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Optimum dosage for growth of dietary dehydroepiandrosterone in hybrid red tilapia fry *Oreochromis niloticus Linn x Oreochromis mossambicus Linn*

Asaad H. Mohamed[1, 2], Rex Ferdinand M. Traifalgar[2] and Augusto E. Serrano. Jr.[2*]

[1]*Department of Fisheries and Wildlife Sciences, Sudan University of Science and Technology, Khartoum- Sudan*
[2]*Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Iloilo-Miagao, Philippines*

ABSTRACT

Effects of dehydroepiandrosterone (DHEA) on growth, survival and utilization efficiency of hybrid red tilapia Oreochromis niloticus x O. mossambicus were determined. Newly hatched larvae were fed with feeds containing four different concentrations OF DHEA: 0, 20, 40, 80 and 160 mg kg^{-1} for 12 weeks. Optimum dosage of 106 mg kg^{-1} was estimated using the quadratic model with weight gain as the response parameter. Larvae fed at 80 mg kg^{-1} of DHEA showed higher growth rate than did those fed the diet with 17α-methyl testosterone. No significant differences in specific growth rate between the DHEA-treated and the negative and positive control groups were observed. However, significant differences among the treatment groups were observed in survival rate and weight gain. These findings suggested that DHEA could be used effectively as growth-promoting agent for the hybrid red tilapia for 12 weeks.

Key words: dehydroepiandrosterone DHEA, optimum dosage, red tilapia, growth, survival

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INTRODUCTION

In tilapia culture, the major drawback is the tendency to breed early and overpopulate culture system. The fish may mature at an early age and produce thousands of offspring every 28 to 45 days [2]; this could lead to overpopulation that results in competition for food and stunted growth. Thus, production of monosex tilapia becomes a desirable practice in its culture.

Exogenous androgens had been used extensively in aquaculture to induce sex reversal and increase growth rate in gonochoristic species such as *Ictalurus punctatus* and *Oreochromis mossambicus* [5, 6]. An androgen, dehydroepiandrosterone (DHEA) is a steroid hormone which is a precursor of both estrogen and androgens with hormonal properties of its own [9]. This 17-keto-steroid is synthesized primarily in higher animals by the adrenal cortex and to a lesser degree by the gonads [10]. To our knowledge, no work has been done on aquaculture fish regarding the application of DHEA as a replacement for the commonly used testosterone or its various forms. Previously, we have shown that this hormone when incorporated in feeds and fed to hybrid red tilapia larvae for 24 d exhibited the highest percentage of male larvae than did those fed 17a-methyltestosterone[1]. Our finding demonstrated that DHEA could be used as a dietary supplement to induce masculinization and improve growth of tilapia larvae.

One of the disadvantages of using methyltestosterone (MT) when administered at high dose is that it leads to sterility and in some cases paradoxical sex reversal and may result in growth suppression [7]. It is thus interesting to know whether DHEA could replace DHEA without these mentioned disadvantages. This paper aims to determine the effects of DHEA on growth and survival of fry of the hybrid red tilapia on a longer period (90 days) and estimate the optimum level of the hormone to be incorporated in feeds.

MATERIALS AND METHODS

The study was conducted in an indoor recirculating-water culture system with a total water volume of 200 L fiberglass tanks for the first phase and in an outdoor concrete tank facilities for the second phase, for a total period of 12 weeks. Healthy swim-up larvae of hybrid red Tilapia *Oreochromis niloticus x O. mossambicus*, with body weight range of 0.013-0.010 g were obtained from the Southeast Asian Fisheries Department Center, Aquaculture Department, Tigbauan, Iloilo. Larvae were weighed before random separation into six treatment groups with three replicates at 100 larvae replicate^{-1}. Water flow rate was maintained at 300 ml min^{-1} and water temperature at 27-28°C; aeration was continuously provided for the entire culture period.

Dietary treatments were offered during 24 d to each group of fry with three replicates. Basal diet was formulated as shown in Table 1. Test diets were prepared containing DHEA at varying concentrations of 0, 20, 40, and 80 mg kg^{-1} as described by Popma and Green [8]. The negative control group was fed the hormone-free basal diet while the positive control treatment group was fed the basal diet mixed with 60 mg kg^{-1} of 17α-methyl testosterone. Fry were fed five times daily (from 0800 to 1800) at a feeding rate of 30% which was later decreased to 20% body weight towards the end of the experiment. Batch weighing of the fish was done weekly. Survival, FCR, specific growth rate, % weight gain, final weight and final length were determined after 24 d culture period. For the second phase, fry were transferred to 18 hapa nets (60 cm x 70 cm x100 cm) corresponding to the treatments in the first phase, installed in outdoor concrete tank (6 m x 6 m x 1 m*)*. Fry were fed three times daily at a decreasing rate of 15%, 10, 5% and 3% body weight during the 12-week growth trial.

Water conditions such as temperature, dissolved oxygen DO were recorded daily; pH, total alkalinity, total ammonia-nitrogen, reactive nitrite and reactive phosphorous were monitored at the beginning and upon 50% water change every week before sampling for the first phase and every two weeks during the second phase.

Table 1. Ingredients Composition and Proximate Composition of the Experimental Diets

Ingredients	Dry Matter (g 100 g^{-1})
Squid meal	50.0
Danish fish meal	36.0
Soy bean oil	1.0
Cod liver oil	1.0
Vitamin mix*	0.8
Mineral mix**	0.8
Bread flour	5.4
Carboxymethylcellulose (CMC)	5.0
Total	100.0
Proximate Composition	**Dry basis %**
Crude protein	55.6
Crude fat	7.6
Crude fiber	0.3
Ash	15.3
Moisture	4.2
NFE	17.3

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 12.000mg, 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.*

Estimation of requirements and statistical analysis.
Data were analyzed by fitting quadratic regression equation used in fish to estimate protein and amino acids [4, 11] and in seahorse[3]. This model was deemed appropriate for the treatment of almost a hyperbolic data in which the response parameters reached a peak and declined at the highest level of the independent variable. In this method, a quadratic equation is used to fit the response data obtained from feeding a dietary series:

$$R = a + bI + cI^2$$

where R is the measured response; I is the dietary nutrient concentration; and a, b, and c are constants that are calculated to provide the best fit of the data. The value of I that produces the maximum response *Imax* is calculated as follows:

$$I_{max} = -0.5\ (b/c)$$

Standard error of the mean (SEM) were calculated for all mean values. Data were subjected to analysis of variance (ANOVA) and Duncan's Mutiple Range Test to determine differences in means ($p<0.05$).

RESULTS AND DISCUSSION

Water conditions such as water temperature, D.O., total alkalinity, total ammonia-N, reactive phosphorus and nitrite were not significantly different in all treatments (data not shown). Survival in all treatments were not significantly different from each other except for those fish fed the diet with 80 mg kg^{-1} diet which gave significantly the highest survival rate (Table 2). Condition factor was also not significantly different in all medicated treatments except in fish fed the negative control diet (0 mg kg^{-1} DHEA) which gave the highest condition factor.

Fish treated with methyl testosterone at 60 mg kg^{-1} were not significantly different in final body weight from those DHEA-treated tilapia except those fish fed diet with 80 mg kg^{-1} DHEA which exhibited significantly heavier fish (Table 2). The same trend was observed in weight gain, final length, SGR and survival in which fish fed the positive control (containing methyl testosterone at 60 mg kg^{-1}) was not significantly different from those DHEA-treated tilapia except those fish fed diet with 80 mg kg^{-1} DHEA which exhibited significantly better values. FCR values were inversely linear with dosage of DHEA and were not significantly different from the values of fish fed the positive control diet except those fed diet containing 80 mg kg^{-1} which exhibited significantly the best conversion ratio. The optimum level of DHEA treatment was estimated to be 106 mg kg^{-1}using the quadratic model for the final body weight since it exhibited the hyperbolic curve ideal for this type of mathematical model (Figure 1).

Table 2. Growth and condition parameters of red tilapia (*Oreochromis niloticus Linn x Oreochromis mossambicus Linn*) fry fed diets with methyltestosterone or varying levels of DHEA for 90 days.

	MT (60 mg kg^{-1})	DHEA (mg kg^{-1} diet)				
		0	20	40	80	160
Initial ABW (g)	0.013±0.000	0.013±0.001	0.011±0.002	0.012±0.001	0.010±0.003	0.011+0.002
Final ABW (g)	17.71±1.50^b	15.56±0.99^c	16.83±1.55^b	16.95±2.29^b	19.28±1.57^a	18.93+5.03a
Weight gain (g)	17.70±0.98^b	15.55±0.78^d	16.82±0.90^c	16.94±1.87^b	19.27±0.99^a	18.92+4.89a
Initial Length (cm)	0.92±0.02^a	0.92±0.02^a	0.92±0.02^a	0.92±0.02^a	0.92±0.02^a	0.92+0.02
Final Length (cm)	9.66±0.26^a	9.12±0.12^a	9.53±0.28^a	9.50±0.45^a	9.96±0.24^a	9.76+0.79a
SGR %	6.30±0.002^c	6.20±0.002^d	5.90±0.001^b	6.30±0.000^c	5.70±0.003^a	6.40+0.010a
CF	1.91±0.02^a	1.97±0.04^b	1.88±0.06^a	1.91±0.05^a	1.92±0.04^a	1.93+0.02
Survival %	63.02±2.64^b	69.36+3.98^b	67.64±3.75^b	58.18±4.90^b	78.95±5.92^a	57.41+10.95^b
FCR	2.18±0.15^a	2.33±0.50^a	2.17±0.29^a	1.91±0.10^a	1.76±0.07^b	2.39+0.16^a

Means within rows without a common superscript are significantly different (P>0.05).

In general, fish treated with synthetic steroid exhibit higher mortality in most species [7]. In the present study, survival ranged from 57 to 79% and fish that were fed with the basal diet with no synthetic hormone had 60% survival rate. Thus, it was apparent that dietary DHEA did not result in higher mortality.

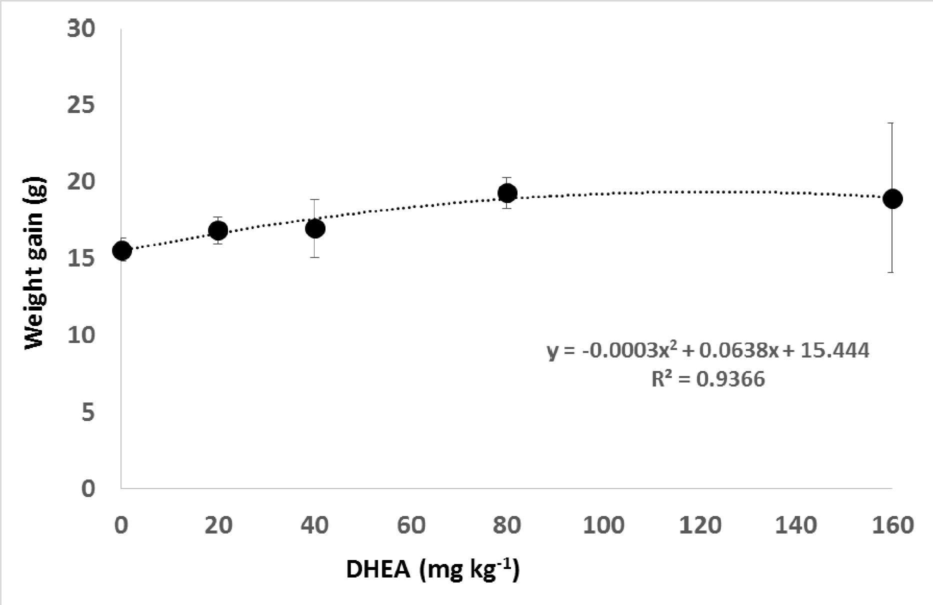

Figure 1. Weight gain of red tilapia at varying DHEA levels fitted in a quadratic model.

One of the objectives of inducing hormonal sex reversal is to realize 100% growth potential. In the present study, we estimated the optimal dose of DHEA as 106 mg kg^{-1} dry feed using the quadratic model, beyond which growth apparently gradually diminished. This observation agreed with the results cited by Pandian and Sheela [7] on the black molly, *Poecilla sphenops*. They have treated the fish with 17 α-MT and have found the relative growth is enhanced in 3-month old treated individuals with increasing steroid dose up to the preoptimal level (for sex reversal) beyond which the increase in relative growth began to diminish. In their study, growth was markedly and consistently suppressed in 18-month old individuals irrespective of treatment intensity. The effect of synthetic hormone in the present study clearly indicated that it accelerated growth in the red tilapia and this could be an advantage in escaping from larger predators during the early stages of development but with penalty of suppressed growth of adults as observed in *P. sphenops* treated with MT. Pandian and Sheela [7] have compared the effects of MT on cyprinids and cichlids and have concluded that in general, the cyprinids show a positive growth response of 2-3 times that of the control, while that for cichlids is 1-2 times faster. This explains the lower values of parameters in the present study between fish fed diets at various dosages of DHEA with only the values of the highest dosage of 160 mg kg^{-1} exhibiting significant superiority in most parameters. Nonetheless, we agree with the observation of Pandian and Sheela [7] that growth response of hormonally sex-reversed tilapia may prove to be a greater advantage to tropical countries where more protein-hungry inhabitants are looking for fast growing fish.

CONCLUSION

Larvae fed at 80 and 160 mg kg^{-1} of DHEA showed higher growth rate response than those fed diets with 17α-methyl testosterone. No significant differences in specific growth rate between the DHEA-treated and the negative and positive control groups were observed. However, significant differences among the treatment groups were observed in survival rate and weight gain, respectively. Optimum dosage of 106 mg kg^{-1} was estimated using the quadratic model with weight gain as the response parameter. These findings suggested that DHEA can be used efficiently as growth-promoting agent for young hybrid red tilapia for at least 12 weeks.

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