-treated populations, both produced 100% males. When tilapia test populations were fed Red Kwao Krua crude extract with ratios of ,120 ,80 ,40and 160mg / kg^{-1} feed, results were significantly higher than control (74.33%, 81.33%, 96.33%, and 97.33% males were obtained), but lower than a treatment of 200 mg Red Kwao Krua with 60 mg MT (Figure 3).

Results showed that after 60 days of experimental period, supplementing Red Kwao Krua crude extract (160 ,120 ,80 ,40and 200 mg / kg^{-1} feed) in diets did not affect mortality and growth of tilapia. No significant differences in percent survival among Red Kwao Krua crude extract treated populations (69.93%, 71.47%, 67.33%, 75.73% and 72%, respectively) were observed, compared with MT-treated populations (72.07 %) and control (71.4 %). No significant differences were also found in mean total length (ranging from 4.98 ± 1.00mm to 5.22 ± 1.06 mm) and weight (ranging from 11.59 ± 2.57mm to12.46 ± 1.86 mm) among all the treatment (Table 1).

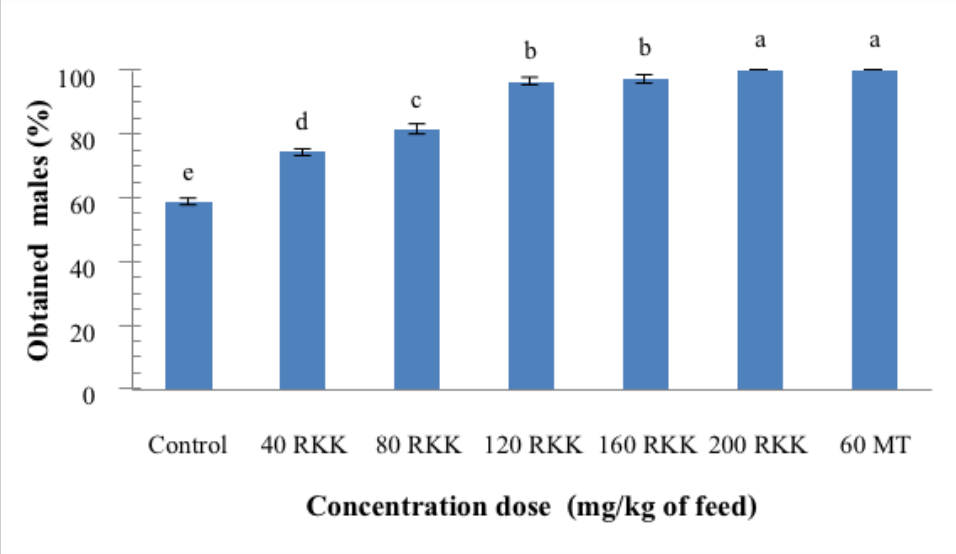

Fig. 3. Mean male sex (%) obtained from Red Kwao Krua crude extract (RKK) treated at different concentration dose, compared to 17α- methyl testosterone treated (MT). Significant differences are indicated by different letters above the error bars within each variable ($P < 0.05$; n=3)

Table 1. Effects of Red Kwao Krua crude extract on growth and survival of Nile tilapia, at 2 months old. Data is expressed as mean ± standard deviation (SD)

Treatment	Survival (%)	Final Total length (cm)	Final weight (g)	Specific growth rate (%/day)
Control	71.40±1.62	5.00±1.16	11.59±2.57	4.05±0.52
40 mg of Red Kwao Krua crude extract	69.93±4.45	5.13±1.02	12.34±2.62	4.12±0.46
80 mg of Red Kwao Krua crude extract	71.47±3.95	5.04±1.00	12.00±2.00	4.13±0.27
120 mg of Red Kwao Krua crude extract	67.33±0.68	5.08±1.02	12.21±1.99	4.11±0.27
160 mg of Red Kwao Krua crude extract	75.73±3.57	4.98±1.00	11.96±2.14	4.05±0.49
200 mg of Red Kwao Krua crude extract	72.00±2.19	5.05±0.82	12.17±2.07	4.12±0.46
60 mg of 17α- methyl testosterone	72.07±2.19	5.22±1.06	12.46±1.86	4.13±0.46

Discussion

In Thailand, many attempts have been made to reduce synthetic hormone use in fish sex reversal; since, it is expensive and the product has to be imported from abroad. Moreover, consumers are becoming more concerned about any use of chemicals or hormone in animals and plants that they eat, including farmed fish. One option is to use a natural substance produced by plants, called phytoandrogen, having similar effects as androgen in animals. Red Kwao Krua, a Thailand herbal plant, containing high amount of phytoandrogens (Cherdshewasart and Nimsakul, 2003) was used in this study, providing a possible alternative for tilapia sex reversal.

When dried power of Red Kraw Krua tuber roots was intermixed with fishmeal and fed directly to tilapia (Mengumphan *et al.,* 2006), the percentage of sex reversed male Tilapia were unsatisfactory compared to the use of 17-α methyltestosterone (Shelton *et al.,* 1981; Phelps and Cerezo, 1992). Perhaps, unacceptable transfer of hormone, from the root powder to fish feed was the major contributory factor, as a result of dusty distribution or excess powdered feed. However, crude extract from Red Kwao Krua has never been used to induce sex reversal in Tilapia and provided more reliable levels of phytoplankton; thus, better able to infiltrate into the feed mix. The formulation and feed mixing procedure used in this study is consistent with that undertaken for 17α- methyltestosterone, the synthetic sex reversal hormone.

Doses of 200 mg Red Kwao Krua crude extract /kg diet produced a 100% male population. Likely, the phytoandrogen from Red Kwao Krua increased exogenous male sex hormone that interferes at early life stage sex characteristics and induced physical male sex direction of the fish. Additionally, results of using Red Kwao Krua crude extract with ratios of ,120 ,80 ,40and 160mg. kg^{-1} feed, showed significantly higher ($p<0.05$) sex reversal than control. Results also showed that using Red Kwao Krua crude extract in all tested diets (160 ,120 ,80 ,40and 200 mg. / kg^{-1} diets) had no affect on mortality and growth of tilapia. This indicates that Red Kwao Krua crude extract can be supplemented in tilapia diet with no apparent toxicity concern.

These findings suggested the use of Red Kwao Krua as an effective replacement for synthetic hormone, 17α- methyltestosterone, currently used in tilapia sex reversal. However, storage of crude extract after extraction needs to be managed to maintain quality control. Crude extract is not a pure substance and improper storage can cause other chemical components in the crude extract to affect its hormone quality. Thus, groundwork is required for further comparative studies on purified extract of red Kwao Krua, to maintain consistent product quality and shelf life.

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References

Beardmore, J. A., Mair, G. C., Lewis, R. I. (2001). Monosex male production in finfish as exemplified by tilapias: applications, problems and prospects. Aquaculture 197:283-301.

Cherdshewasart, W. and Nimsakul, N. (2003). Clinical trial of *Butea superba*, An Alternative herbal treatment for erectile dysfunction. Asian Journal Andrology 5:243- 246.

Guerrero, R. D. and Guerrero, L. A. (1988). Feasibility of commercial production of Nile tilapia fingerlings in Philippines. The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines. pp. 183-186.

Guerrero, R. D. and Shelton, W. L. (1974). An aceto-carmine squash technique for sexing juvenile fishes. The Progressive Fish-Culturist. 56 pp.

Mengumphan, K., Samitasiri, Y. and Carandang, R. (2006). 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). Asian Fish Science 19:271-279.

Penman, D. J. and McAndrew, B. J. (2000). Genetics for the management and improvement of cultured tilapia. Tilapias: Biology and Exploitation. Kluwer Academic Publishers, Dordrecht/Boston/London. pp. 227-266.

Phelps, R. P. and Cerezo, G. (1992). The effect of confinement in hapas on sex reversal and growth of *Oreochromis niloticus*. Journal of Applied Aquaculture 1:73-81.

Sanches, L. E. F. and Hayashi, C. (2001). Effect of feeding frequency on Nile tilapia *Oreochromis niloticus* (L.) fries performance during sex reversal in hapas. Acta Scientiarum 23:871-876.

Shelton, W. L., Rodrigues-Guerrero, D. and Lopez-Macias, J. (1981). Factor affecting androgen sex reversal of *Tilapia aurea*. Aquaculture 25:59-65.

Tocharus, C., Jeenapongsa, R., Thanasak Teakthong, T. and Smitasiri, Y. (2005). Effects of long-term Treatment of *Butea superba* on sperm motility and concentration. Naresuan University Journal 13:11-17.

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