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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, survival rate, feed conversion ratio and gain in weight on red, ghana and chitralada strains of Nile tilapia. Red kwao kreua, 100 g kg^{-1}, 200 g kg^{-1} and 300 g kg^{-1}, 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 ± 25.5 % male sex ratio was obtained in treatment 3 of the chitralada strain. As to the effect of the treatments on the three strains it was observed that they generally have comparable effects.

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Introduction

The more common method of generating mostly male populations is through the use of steroids fed to sexually undifferentiated fry. Typically the desire is to produce all males, so MT is included in the diet for several weeks when the fish start eating.

During the development of sex reversal treatment procedures, concern was expressed for health and environmental issues. Fish for food exposed to steroids is recognized as a controversial issue more so, its effect in the environment. The possible loss of this tool for sex reversal is serious. Fortunately, the development of the Genetically Male Tilapia technology provides a very good alternative however, the application may not be applicable on all strains of tilapia. (Tuan *et.al*. 1998).

Researches in generating all male population that are practical and convenient should be continued especially from plant sources with reproductive endocrine disrupting mechanisms. Cherdshewasart *et.al*. (2004) found that red kwao kreua (*B. superba*) plant extractmight either have an anti-estrogen mechanism or a potent cytotoxic effect because of its anti-proliferation effects on the growth of MCF-7 cells. Red kwao kreua is endemic in Thailand and has been used by the Thais for its various medicinal benefits especially sex invigoration in males (www.adamhawa.com).

Red kwao kreua obtained from Chiang Mai were examined for some bioactive compounds using standard isoflavonoids-Puerarin, Daidzein and Genistein from which 1.9 mg/kg, 37.2 mg/kg, 4.5 mg/kg respectively were obtained (Manosroi and Manosroi 2005). Isoflavones are flavonoids acting as phytoestrogens that are considered by many as useful in treating cancer. Isoflavones are polyphenolic compounds produced almost exclusively by the members of the *Leguminosae* family (bean-family). They are long known for their estrogen-like effect on mammals. The term flavonoid refers to a class of plant secondary metabolites based around a phenylbenzopyrone structure. Flavonoids are most commonly known for their antioxidant activity (www.wikipedia.com). Genistein and Daidzein are believed to be Aromatase Inhibitors (AI). AIs are compounds that inhibit aromatase. Aromatase is an enzyme produced in the liver responsible for the conversion of androgens into estrogens (www.dadamo.com). Genistein is found to have both weak estrogen and weak anti-estrogen effects (www.genistein.com). Pifferer and Donaldson, (1989) as cited by PDACRSP suggested that paradoxical feminization could be due to aromatization than inhibition of in-vivo synthesis of androgens. Isoflavonoids such as genistein act as estrogen agonists via estrogen receptors in cultured cells (Miksicek 1995; Sandell et al. 1997 as cited by PDACRSP). However, Guy et al. (1995) as cited by PDACRSP reported that following genistein treatment of rats during early pregnancy, the number of males was higher than females among progenies although the sex ratios were not different from the Control.

Therefore, we deemed it necessary to investigate the potential of this herbal plant for sex reversal and its effect on survival rate (SR), feed conversion ratio (FCR) and gain in weight (GW) and also to assess if the two MT dosages will yield comparable results.

Materials and Methodology

Experimental fry

A total of 54 (1x1x1 m) hapas were suspended in an approximately 30x20 m^2 earthen pond. The hapas were maintained at 0.6 m water depth. Random distribution of recently hatched tilapia fries was done: T0 (control) pure fishmeal; T1 (40 mg kg^{-1} MT); T2 (60 mg kg^{-1} MT); T3 (100 g kg^{-1} red kwao kreua*)* T4 (200 g kg^{-1} red kwao kreua) and T5 (300 g kg^{-1} red kwao kreua). Each treatment had three tilapia strains (red, ghana, chitralada) replicated thrice with 100 fries per replication. Mortality, initial and final weights of the fries were obtained.

Feed preparation

A 40 and 60 mg kg^{-1} MT was prepared by dissolving them into separate 200 ml ethanol (95%), sprayed onto one kilogram fishmeal, air dried for 24 hours before feeding. Red kwao kreua roots were prepared by drying and pounding until reaching powdery consistency then mixed with fishmeal to produce 100, 200 and 300 grams kg^{-1} dosage levels.

Feeding regimen

The amount of feed given (grams) was fixed based on the assumed average biomass (grams) per fry per week (week 1= 0.01, 2= 0.06, 3= 0.20, 4=0.30). During the first week the fries were given feed at 30% of their biomass at a feeding rate of 5 times per day. On the second week they were given feed at 20% of their biomass 4 times per day. On the third week they were given feed at 15% of their biomass 3 times per day and on the 4th week they were given feed at 10% of their biomass 2 times per day.

Gonad examination

A total of 10% fish samples were randomly selected in each replication from all the treatments after 60 days for percent sex reversal computation. The gonads were surgically removed, prepared using the squash method (Guerrero III and Shelton 1974) and examined microscopically (40x). The remainders of the fish were grown until selection for broodstock.

Statistical analysis

One-way analysis of variance and Duncan's multiple range test of the SPSS 9.0 were utilized for statistical analysis.

Results

Presented in table 1 is the percent male of the treatments from the three strains. Treatments 1 and 2 (MT treatments) were statistically comparable. Furthermore, MT treatments were significantly different from the control except in the chitralada strain. It was also evident however that the target ≥95% male sex reversal was not observed in the MT treatments. The percentage of intersex was consistently observed and was high in Treatments 1 (29.9), 2 (26.7) and 4 (13.0). Treatments 3, 4 and 5 (red kwao kreua treatments) were also statistically comparable to Treatments 1 and 2 except between Treatments 2 and 3 of ghana and chitralada strains respectively and Treatments 2 and 5 of chitralada strain. The range of percent male sex from the red kwao kreua 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 red kwao kreua treatments although highest sex reversal was observed in Treatment 4 of the 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 means of male sex (%) ± SD between treatments and strains of *O. niloticus*

Treatments	red	ghana	chitralada
T0	29.5 ± 26.4^{Aa}	47.2 ± 21.0^{Aa}	69.2 ± 8.1^{Aab}
T1	69.3 ± 31.2^{Ab}	83.3 ± 15.3^{Abc}	70.1 ± 7.6^{Aab}
T2	73.3 ± 23.0 ^{Ab}	93.3 ± 5.8^{Ac}	94.4 ± 9.6^{Ab}
T3	57.9 ± 11.8^{Aab}	62.2 ± 3.8^{Aab}	60.2 ± 13.1^{Aa}
T4	48.2 ± 9.9^{Aab}	72.2 ± 25.5^{Aabc}	70.9 ± 16.3^{Aab}
T5	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 (lower case letters) are significant at $P<0.05$.

Shown in tables 2, 3 and 4 are the SR, FCR and GW between species respectively.

In table 2 the red strain was statistically significant to the other strains in T2 while significant difference in T4 was observed among the three species. The unusual low average survival rate of red species in T2 could have been brought about by the escape of 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 survival rate was observed in T2 and T5 respectively as compared to the Control. In ghana strain, only T1 was statistically significant with the Control. No significant difference was observed among the Treatments in the chitralada strain.

Table 2. Comparison of average SR (%) ± SD between treatments and strains of *O. niloticus*

Treatments	red	ghana	chitralada
T0	77.3 ± 6.4^{Abc}	81.3 ± 13.4^{Aa}	78.0 ± 6.0^{Aa}
T1	67.7 ± 21.0^{Ab}	98.0 ± 3.5^{Ab}	85.7 ± 24.8^{Aa}
T2	33.3 ± 0.2^{Aa}	92.3 ± 13.3^{Bab}	72.0 ± 38.6^{ABa}
T3	86.7 ± 6.0^{Abc}	92.3 ± 2.5^{Aab}	88.7 ± 2.0^{Aa}
T4	85.0 ± 4.4^{Bbc}	92.3 ± 2.5^{Cab}	76.3 ± 3.2^{Aa}
T5	89.3 ± 2.3^{Ac}	93.3 ± 1.5^{Aab}	90.0 ± 8.7^{Aa}

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

As shown in table 3 the chitralada strain was significantly different from the other two species in Treatments 4 and 5 while the ghana strain was significantly different from the other species in Treatment 3. As can be noted in the red kwao kreua treatments, highest FCR was observed in ghana and chitralada strains in Treatments 3 and 5 respectively. Between the MT treatments no statistical significance was observed between species.

Comparison between Treatments in the red strain revealed no significant difference. In the ghana strain, only T3 was statistically different from the Treatments, while T2 and T3 were significantly different from all treatments in the chitralada strain.

Table 3. Comparison of average FCR $\pm$ SD between treatments and strains of *O. niloticus*

Treatments	red	ghana	chitralada
T0	0.9 ± 0.1^{Aa}	1.0 ± 0.3^{Aa}	1.3 ± 0.2^{Ab}
T1	1.3 ± 0.5^{Aa}	0.9 ± 0.0^{Aa}	0.8 ± 0.3^{Aa}
T2	1.4 ± 0.6^{Aa}	0.8 ± 0.2^{Aa}	0.8 ± 0.3^{Aa}
T3	1.2 ± 0.2^{Aa}	1.4 ± 0.0^{Bb}	1.0 ± 0.1^{Aab}
T4	0.9 ± 0.0^{Aa}	0.9 ± 0.0^{Aa}	1.0 ± 0.0^{Bab}
T5	1.1 ± 0.2^{Aa}	0.9 ± 0.0^{Aa}	1.4 ± 0.2^{Bb}

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

In table 4, only the GW of ghana and chitralada strains in Treatments 3 and 5 were significantly different. The ghana strain had a better GW in Treatment 5 while the chitralada had a higher GW in Treatment 3. The chitralada strain had a better total GW in Treatment 3 while ghana was better in Treatment 5. Highest total GW was noted in the ghana strain of Treatment 2.

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. Finally, MT treatments were found to be statistically significant among the Treatments in the chitralada strain.

Table 4. Comparison of average GW (grams) $\pm$ SD between treatments and strains of *O. niloticus*

Treatments	Red	ghana	chitralada
T0	56.0 ± 7.5^{Aa}	53.9 ± 13.0^{Ab}	39.9 ± 6.2^{Aa}
T1	42.9 ± 16.9^{Aa}	57.8 ± 2.0^{Ab}	74.3 ± 27.3^{Ab}
T2	43.7 ± 24.4^{Aa}	76.3 ± 14.3^{Ac}	68.6 ± 20.9^{Ab}
T3	45.6 ± 8.1^{ABa}	36.0 ± 1.0^{Aa}	50.5 ± 7.0^{Ba}
T4	58.4 ± 6.4^{Aa}	57.5 ± 5.3^{Ab}	47.5 ± 4.0^{Aa}
T5	48.5 ± 8.4^{Aba}	58.2 ± 6.3^{Bb}	38.3 ± 6.1^{Aa}

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

Discussion

The percent male sex reversal of red kwao kreua treatments in some instances statistically did not differ among 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 a male sex ratio similar 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 the 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 $\pm$1.5% male tilapia for a period of 21 days. This practice is currently used in commercial mass production of sex reversed 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. There were also varying results as to the effect of red kwao kreua 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 a natural environment unlike when it was done in a closed or recirculating water system (Jo *et al*. 1988).

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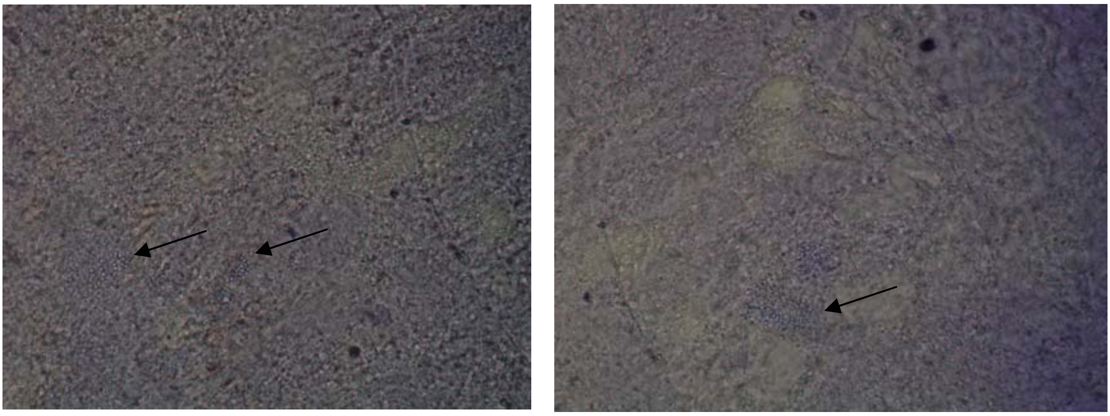

Figure 1. Male gonad of Nile tilapia showing the presence of sperm cells (arrows).

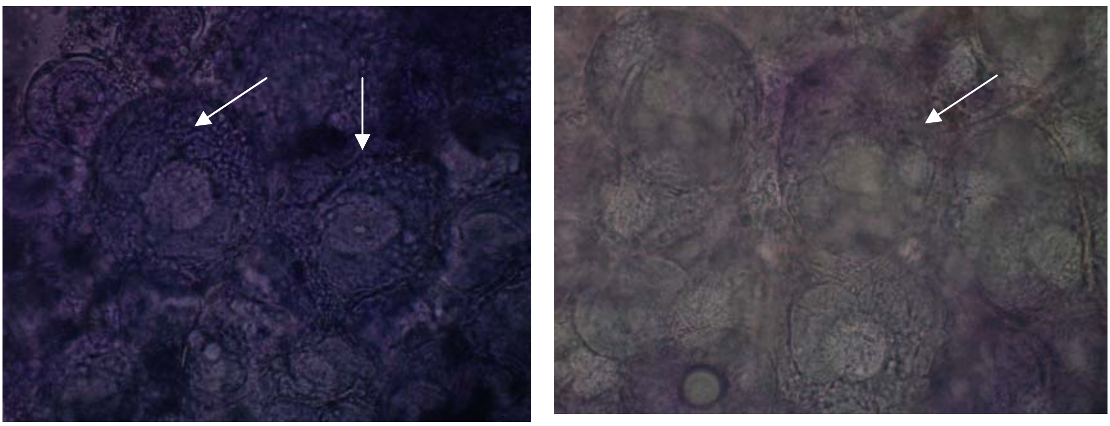

Figure 2. Female gonad of Nile tilapia showing the presence of egg cells (arrows).

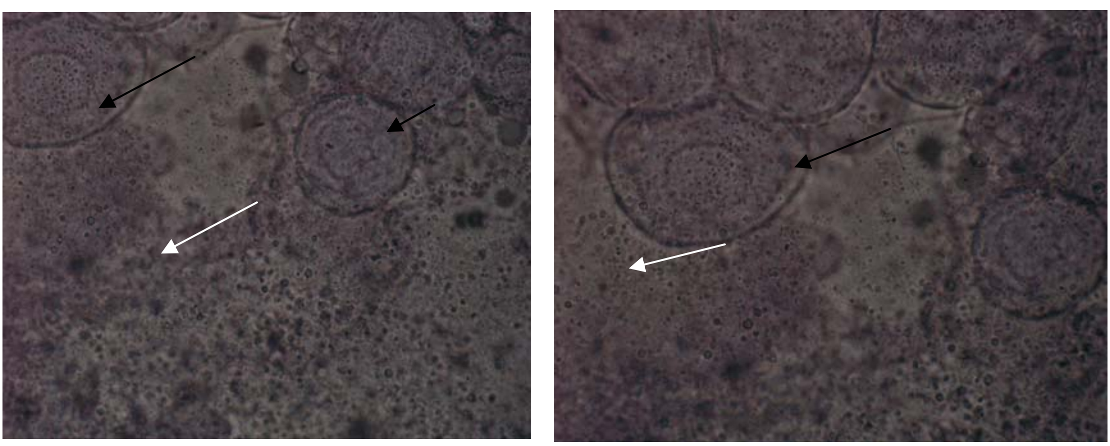

Figure 3. Intersex gonad of Nile tilapia showing the presence of egg cells (black arrows) and sperm cells (white arrow).

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