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# Dietary Administration of Dehydroepiandrosterone Hormone Influences Sex Differentiation of Hybrid Red Tilapia (*O. niloticus*× *O. mossambicus*) Larvae

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## ABSTRACT

Effects of a steroid hormone Dehydroepiandrosterone (DHEA) on sex differentiation of hybrid red Tilapia *Oreochromis niloticus*×*O. mossambicus* larvae were investigated. Three day-old tilapia larvae were fed diets supplemented with varying concentrations of DHEA (0, 20, 40, 80 and 160 mg $kg^{-1}$ feed) for 24 days. A positive control group fed with diet containing 60 mg $kg^{-1}$ of 17α-methyl testosterone was also included in the experimental run. Results indicate that among the DHEA treatment groups, larvae fed with 160 mg $kg^{-1}$ DHEA showed the highest percentage of males that is comparable to the number of differentiated male fish observed in treatment group receiving the 17α-methyl testosterone as the positive control group. DHEA supplementation also improves weight gain and enhances feed conversion ratio. These findings suggest that DHEA can be used as a dietary supplement to induce masculinization and can improve the growth performance of tilapia larvae.

**Key words:** Masculinization, hybrid red tilapia *Oreochromis niloticus*×*O. mossambicus*, dehydroepiandrosterone

## INTRODUCTION

Tilapia is an important food commodity that is widely cultured in tropical and subtropical developing countries. Since the culture technology for tilapia is well developed, the major drawback in culture is the tendency of the species to proliferate rapidly and overpopulate the culture system. This condition leads to stunting and production of under-sized fish that not suitable for marketing and decreases the economic gains of the grow-out operation (Balarin and Haller, 1983). Mono sex tilapia-all males' culture- has been found as a solution. Moreover, all male culture also increases the productivity and yield during harvest since male tilapia can grow better and can utilize feed nutrients efficiently than the females (Paiva, 1988; Mair *et al*., 1997).

Inversion of sex to produce all male tilapia is commonly practiced using androgenic hormones. Synthetic derivatives of testosterone including 17α-methyl testosterone, α-ethyltestosterone and

1-dihydrotestoseterone are commonly used synthetic steroids showing potent effects in inducing male differentiation in tilapia. The high pharmacological potency of these testosterone derivatives in inducing masculinization as compared to the natural testosterone is attributed to the slower tissue elimination and metabolism of these compounds in fish (Vorasayan and Petchrich, 2004; Nuanmanee *et al*., 2004). However, some countries allow the use of these synthetic hormones in fish but currently there are growing concerns on the risk associated with the potential accumulation of these compounds to the environment and the contamination of the biota (Contreras-Sanchez *et al*., 2001; Cek *et al*., 2004).

The high cost of these synthetic androgenic derivatives plus the problems in international trading adds additional economic burden to tilapia growers. The use of natural androgenic steroid precursor for masculinization in fish has been suggested as a possible alternative to overcome the problems associated with synthetic testosterone derivatives.

Androstenedione (11 β-hydoxyandrostenedione), a testosterone precursor molecule has been reported to be as potent as testosterone in the masculinization of tilapia larvae (Baroiller *et al*., 1988). Dehydroepiandrosterone, DHEA is another testosterone precursor molecule with potential use in the masculinization of fish larvae. Application of this hormone in higher vertebrate organisms has been documented to elevate testosterone levels, increase muscle mass, strength, enhance immunological responses and improves tolerance to stress (Phillips *et al*., 1994; Barrett-Connor *et al*., 1999).

Although investigation on the biological effects of this hormone in aquatic animals has been limited but initial report shows that DHEA application in the scallop, *Placopecten magellanicus* larvae has resulted to masculinization of the larvae indicating a potent androgenic action of this natural testosterone precursor (Wang and Croll, 2004).

To date there has been no previous work investigating the potential use of this steroid, DHEA on finfish, particularly on its influence in the sex differentiation of the larvae. This study was conducted to asses the effects of dietary supplemented DHEA on sex differentiation, survival and biological growth performance of the red Tilapia *Oreochromis niloticus*×*O. mossambicus* larvae.

## MATERIALS AND METHODS

**Experimental fish:** The study was conducted in an indoor fiberglass tanks recirculating-fresh water system in the rearing facilities of the Brackish water Aquaculture Center, University of The Philippines Visayas, Leganes, Iloilo City, Philippines. Swim-up fry of the hybrid red Tilapia *Oreochromis niloticus*×*O. mossambicus*, with a size of 0.013 g were obtained from a Tilapia hatchery of the Southeast Asian Fisheries Department Center, Aquaculture Department, Tigbauan-Iloilo City Philippines, from the period of October 15.2011 to January 14.2012. One thousand eight hundred larvae were randomly distributed to 18 fiberglass tanks (200 L) at a density of 100 larvae per tank. This constitutes the six dietary treatments run in triplicates in a completely randomized design. The water flow rate was maintained at approximately 100 mL $min^{-1}$, water temperature at 27-28°C and a natural photo period. The set up were provided with aeration that maintained dissolved oxygen above 5 mg $L^{-1}$ for the entire culture period.

## EXPERIMENTAL DIETS AND FEEDING TRIAL

Basal diet containing fish meal and squid meal as the main protein source was formulated based on the optimum nutrient requirement of tilapia larvae (Table 1). Hormone incorporated diets

Table 1: Ingredients composition and proximate composition of the experimental diet

| Ingredients | Dry matter (%) |
|---|---|
| Squid meal | 50 |
| Danish fish meal | 36 |
| Soy bean oil | 1 |
| Cod liver oil | 1 |
| Vitamin mix* | 0.75 |
| Mineral mix** | 0.75 |
| Vitamin C (Ascorbic acid) | 0.05 |
| Bread flour | 5.45 |
| Carboxymethyl cellulose (CMC) | 5 |
| Total | 100 g |
| **Proximate composition** | **Dry basis (%)** |
| Crude protein | 55.57 |
| Crude fat | 7.62 |
| Crude fiber | 0.26 |
| Ash | 16.29 |
| Moisture | 4.20 |
| NFE | 20.26 |

The vitamin and mineral premix provide the following quantities per kilogram of diet, *Vitamin A: 10.000.000 IU, Vitamin D3: 500.000 IU, Vitamin E: 11.000 IU, Vitamin K3: 1.000 mg, Vitamin B1: 1.000 mg, Vitamin B6: 3.000 mg, Vitamin B12: 22 mg, Nicotinic acid: 22.000 mg, **Pantothenic acid: 12.000 mg, Folic acid: 500 mg, Biotin: 75 mg, Iron: 120.000 mg, Zinc: 75.000 mg, Manganese: 20.000 mg, Copper: 10.000 mg, Iodine: 1.000 mg, Cobalt: 375 mg, Selenium: 150 mg

were prepared following the procedures described by Popma and Green (1990). The diets were prepared by mixing the dry ingredients with feed oil followed by the addition of varying doses of DHEA hormone (Hubei Danjiangkou Kaitai Hormone Co., Ltd. 99.52% Purity, Mainland-China) as (0, 20, 40, 80 and 160 mg $kg^{-1}$) dissolved in adequate amount of 95% ethanol. The diet mixtures were then air dried to eliminate the alcohol and added with appropriate amount of water. The resulting moist mash were then mixed, dried at 60°C, crumbled and sieved to appropriate sizes. Diets were then stored at -28°C until used.

The same preparation were employed in the formulation of the positive control diet containing 60 mg $kg^{-1}$ of 17$\alpha$-methyl testosterone. Each of treatment groups were fed their respective diets at a rate of 20% biomass subdivided into five equal feeding frequencies from 8:00 to 18:00 h at 2 h interval for 24 days. At the end of the feeding trial fish were collected, weighed; survival, FCR and specific Growth Rate (SGR) were determined. Following the sampling after 24 days of culture, the experimental fish were grown for another 66 days and maintained with the basal diet. This was done so that gonads will be clearly identified as that of a male or a female.

Sex determination was done by sacrificing 40% of the surviving fish at each replicate. Fish were killed by cold shock (water temperature of 0°C) and were cut ventrally from the genital papilla to the base of the pectoral fin using a scalpel. A window on the lateral side was opened and the viscera were removed, leaving gonads, swim bladder and kidneys in place. A few drops of Bouin's solution were then applied topically to the gonads.

This procedure hardened the gonadal tissue and the anterior and posterior ligaments were cut and gonads were removed using a forceps and placed on a glass slide. Gonadal squash using malachite green as stain were used to identify the gonad tissues (Guerrero and Guerrero, 1988; Afonso *et al*., 2001). Malachite green stained gonads were examined and characterized under a compound microscope.

**Statistical analysis:** Comparison between treatment means and parameters was analyzed using one-way analysis of variance (one-way ANOVA) and Duncan's multiple range tests for least significant difference was applied. Statistical analyses for survival rate were performed on data after square root transformation.

## RESULTS

Based on histological examinations, the number of masculinized fish was found highest in treatment receiving diet supplemented with 17α-methyl testosterone. The number of male percentage in each dietary treatment increases with increasing levels of dietary DHEA supplementation. Among the DHEA treated groups, treatment receiving diets with 160 mg $kg^{-1}$ DHEA showed the highest percentage of male fish that is statistically similar to that obtained in treatment receiving the 60 mg $kg^{-1}$ of 17α-methyl testosterone supplemented diet. The control group (basal diet) exhibited the lowest number of male percentage that is statistically similar to that obtained in treatment groups receiving the 20, 40 and 80 mg $kg^{-1}$ DHEA diet (Table 2). No intersex fish was found among the treatment groups receiving the experimental diets.

Survival of the larvae as a response to the hormone supplementation is shown in Table 3. Levels of DHEA supplementation were found not to affect the larvae survival and no significant differences were observed as compared to the control and the positive control treatments. However, weight gain was found to be influenced by the levels of supplemented DHEA. Significantly higher weight gain as compared to the control (Without DHEA) was observed in treatment receiving the 80 mg $kg^{-1}$ DHEA diet.

Further, weight gains from the other dietary treatments were not significantly different from the control and that of the 80 mg $kg^{-1}$ DHEA diet. Moreover, feed conversion ratio (FCR) was influenced by the dietary treatments. Highest FCR was exhibited in the control treatment that is not significantly different from that of the treatments receiving the 20 and 40 mg $kg^{-1}$ DHEA diet.

Table 2: Sex composition of hybrid red tilapia fry fed diets treated with varying doses of DHEA and 17α-methyl testosterone hormones for 24 days duration

| Treatments | Males (%) | Females (%) |
|---|---|---|
| Control | 61.667±10.408$^{a}$ | 38.333±10.408$^{a}$ |
| MT-Control | 99.167±1.443$^{b}$ | 00.833±1.443$^{b}$ |
| DHEA 20 mg $kg^{-1}$ | 69.167±11.273$^{a}$ | 30.833±11.273$^{a}$ |
| DHEA 40 mg $kg^{-1}$ | 70.833±8.036$^{a}$ | 29.167±8.036$^{a}$ |
| DHEA 80 mg $kg^{-1}$ | 73.333±8.036$^{a}$ | 26.667±8.036$^{a}$ |
| DHEA 160 mg $kg^{-1}$ | 96.667±3.819$^{b}$ | 03.333±03.819$^{b}$ |

Means within rows without a common superscript are significantly different at $p>0.05$

Table 3: Mean growth indices of hybrid red tilapia fry achieved after 24 days fed with DHEA and 17α-methyl testosterone inclusion treatment

| | Control treatments | | Phytoandrogen DHEA treatments doses (mg $kg^{-1}$ diet) | | | |
|---|---|---|---|---|---|---|
| Growth indices | *Control | **P-control | 20 | 40 | 80 | 160 |
| Weight gain % | 0956.91±78.35$^{a}$ | 1387.40±169.38$^{ab}$ | 1064.60±303.96$^{ab}$ | 1019.80±169.8$^{ab}$ | 1659.75±57.96$^{b}$ | 1109.06±159.23$^{ab}$ |
| SGR (%) | 09.82±0.31 | 11.23±0.48 | 10.16±1.01 | 10.06±0.15 | 11.90±0.16 | 10.37±0.5 |
| Survival (%) | 85.00±2.83 | 81.50±0.71 | 85.33±4.72 | 76.50±0.71 | 94.00±4.24 | 79.00±9.9 |
| FCR | 01.24±0.10$^{b}$ | 01.02±0.078$^{a}$ | 01.12±0.061$^{b}$ | 01.11±0.12$^{b}$ | 00.99±0.04$^{a}$ | 01.04±0.08$^{a}$ |

Means within rows without a common superscript are significantly different at $p>0.05$, *Basal diet with 500 mL of 95% ethanol per kg diet. **Positive-control treatment with basal diet containing 60 mg $kg^{-1}$ 17α-methyl testosterone hormone

DHEA supplementation at 80 mg $kg^{-1}$ diet resulted to the best FCR but did not differ significantly from that of the treatments receiving the 60 mg $kg^{-1}$ of 17α-methyl testosterone (positive control) and the treatment with 160 mg $kg^{-1}$ DHEA diet, respectively. Specific Growth Rates (SGR) were not observed to differ among the dietary treatments.

## DISCUSSION

The present study demonstrates that similar to testosterone, DHEA supplementation can also influence the sex differentiation or masculinization of hybrid red Tilapia *Oreochromis niloticus*× *O. mossambicus* larvae. Testosterone and its derivatives are commonly used hormones in the sex reversal of tilapia. In the present study, treatment group fed with 60 mg $kg^{-1}$ 17α-methyl testosterone supplemented diet showed the highest number of male fish (99.17%) confirming the high masculinization effects of this hormone documented in other species of tilapia (Jae-Yoon *et al*., 1988; Tayament and Shelton, 1978; Guerrero and Guerrero, 1988).

Moreover, the present results indicate that DHEA supplementation at a dose of 160 mg $kg^{-1}$ diet elicited similar effects as that of testosterone supplementation in inducing masculinization in tilapia larvae. The present finding of the masculinization effects of dietary supplemented DHEA is the first time to be documented in Tilapia. DHEA is an active steroid that is a precursor of testosterone. This steroid is directly converted to testosterone with the action of 17β-hydroxysteroid dehydrogenase and is also converted to an active steroid androstenedione with the action of 3β-hydroxysteroid dehydrogenase (Labrie *et al*., 2005).

Androstenedione is also an active precursor of testosterone and the high masculinizing effects of DHEA on tilapia in the present study could be attributed to the transformation of this hormone into testosterone. Similar to the current findings, DHEA injection to juvenile sea scallop, *Placopecten magellanicus* resulted to the masculinization of this organism, suggesting an androgenic effect of DHEA on this species (Wang and Croll, 2004). Also in the developing European starlings, *Sturnus vulgaris*, DHEA was found to be high in testes than the ovary and it has been suggested to play an important role in sexual differentiation of the birds brain (Shah *et al*., 2011).

Survival and growth rate of fish receiving DHEA supplementation were comparable to the control suggesting that this hormone at the levels tested is not detrimental to the overall physiological condition of the larvae. Prominent improvement of weight gain is exhibited in treatment receiving DHEA dose of 80 mg $kg^{-1}$ diet elucidating the growth enhancing effect of this hormone in fish. Additionally, significant improvement of FCR is exhibited in treatment groups receiving the testosterone supplemented diet and those supplemented with DHEA at 80 and 160 mg $kg^{-1}$ diet, respectively.

Anabolic effects resulting to higher weight gains are commonly observed in tilapia larvae fed with diets supplemented with androgenic steroid hormones. Sparks *et al*. (2003) reported that *O. mossambicus* fry fed with 17α-methyl testosterone supplemented diet grew significantly larger than their respective controls. Significant increase in growth in 17α-methyl testosterone treated group of tilapia was also recorded by Ridha and Lone (2008).

The increase in growth as a result of androgenic steroid supplementation has been suggested to be an effect of enhanced feed digestion and nutrient absorption (Adel *et al*., 2006) and could explain the growth enhancement effects of DHEA supplementation observed in the present study. Maintenance of muscle mass and prevention of molecular mechanisms involved in muscle atrophy is also known to be a biological function of DHEA (Ceci *et al*., 2011). Also, as documented in human clinical trials, DHEA supplementation elevates circulating levels of the thyroid hormone, increases

cellular metabolic activities and improves lipid metabolism (Colker *et al*., 1999). These reports on the biological actions of DHEA may explain the present findings of the growth enhancement and improvement of FCR in tilapia larvae as a result of dietary DHEA supplementation. However, the detailed mechanism on the growth promoting effect of this steroid hormone in fish needs further investigations.

Collectively, findings of this study indicate that dietary supplementation of DHEA at a dose of 160 mg $kg^{-1}$ diet is as effective as supplementation with 17α-methyl testosterone in the masculinization of hybrid red Tilapia *Oreochromis niloticus*×*O. mossambicus* larvae. In addition DHEA supplementation can also improve weight gain and enhance FCR of the larvae. DHEA could be used as a male inducing dietary supplement in the larval rearing stage of tilapia.

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