The Israeli Journal of Aquaculture – Bamidgeh • ISSN 0792-156X • IJA.74.2022.1820113, 13 pages
CCBY-NC-ND-4.0 ● https://doi.org/10.46989/001c.55654

17α-Methyltestosterone enhances growth and reproductive performance of immature male Asian catfish (*Clarias macrocephalus*)

Lemark M. Bautista[1,2], Barry Leonard M. Tumbokon[3], Augusto E. Serrano, Jr.,[1,3*]

[1]Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines
[2]Pangasinan State University–Binmaley Campus, San Isidro Norte, Binmaley, Pangasinan, Philippines
[3]National Institute of Molecular Biology and Biotechnology, University of the Philippines Visayas, Miagao, Iloilo, Philippines

(Received Oct 31, 2022; Accepted Nov 07, 2022; Published Nov 15, 2022)

Keywords: anabolic hormone, gonadosomatic index, hepatosomatic index, testis weight, steroid

Abstract

This study aimed to assess the effects of 17α-methyltestosterone (MT) on the growth and reproductive performance of male Asian catfish (*Clarias macrocephalus*). A total of 36 immature male Asian catfish (ABW = 125.97 g) were randomly distributed into 12 circular tanks, and fed with either the control diet (with no MT) or diets containing MT at 60 mg·kg$^{-1,}$ 90 mg·kg^{-1}, or 120 mg·kg^{-1} for 45 days. Results showed that weight gain (WG), specific growth rate (SGR) and feed intake (FI) were significantly higher in the male catfish fed with diets containing 60 and 90 mg·kg^{-1} MT than in those fed with the control diet. Also, the diet attractability test revealed that the 60 and 90 mg·kg^{-1} MT groups significantly attracted more catfish than did the control diet; however, increasing MT to 120 mg·kg^{-1} reduced its attractability to the immature male catfish. MT-treated male catfish exhibited significantly heavier and significantly longer testes than male catfish fed the control diet at the termination of the feeding trial. These male catfish also exhibited significantly higher gonadosomatic indices (GSI) than catfish in the control group. Following induced spawning of nontreated female catfish and artificial fertilization of its eggs using testis preparation from the experimental male catfish at the termination of the feeding trial, results showed that testis preparations from all MT-treated males resulted in significantly higher fertilization (FR) and hatching rates (HR) of the eggs. In conclusion, incorporating MT to the diet improved both the growth and reproductive performance of the male *Clarias macrocephalus*. Precisely, dosages of optimal dietary MT using a quadratic model for maximal SGR, GSI, FR, and HR values were estimated to be 58.3, 75.0, 90.6, and 78.2 mg·kg^{-1}, respectively.

* Corresponding author. e-mail: aeserrano@up.edu.ph

Introduction

Asian native catfish (*Clarias macrocephalus*) is one of the six Clarias species reported to be naturally occurring in the Philippines (Santos et al., 2015). Currently, the abundance of *C. macrocephalus* species is significantly reduced due to habitat loss, poor water quality, and larger competitors (Vidthayanon & Allen, 2013). Several efforts have been made in the breeding, hatchery, and grow out of *C. macrocephalus.* However, these did not successfully sustain the industry of native catfish in the country. One of the main reasons is the reduced reproductive success of native catfish in the wild and in captivity. Unsuitable environmental conditions, ineffective feed diets, and failure in the captive maturation of breeders are several factors contributing to the problem. In our previous study, we found that adding benfotiamine to our high carbohydrate formulation enhanced the growth and reproductive performance of *C. macrocephalus* (Bautista et al., 2022). Still, the sexual performance of male catfish remains to be further enhanced. Great efforts should be made to understand and improve its reproductive processes in captivity.

It is known that semen production of Asian catfish males in captivity is limited. Thus, research is needed about the development of the testes and the action of androgen hormones on gametogenesis in captivity. Studies that evaluated gonadal steroids are very few, and an example of such studies was reported in common snook (*Centropomus undecimales*) in the natural environment. These studies reported an increase in androgen hormones and the development of the testes concerning the reproductive season (Rhody et al., 2015; Roberts et al., 1999). Androgen hormones can induce spermatogenesis in immature fish and during puberty (Borg, 1994). These hormones are mainly produced in the gonads, and variations in plasma concentrations regulate gonadal development (Schulz et al., 2010) and stimulate gonadotropin-releasing hormone (GnRH) production in the brain (Schulz et al., 1999; Taranger et al., 2010). Testosterone is an essential precursor in steroidogenesis and may stimulate the production of gonadotropins via the pituitary gland (Shulz et al., 2002). The synthetic androgen MT is widely used in aquaculture for research and control of reproductive processes. Among its uses are masculinization, sexual inversion, and stimulation of semen production in adult males (Henry et al., 1998; Aizen et al., 2005; Sarter et al., 2006). MT promotes both muscle growth and the development of male sexual characteristics (Khalil et al., 2011). Steroid hormones have increased growth, protein synthesis, and efficient utilization of feeds in several cultured animals (Adenigba et al., 2017; Ajiboye et al., 2015; Matty and Cheema, 1978). The research on the effects of anabolic steroids (e.g., methyltestosterone or ethynyltestosterone) in fish has been mainly directed toward sex reversal (Adenigba et al., 2017; Nuanmanee et al., 2004; Vorasayan and Petchrich, 2004) or to the inducement of sterility (Kaliba et al., 2007; Straus et al., 2013). But based on many reports, different supplementations of steroids, both androgenic and estrogenic, added to feeds also stimulate the growth parameters and feed conversion efficiency of fish (Kumar et al., 2016; Muniasamy et al., 2019; Yu et al., 1979). Studies have been carried out on several important cultured fish species, such as salmonids (McBride & Fagerlund, 1973; Yu et al., 1979), cyprinids (Matty & Lone, 1979; Venkateshvaran et al., 1993), and Nile tilapia (Turan et al., 2007; Khalil et al., 2011).

Since steroid supplementation increases fish growth, the possibility of incorporating male steroid hormones in the diets of male native catfish might enhance both its growth and reproductive performance. This study was performed to evaluate the effects of MT on the growth and reproductive performance of hormone-treated male Asian catfish (*Clarias macrocephalus*) during first maturation.

Materials and Methods

Experimental Fish

Healthy immature native catfish (*Clarias macrocephalus*) were procured from catfish collectors in Aborlan, Palawan, Philippines. The fish species was morphologically identified as *C. macrocephalus* based on its short, rounded occipital process, long dorsal fins, and the presence of white spots on the sides of its body (Na-Nakorn, 2004; Tan et al., 2016; Whan-Air et al., 2018). Purchased catfish were transported to Salem Aquafarm

in Puerto Princesa City, Palawan. Fish were acclimatized to the controlled condition and were fed with the commercial diet before the feeding experiment.

Experimental Diet Preparation

Four experimental diets were prepared: a control diet with no steroid hormone; and diets incorporated with graded concentrations of the steroid (MT) at 60 mg·kg^{-1}, 90 mg·kg^{-1}, and 120 mg·kg^{-1}, respectively. Commercial feeds (Tateh Aquafeeds: Catfish Surfer Pellet) were pulverized and then steamed for about 60 min. Steamed feeds were placed into trays and left to dry in the air for several minutes. Pre-measured MT hormone was dissolved in ethyl alcohol and mixed thoroughly with the prepared air-dried feeds. The mixture was extruded through a grinder, air-dried, and cut into desired sizes. The control diet was pulverized and prepared in the same manner as that for the experimental diets with the addition of distilled water instead of the hormone preparation. The prepared feeds were stored in the freezer until use.

Feeding trials

A total of 36 male subadult Asian native catfish (3 fish per tank $^{-1}$ with an ABW = 125.97 g) were randomly distributed into 12 circular tanks (70 cm diameter x 25 cm height) filled with 50 L freshwater (salinity of 0 parts per thousand, ‰). Each tank was provided with a continuous supply of aeration. The groups of fish in the randomly assigned tanks were fed with one of the 4 dietary treatments and reared for a total of 45 days.

Feeding was *ad libitum,* wherein the amount of feed was measured every first day of the week and was used as the basis for the feeding rate for the whole week. Feeding was closely monitored at each feeding time and adjusted accordingly to stop feeding when no feeding activity was detected or add more feeds when feeds were consumed in a short period. Diets were given thrice daily at 0800h, 1400h, and 2000h. Water temperature, pH, and salinity were measured three times a week, while nitrite and ammonia were monitored once a week. Sampling was done on the first day of stocking and every 15 days by bulk-weighing the experimental fish per rearing tank until the end of the experiment.

Diet Attractability Test

A customized wooden tank with multiple chambers was used to assess the attractability of the experimental diets (Suresh et al., 2011). A tank (4 ft x 4 ft x 1 ft) was divided into three main chambers: an acclimatization chamber, a middle chamber, and a feeding chamber. A removable wooden shutter separates the acclimatization and middle chambers. The feeding chamber is divided into four sub-chambers with an opening to allow free catfish access to experimental feeds placed in each chamber. The tank was filled with 150 L of freshwater (0 ppt) input. Three attractability tests were performed using 20 male catfish (ABW = 127.6 g). At the start of the test, fish were stocked in the acclimatization chamber for 1 h before the placement of experimental diets. Five grams of each diet were randomly placed in each feeding chamber. The shutter was removed 3 min after feed placement to allow access of catfish to the feeds. Feed preference was quantified by counting the number of catfish in the feeding chamber at 1, 5, and 10 min following the raising of the shutter. The percentage of catfish per feeding chamber at different time intervals was calculated.

Growth Performance

During the experiment, weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate (SR) were computed using the following formula:

Weight Gain (WG, g) = Final Average Body Weight (FABW) – Initial Average Body Weight (IABW)

Specific Growth Rate (SGR, %·day^{-1}) = 100*[Ln (FABW(g) – Ln (IBW (g)] / (No. of days)

Feed Intake (FI, g) = sum of daily feed offered for the whole culture period (g)

Feed Conversion ratio (FCR) = Total FI (g) / WG (g)

Survival Rate (%) = (Total no. of fish survived) / (Total no. of fish stocked) x 100

GSI and HSI of male Asian catfish

After rearing for 45 days, experimental male catfish were subjected to morphometric, gonadosomatic, and hepatosomatic index analysis. Before the examination, fish were subjected to pithing, a technique used to immobilize the fish by inserting a pointed metal rod into its occipital process. Fish were then placed in a basin containing water and ice. The water's cold temperature has been observed to be an effective method of making the fish inactive. Dissection of the liver and testes of the fish was done by cutting the ventral part of the fish's body using a scalpel and scissors. Dissected liver and pair of testes were placed in a petri dish. Subsequently, we measured their lengths and weights. The formula used to compute gonadosomatic and hepatosomatic indices was as follows:

Gonadosomatic Index (GSI, Testes) (%) = 100*(Testis weight (g) / Body weight (g))

Hepatosomatic Index (HSI, %) = 100*(Liver weight (g) / Body weight (g))

Reproductive performance

Another feeding trial was conducted for 45 days to determine MT's effects on immature male catfish's reproductive performance. A total of 16 immature male catfish were distributed in 16 circular tanks (i.e., one individual fish per replicate at 4 replicates per treatment (treatments were 0, 60, 90, and 120 $mgkg^{-1}$ MT). At the termination of the experiment in which all experimental male catfish have reached sexual maturity, all male catfish were sacrificed and testes excised. Testes from two replicates (i.e., two individual male catfish) were macerated and used to fertilize artificially all stripped eggs of one non-MT treated female; this made the total number of non-treated female catfish used in the artificial fertilization to be 8 individuals. Twelve hours after mixing the macerated testes and stripped eggs, 0.5 g of eggs were collected, from which fertilization rate was determined, and subsequently hatching rate; larval survival rate was determined 7 days after hatching. The following formulae were used:

Fertilization Rate (%) = 100*(No. of fertilized eggs / Total no. of eggs in a batch)

Hatching Rate (%) = 100*(No. of eggs hatched / Total no. of fertilized eggs)

Survival Rate (%) = 100*(Total no. of survived larvae until day 7 / Total no. of larvae at day1

Estimation of optimum hormone dosage and statistical analysis

Data were analyzed by fitting a quadratic regression equation used in fish to estimate protein and amino acids (Chiu et al., 1988; Zeitoun et al., 1976). This model was deemed appropriate for treating almost all hyperbolic data in which the response parameter reaches a peak and finally declines from 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 calculated to provide the best fit of the data. The value of I that produces the maximum response I_{max} is calculated as follows:

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

The standard error of the mean (SEM) was calculated for all mean values. Data were subjected to analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT)

to determine differences in means ($p<0.05$). All statistical analyses were done with the Statistical Package for the Social Sciences (SPSS) Version 24 software (Chicago, Illinois, USA).

Results

Growth, Feed Utilization, and Survival

WG, SGR, and FI values were significantly higher in male catfish in the 60 mg·kg^{-1} and 90 mg·kg^{-1} groups than in those in the control group ($P<0.5$) (**Table 1**). FCR values were significantly better in the 60 mg·kg^{-1} and 90 mg·kg^{-1} groups than in the control and 120 mg·kg^{-1} groups ($P<0.5$). FABW values and survival rates were not significantly different among dietary groups. The 60 mg·kg^{-1} and 90 mg·kg^{-1} diets attracted more male catfish than the control diet (**Table 2**). However, increasing up to 120mg hormone level resulted in decreased attractability ($P<0.5$).

Table 1 Growth, feed utilization, and survival of male native catfish fed the experimental diets for 45 days culture period.

Diet 17aMT (mg)	*IABW (g)*	*FABW (g)*	*WG (g)*	*SGR (% day^{-1})*	*FI (g)*	*FCR*	*Survival (%)*
0	125.55±0.40^{a}	132.06±1.27^{a}	6.50±1.3^{a}	0.11±0.02^{a}	27.53±0.57^{a}	4.5±0.84^{a}	88.89±11.11^{a}
60	127.89±3.11^{a}	140.33±3.35^{a}	12.44±0.29^{b}	0.21±0.00^{b}	29.94±0.76^{b}	2.4±0.03^{b}	100.0±0.00^{a}
90	126.33±0.84^{a}	137.00±1.02^{a}	10.66±0.33^{b}	0.18±0.01^{b}	29.26±0.43^{ab}	2.7±0.09^{b}	100.0±0.00^{a}
120	124.11±4.75^{a}	129.22±5.49^{a}	5.11±1.17^{a}	0.09±0.02^{a}	26.93±0.26^{a}	5.8±1.30^{a}	88.89±11.11^{a}

Table 2 Attractability of male Asian native catfish (*C. macrocephalus*) to the experimental diets.

Diet 17aMT (mg·kg-1)	*Percent of catfish attracted (%)*			
	1 min	*5 min*	*10 min*	*Average*
0	8.33±1.67^{a}	20.00±2.89^{b}	16.67±3.33^{b}	15.00±1.66^{b}
60	11.67±1.67^{a}	31.67±4.41^{b}	25.00±2.89^{b}	22.77±0.55^{c}
90	13.33±4.41^{a}	23.33±1.66^{b}	21.66±1.67^{b}	19.45±1.46^{c}
120	3.33±1.67^{a}	11.66±1.66^{a}	8.33±1.67^{a}	7.77±1.46^{a}

Gonadosomatic and Hepatosomatic Index

Male Asian catfish fed diets containing MT exhibited significantly heavier and significantly longer testes than those fed the control diet ($P<0.5$, **Table 3**). HSI values were not significantly different among dietary groups. In contrast, male catfish fed diets with varying levels of MT showed significantly higher gonadosomatic indices (GSI) than those in the control group ($P<0.5$). The dosage that elicited the maximum GSI was 58.3 mg·kg^{-1}, estimated from the quadratic model (**Figure 1**).

Table 3 GSI and HSI indices of male catfish fed experimental diets containing varying levels of MT

Dietary MT (mg·kg^{-1})	*Testes weight (g)*	*Testes length (cm)*	*GSI (male)*	*Liver weight (g)*	*Liver length (cm)*	*HSI (male)*
0	0.27±0.03^{a}	1.63±0.15^{a}	0.23±0.01^{a}	0.60±0.17^{a}	1.50±0.28^{a}	0.51±0.13^{a}
60	0.47±0.03^{b}	2.10±0.10^{b}	0.38±0.02^{b}	0.76±0.18^{a}	1.80±0.15^{a}	0.63±0.16^{a}
90	0.50±0.05^{b}	2.30±0.06^{b}	0.41±0.04^{b}	0.77±0.19^{a}	1.93±0.29^{a}	0.62±0.14^{a}
120	0.40±0.00^{b}	2.07±0.18^{b}	0.34±0.01^{b}	0.70±0.15^{a}	1.76±0.12^{a}	0.55±0.09^{a}

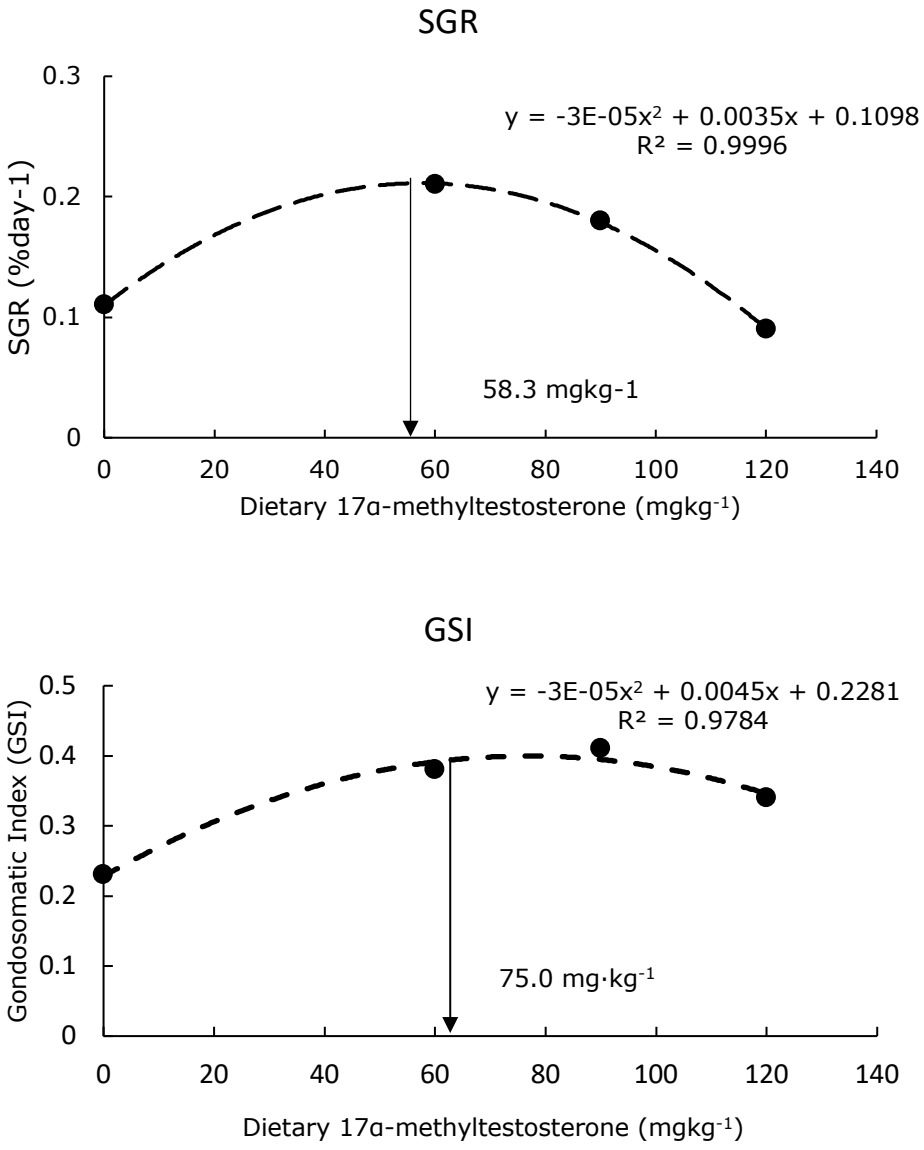

Figure 1 SGR (top) and GSI (bottom) of male *Clarias macrocephalus* fed diets containing graded levels of MT, fitted in a quadratic model. Maximum SGR and GSI values were elicited at 58.3 mg·kg^{-1} and 75 mg·kg^{-1} MT, respectively.

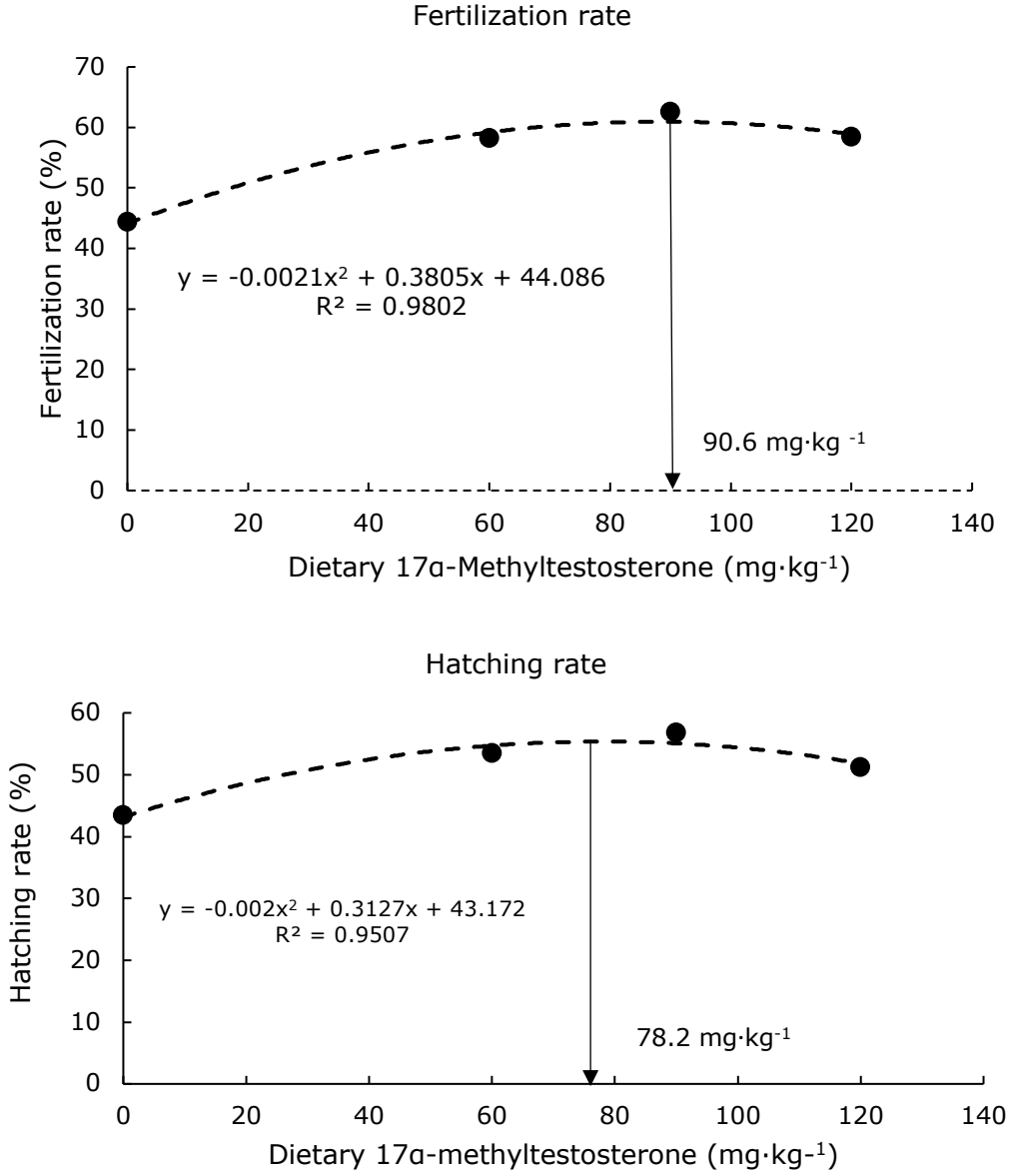

Figure 2 FR (top) and HR (bottom) values of sexually mature male *Clarias macrocephalus* fed diets containing graded levels of MT, fitted in a quadratic model. Maximum FR and HR values were elicited at 90.6 mg·kg^{-1} and 78.2 mg·kg^{-1} MT, respectively.

Reproductive performance

FR and HR values in the artificial fertilization of eggs of nontreated female catfish were significantly higher when fertilized with testes of MT-treated males than those of the control group (P<0.5, **Table 4**). Highest FR and HR values were observed in the 90 mg·kg^{-1} group, followed by those in the 60 mg·kg^{-1} g group, 120 mg·kg^{-1} group, and the control group in this decreasing order. The maximum FR and HR values were obtained at estimated dosages of 90.6 mg·kg^{-1} and 78.2 mg·kg^{-1} MT, respectively, when the quadratic model was

employed (**Figure 2**). Larval survival rates (LSR) were not significantly different among treatments but were numerically higher in the MT-treated groups.

Table 4 Effects of various MT treatments on male catfish reproductive performance in terms of fertilization rate, hatching rate, and larval survival rate for 7 days.

Treatment	*No of Females*	*Female BW(g)*	*Stripped egg weight (g)*	*Fecundity (no. Stripped Eggs)*	*No. of Fertilized eggs (/ 0.5 g eggs)*	*No. of fertilized eggs (/0.5 g eggs)*	*No. of hatched Larvae*	*No. of larvae survived after 7 days*	*Fertilization Rate (%)*	*Hatching Rate (%)*	*Survival Rate % (7 days)*
1_1, 0											
	1	157	9.0	4968	276	121	53	21	43.84	43.80	39.62
1_2, 0											
1_3, 0											
	1	150	7.3	4409	302	135	58	25	44.70	42.96	43.10
1_4, 0											
Ave.					289	128	56	23	44.27[a]	43.38a	41.36[a]
2_1, 60											
	1	148	7.0	3570	255	136	71	32	53.33	52.21	45.07
2 _2, 60											
2 _3, 60											
	1	155	7.5	4320	288	181	99	55	62.85	54.70	55.56
2_4, 60											
Ave.					272	159	85	44	58.09[b]	53.45[b]	50.31[a]
3_1, 90											
	1	152	7.8	4399	282	168	90	46	59.57	53.57	51.11
3_2, 90											
3_3_90,											
	1	147	8.0	4336	271	177	106	57	65.31	59.89	53.77
3_4, 90											
Ave.					277	173	98	52	62.44[b]	56.73[b]	52.44[a]
4_1, 120											
	1	150	8.2	4379	267	153	82	37	57.30	53.59	45.12
4_2, 120											
4_3, 120											
	1	155	7.5	4095	273	162	79	40	59.34	48.77	50.63
4_4, 120											
Ave.					270	158	81	39	58.32[b]	51.18[ab]	47.88[a]

Discussion

The present study showed that MT significantly improved the growth performance of the native catfish. Increased WG, SGR, and FI values were observed in the treatments fed experimental diets supplemented with 60 mg and 90 mg of MT. Similar observations were reported by Yu et al. (1979) when steroid hormones were incorporated in test diets fed to coho salmon. Their study revealed that MT supplementation promotes more significant fish weight gain and feed efficiency. Lone and Matty (1980) also observed that fish in the experimental groups that received MT grew faster in weight than the controls throughout the study. The results of their study show that MT significantly increases growth in carp. Significant increases in weight and length were also observed in chinooks fed hormone diets (McBride and Fagerlund, 1973). Muniasamy et al. (2019) also evaluated the effect of different concentrations of MT-incorporated diet on the growth performance of *Channa punctatus* and *Cirrhinus mrigala*. The growth performance in terms of length and weight gain of the fish receiving 100 mg·kg^{-1} in *C. punctatus* and 60 mg·kg^{-1} in *C. mrigala* were significantly higher than those receiving 80, 120, and 0 (untreated control) mg·kg^{-1} of the hormone. High specific growth rates were observed in *C. punctatus* and C. *mrigala* showing a positive influence of hormone-incorporated diet on growth performance.

In the present study, oral administration of the synthetic androgen MT for 45 days promoted reproductive performance in male catfish, leading to increased GSI, FR, and HR values in the MT-treated male catfish. In the common snook, Passini et al. (2018) reported that MT implants promoted the growth of the gonad and stimulated spermatogenesis

during first maturation. Still, they observed suppression in steroid production with the highest MT concentrations. The increase in the GSI values as dietary MT increased in the present study indicated the viability of dietary MT in stimulating the development of first maturation in male Asian catfish. A significant increase in GSI is also observed in Nile tilapia treated with MT (Khalil et al., 2011). Yamazaki (1976) also found that administration of MT in the diet combined with injection of salmon gonadotropin effectively accelerated the sexual maturation of rainbow trout. MT is recognized as an androgen agonist in fish as it induces regressed testes to produce sperm in hypophysectomized mummichog *Fundulus heteroclitus* (Lofts et al., 1966). MT binds with high affinity to fish androgen receptors (Sperry & Thomas, 1999) and induces the development of male nuptial tubercles in fathead minnow, *Pimephales promelas* (Ankley et al., 2001).

In other fish species, dietary MT presented unfavorable effects on the gonads. For one, there is a phenomenon called paradoxical feminization, which occurs at a high dosage of MT or prolonged treatment in which male fish are induced to convert to female (i.e., gonadal intersexuality) (Papoulias et al., 2000). After forty-five days of oral administration of MT and at the dosages used, paradoxical feminization had not been observed in the present study. Macintosh (1988) showed that 60 mg·kg^{-1} MT produced some testicular degeneration, lowering the GSI value. Ahmad et al. (2002) found that male and female GSI was significantly decreased at high MT doses (5, 10, 20, and 40 mg MT·kg^{-1} feed), while non-significant change was observed at low MT doses (0.5, 1.0, and 2.5 mg MT·kg^{-1} feed). Shen et al. (2015) observed that in *Pelteobagrus fulvidraco*, high doses caused large amounts of vacuolated seminiferous lobules that could be due to the degeneration of testes. High doses and long-term administration of MT produced sterile males with a malformation in grouper and milkfish (Lee et al.,1986; Tan-Fermin et al.,1994). Yamazaki (1972) in pink and chum salmon, Hirose & Hibiya (1968a, 1968b) in goldfish and rainbow trout, and Sayed et al. (2018) in *Oreochromis niloticus* reported that MT administration of 2.5 mg MT·kg^{-1} induced the degenerative changes in testes. Higgs et al. (1977) noted clear signs of gonad degeneration in coho salmon affected by MT, causing fish sterility, which might be considered advantageous in fish culture.

Feed intake of male catfish fed experimental diets containing 60 mg·kg^{-1} MT was significantly higher than those fed the other diets. Yamazaki (1976) observed that supplementation of MT in the rainbow trout and goldfish diet resulted in better food consumption and appeared to have improved appetite and feeding behavior. Muniasamy et al. (2019) also demonstrated a considerable increment in consumption rate in *C. punctatus* fed with 100 mg·kg^{-1} and C*. mrigala* 60 mg·kg^{-1} hormone. Furthermore, Yu et al. (1979) stated that fish fed with a steroid-supplemented diet appeared to be more active in feeding. The MT appeared to be the most effective among the three steroids tested. Fish fed on the said diet had more significant weight gain, feed conversion efficiency, and protein conversion efficiency.

The present study's liver length, weight, and HSI values remained unaffected by MT treatment in the male catfish for 45 days. This is parallel to the other studies in which no significant pathological differences were observed among treatments after exposure to MT (Kefi et al., 2013; Simone, 1990). In contrast, Sayed et al. (2018) reported that different histopathological alterations were recorded in fish liver collected from Nile tilapia monosex farms produced by MT administration. Khater (1998) observed that liver tissue of Nile tilapia treated with 60mg MT for 14 days showed diffused hydropic degeneration, congested central vein, and hemorrhage in the hepatic parenchyma. Hasheesh et al. (2011) noted diffused vacuolar degeneration followed by mild and severe hepatic vacuolations.

In the present study, FR and HR of eggs from non–treated female Asian catfish in the present study were significantly higher when fertilized with testes from MT-treated male catfish. Success in fertilization and hatching rate is affected by the quantity and quality of the testes. It was observed in the present study that the testes of male catfish fed diets containing MT were significantly heavier and longer than the control group. The study of Yamazaki (1976) illustrated the effect of MT on the quality of testes of rainbow trout. Horai masu rainbow trout administered with MT reached its testis maturity at the

end of their experiment. The testes of the treated fish were filled with spermatocytes and spermatozoa, while no spermatogenesis was found in any control males. Additionally, Bera et al. (2021) investigated the scope of co-administration of the androgen MT in combination with gonadotropin-releasing hormone analog (GnRHa) as a single hormone pellet at varied frequencies in male and female milkfish to affect maturation, egg quality, spawning, and subsequent larval fitness. Their results revealed that applying gonadotropin-releasing hormone analog (GnRHa) and androgen (MT) induced milkfish breeders' reproductive axis, resulting in extended spawning frequency.

In conclusion, incorporating MT into the diet improved the growth and reproductive performance of the male *Clarias macrocephalus*. Optimal dietary MT was estimated using a quadratic regression model for maximal SGR, GSI, FR, and HR values to be 58.3, 75.0, 90.6, and 78.2 $mg \cdot kg^{-1}$, respectively.

Acknowledgments

The authors would like to express their sincerest gratitude for the financial and scholarship grant provided by the DOST – ASTHRDP and DOST – PCAARRD GREAT Program; Pangasinan State University – Binmaley Campus for its financial support; and for the support of Salem Aquafarm in Palawan that served as an annex facility of the Western Philippines University (WPU). They are also grateful to UP Visayas Office of the Vice Chancellor for Research and Extension (OVCRE) for the partial research and publication grants.

References

Adenigba, I., Tumbokon, B.L.M., Serrano Jr., A.E., 2017. Androgenic and anabolic effects of *Pinus tabulaeformis* Carr. pollen in *Clarias gariepinus*. *The Israeli Journal of Aquaculture - Bamidgeh, IJA*_69.2017.1388: 8 pages. https://doi.org/0.46989/001c.20875

Ahmad, M.H., Shalaby, A.M.E. Khattab, Y.A.E., Abdel-Tawwab, M., 2002. Effects of 17α-methyltestosterone on growth performance and some physiological changes of Nile tilapia fingerlings (*Oreochromis niloticus L.). Egypt. J. Aquat. Biol. Fish*, 4: 295-311. https://doi.org/10.21608/EJABF.2002.1736

Aizen, J., Meiri, I., Tzchori, I., Levavi-Sivan, B., Rosenfeld, H., 2005. Enhancing spawning in the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition. *Gen. Comp Endocrinol.* 142:212-21. https://doi.org/10.1016/j.ygcen.2005.01.002.

Ajiboye, O. O., Qari, R., Yakubu, A. F., & Adams, T. E. 2015. Growth performance of Nile Tilapia, *Oreochromis niloticus* L. reared in glass aquaria tanks under different treatments and durations. *Int. J. Biol. Biotechnol.*, *12*(2): 203–209.

Ankley, G.T., Jensen, K.M., Kahl, M.D., Korte, J.J., Makynen, E.A., 2001. Description and evaluation of a short-term reproduction test with the fathead minnow (*Pimephales promelas*). *Environ. Toxicol. Chem.* 20: 1276–1290. https://doi.org/10.1002/etc.5620200616

Bautista, L.M., Tumbokon, B.L.M., Plasus, M.G., Serrano Jr., A.E., 2022. Benfotiamine in high carbohydrate diet enhances growth and reproductive performance of the Asian native catfish *Clarias macrocephalus* subadults. *The Israeli Journal of Aquaculture – Bamidgeh IJA.*74.2022.1716727, 11 pages. https://doi.org/10.46989/001c.36448.

Bera, A., Kailasam, M., Mandal, B., Padiyar, A., Ambasankar, K., Sukumaran, K., Makesh M., Kumararaja, P., Subburaj, R., Thiagarajan, G. Vijayan, K., 2021. Maturity induction and extended spawning kinetics of milkfish (*Chanos chanos*) administered with combined GnRHa and 17α-methyltestosterone pellet at varied frequencies. *Aquaculture*, *543*. https://doi.org/10.1016/j.aquaculture.2021.736993

Borg, B., 1994. Androgens in teleost fishes. *Comp Biochem Physiol.* 109(3):219-45. https://doi.org/10.1016/0742-8413(94)00063-G.

Chiu, Y. N., Austic, R. E., Rumsey, G. L., 1988. Effect of feeding level and dietary electrolytes on the arginine requirement of rainbow trout (*Salmo gairdneri*). *Aquaculture* 69:79-91. https://doi.org/10.1016/0044-8486(88)90188-3.

Hasheesh, W. S., Marie, M. A. S., Abbas, H. H., Eshak, M. G., Zahran, E. A., 2011. An evaluation of the effect of 17α-methyltestosterone hormone on some biochemical, molecular and histological changes in the liver of Nile tilapia *Oreochromis niloticus*. *Life Sci. J.* 8(3):343-358.

Henry, J.C., McLean, E., Mayer, I., Donaldson, E.M., 1998. Induction of precocious maturation in masculinized Atlantic salmon by treatment with sustained-release LHRHa and testosterone. *Aquacult. Int.* 6:261-8. https://doi.org/10.1023/A:1009255815481

Higgs, D.A., Fagerlund, U.H.M., McBride, J.R., Dye, H.M., Donaldson, E.M., 1977. Influence of combinations of bovine growth hormone, 17α-methyltestosterone and L-thyroxine on growth of young coho salmon (*Oncorhynchus kisutch*). *Can. J. Zool*., 55: 1048-1056. https://doi.org/10.1139/z77-133

Hirose, K., Hibiya, T., 1968a. Physiological studies on growth promoting effect of protein anabolic steroids on fish. I. Effects on goldfish. *Bull. Jap. Soc. Sci. Fish.* 34: 466-472.

Hirose, K., Hibiya, T., 1968b. Physiological studies on growth promoting effect of protein anabolic steroids on fish. I. Effects of 4-chlorotestosterone acetate on rainbow trout. *Bull. Jap. Soc. Sci. Fish.* 34: 473-481.

Kaliba, A.R., C.C. Ngugi, J. Mackambo and K.K. Quagrainie 2007. Economic profitability of Nile tilapia (*Oreochromis niloticus* L.) production in Kenya. *Aquaculture Research*. 38: 1129-1136. https://doi.org/10.1111/j.1365-2109.2007.01772.x

Kefi, A.S., Kang'ombe, J., Kassam, D., Katongo, C., 2013. Effect of 17α-methyltestosterone on haematology and histology of liver and heart of *Oreochromis andersonii* (Castelnau, 1861). *J Marine Sci Res Dev,* 3: 130.

Khalil, W.K.B., Hasheesh, W.S., Marie, M.A.S., Abbas, H.H., Zahran, E.A., 2011. Assessment the impact of 17α-methyltestosterone hormone on growth, hormone concentration, molecular and histopathological changes in muscles and testis of Nile tilapia; *Oreochromis niloticus. Life Sci. J.* 8: (3). https://doi.org/0.4103/2305-0500.275527

Khater, A.M.M., 1998. Sex reversal in *Tilapia nilotica*. Ph.D. Thesis. Zagazig University, Agricultural Science. 144 pp.

Kumar, S., Behera, S., Nagesh, T.S., Gogoi, R., Prakash, C.B., 2016. Effect of 17α-methyltestosterone on the growth performance of a fighting fish (*Betta splendens*). *J. Exp. Zool. India* 19: 89–93. https://doi.org/10.1016/j.sjbs.2018.07.006

Lee, C.S., Weber, G.M., Tamaru, C.S., 1986. Effect of orally-administered 17α-methyltestosterone on spermatogenesis in immature milkfish, *Chanos chanos* Forsskal. *J. Fish Biol.* 29: 567-572. https://doi.org/10.1111/j.1095-8649.1986.tb04973.x.

Lofts, B., Pickford, G.E., Atz, J.W., 1966. Effects of methyltestosterone on the testes of a hypophysectomized cyprinodont fish, *Fundulus heteroclitus*. *Gen. Comp. Endocrinol.* 6: 74–88. https://doi.org/10.1016/0016-6480(66)90109-2.

Lone, K. P., and Matty, A. J. 1980. The effect of feeding methyltestosterone on the growth and body composition of common carp (*Cyprinus carpio* L.). *General and Comparative Endocrinology*, *40*(4): 409–424. https://doi.org/10.1016/0016-6480(80)90004-0

Macintosh, D.J., Singh, T.B., Little, D.C. and Edwards, P., 1988. Growth and sexual development of 17α-methyltestosterone and progesterone-treated Nile tilapia (*Oreochromis niloticus*) reared in earthen ponds. In Pullin, R.S.V., Bhukaswan, T., Tongothai, K., Maclean, J.L., (Ed.). *The Second International Symposium on Tilapia in Aquaculture, Bangkok, Thailand, Manila*, Philippines. pp. 457- 463.

Matty, A. J., Lone, K. P. 1979. The effect of androgenic steroids as dietary additives on the growth of carp (*Cyprinus carpio*). *Proceedings of the World Mariculture Society*, *10*(1–4): 735–745. https://doi.org/10.1111/j.1749-7345.1979.tb00072.x.

Matty, A.J., Cheema, L.R., 1978. The effect of some steroid hormones on the growth and protein metabolism of rainbow trout. *Aquaculture* 14: 163-178. https://doi.org/10.1016/0044-8486(78)90027-3.

McBride, J. R., Fagerlund, U.H.M., 1973. The use of 17α-methyltestosterone for promoting weight increases in juvenile Pacific salmon. *J. Fish. Res. Board Can.* 30:1099-1104. https://doi.org/10.1139/f73-180.

Muniasamy, S., Allen Benziger, P. S., Ananth Kumar, Y., Haniffa, M. A., Paray, B. A., Albeshr, M. F., & Al-Umri, S. 2019. Effect of 17α-methyltestosterone incorporated diets on growth of spotted snakehead, *Channa punctatus* and white carp, *Cirrhinus mrigala*. *Saudi J. Biol. Sci. 26*(3): 541–546. https://doi.org/10.1016/j.sjbs.2018.07.006.
Na-Nakorn, U. 2004. A perspective on breeding and genetics of walking catfish in Thailand. *Aquaculture Asia Magazine*, 9 (4): 10-12. URL: http://library.enaca.org/AquacultureA.
Nuanmanee, P., S. Siangwan and J. Nitharn. 2004. Effects of androgens on sex reversal of Nile tilapia. *Fisheries Magazine*. 57: 251, September 2004.
Papoulias, D.M., Noltie, D.B., Tillitt, D.E., 2000. Effects of methyltestosterone exposure on sexual differentiation in medaka, *Oryzias latipes*. *Mar. Environ. Res.,* 50: 181-184. https://doi.org/10.1016/s0141-1136(00)00076-3
Passini, G., Sterzelecki, F.C., de Carvalho, C.V.A., Baloi, M.F., Naide, V., Cerqueira, V.R., 2018. 17α-methyltestosterone implants accelerate spermatogenesis in common snook, *Centropomus undecimalis*, during first sexual maturation. *Theriogenology* 106: 134-140. https://doi.org/10.1016/j.theriogenology.2017.10.015
Rhody, N.R., Davie, A., Zmora, N., Zohar, Y., Main, K.L., Migaud, H., 2015. Influence of tidal cycles on the endocrine control of reproductive activity in common snook (*Centropomus undecimalis*). *Gen Comp Endocrinol.* 224: 247-59. https://doi.org/10.1016/j.ygcen.2015.08.007.
Roberts, S.B., Jackson, L.F., King, V.W., Taylor, R.G., Grier, H.J., Sullivan, C.V., 1999. Annual reproductive cycle of the common snook: endocrine correlates of maturation. *Trans. Am. Fish. Soc.* 128:436-45**.**
Santos, S.B. Vesagas, F.P., Tan, M.C., Jumawan, J.C., Quilang, J.P., 2015. Status assessment of *Clarias* species in the Philippines: insights from DNA barcodes. *Science Diliman* 27: 21–40.
Sarter, K., Papadaki, M., Zanuy, S., Mylonas, C.C., 2006. Permanent sex inversion in 1-year-old juveniles of the protogynous dusky grouper (*Epinephelus marginatus*) using controlled-release 17α-methyltestosterone implants. *Aquaculture* 256:443-56. https://doi.org/10.1016/j.aquaculture.2006.01.034
Sayed, A.E.D.H., Farrag, M.M.S., Abdelaty, B.S., Toutou, M.M.M., Muhammad, O.I., 2018. Histological alterations in some organs of monosex tilapia (*Oreochromis niloticus,* Linnaeus, 1758) produced using methyltestosterone. *Egypt. J. Biol. Fish.* 22(4): 141- 151. https://doi.org/10.21608/EJABF.2018.13272
Schulz R.W., França, L.R., Lareyre, J.J., LeGac, F., Chiarini-Garcia, H., Nobrega, R.H., Miura, T., 2010. Spermatogenesis in fish. *Gen Comp Endocrinol*. 165: 390-411. https://doi.org/10.1016/j.ygcen.2009.02.013
Schulz, R.W., Goos, H.J.Th., 1999. Puberty in male fish: concepts and recent developments with special reference to the African catfish (*Clarias gariepinus*). *Aquaculture* 177: 5-12. https://doi.org/10.1016/S0044-8486(99)00064-2.
Schulz, R.W., Miura, T. 2002. Spermatogenesis and its endocrine regulation. *Fish Physiol. Biochem.* 26:43-56. https://doi.org/10.1023/A:1023303427191
Shen, Z.G., Fan, Q.X., Yang, W., Zhang, Y.L., Wang, H.P., 2015. Effects of 17α-methyltestosterone and aromatase inhibitor letrozole on sex reversal, gonadal structure, and growth in yellow catfish *Pelteobagrus fulvidraco*. *Biol. Bull.,* 228: 108-117. https://doi.org/10.1086/BBLv228n2p108
Simone, D.A., 1990. The effects of the synthetic steroid 17-alpha-methyltestosterone on the growth and organ morphology of the channel catfish (*Ictalurus punctatus*). *Aquaculture* 84: 81-93. https://doi.org/10.1016/0044-8486(90)90302-4.
Sperry, T.S., Thomas, P., 1999. Identification of two nuclear androgen receptors in kelp bass (*Paralabrax clathratus*) and their binding affinities for xenobiotics: comparison with Atlantic croaker (*Micorpogonias undulatus*) androgen receptors. *Biol. Reprod.* 61: 1153–1161. https://doi.org/10.1095/biolreprod61.4.1152

Straus, D.L., J.D. Bowker, M.P. Bowman, D.G. Carty, A.J. Mitchell, B.D. Farmer, B.D., Ledbetter, C.K., 2013. Safety of feed Treated with 17α-methyltestosterone (17MT) to larval Nile Tilapia. The *North American Journal of Aquaculture*. 75(2): 212-219. https://doi.org/10.1080/15222055.2012.758211

Suresh, A.V., Kumaraguru vasagan, K.P., Nates, S. 2011. Attractability and palatability of protein ingredients of aquatic and terrestrial animal origin and their practical value for blue shrimp, *Litopenaeus stylirostris* fed diets formulated with high levels of poultry byproduct meal. *Aquaculture*, 319: 132-140. https://doi.org/10.1080/15222055.2012.758211

Tan-Fermin, J.D., Garcia, L.M.B., Castillo Jr., A.R., 1994. Induction of sex inversion in juvenile grouper, *Epinephelus suillus*, (Valenciennes) by injections of 17α-Methyltestosterone. *Bull. Environ. Contam. Toxicol. 40: 413-420. http://hdl.handle.net/10862/1465*

Tan, M. T. C., Jumawan, J. C., and Quilang, J. P. 2016. Low genetic diversity in *Clarias macrocephalus* Günther, 1864 (Siluriformes: Clariidae) populations in the Philippines and its implications for conservation and management. *Journal of Threatened Taxa*, *8*(6), 8849–8859. https://doi.org/10.11609/jott.2261.8.6.8849-8859

Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F.A., Dufour, S., Karlsen, O., Norberg, B., Andersson, E., Hansenet, T., 2010. Control of puberty in farmed fish. *Gen Comp Endocrinol.* 165:483-515. https://doi.org/10.1016/j.ygcen.2009.05.004

Turan, F., and Ihsan Akyurt. 2005. Effects of androstenedione, a phytoandrogen, on growth and body composition in the African *catfish Clarias* gariepinus. *Israeli Journal of Aquaculture - Bamidgeh* 57 (1): 62-66. https://doi.org/10.46989/001c.20391.

Turan, F., Gurlek, M., and Turan, C. 2007. Effects of dietary androstenedione concentration on growth of tilapia fry (*Oreochromis aureus* Linnaeus). *Israeli Journal of Aquaculture - Bamidgeh*, *59* (1): 32–35. https://doi.org/10.46989/001c.20506

Venkateshvaran, K., Venijgopal, G., Reddy, R.R.K., 1993. Field Application of Anabolic Steroids. In: Carp seed production I rearing of fry to fingerling stage. *J. Indian Fish. Assoc.* 23: 65-71. http://hdl.handle.net/1834/32167.

Vidthayanon, C., Allen D.J., 2013. *Clarias macrocephalus*. The IUCN Red List of Threatened Species. Version 2014.2. Available from: www.iucnredlist.org.

Vorasayan, P., Petchrich, N., 2004. Sex reversed tilapia seed production by using hormone mixed feed. *Fisheries Magazine*.57: 251. September 2004.

Whan-Air, W., Thongprajukaew, K., Salaeharae, T., Yoonram, K., 2018. Identification of wild and farmed broadhead catfish (*Clarias macrocephalus* Günther, 1864) based on morphometry, digestive indexes and flesh quality. *Journal of Oceanology and Limnology*, *36*(5): 1788–1797. https://doi.org/10.1007/s00343-018-7205-7.

Yamazaki, F. 1976. Application of hormones in fish culture. J. Fish. Res. Board Can. 33: 948-958. https://doi.org/10.1139/f76-122.

Yamazaki, F., 1972. Effects of methyltestosterone on the skin and the gonads of salmonids. *Gen. Comp. Endocrinol. Suppl.,* 3: 741-750. https://doi.org/10.1016/0016-6480(72)90203-1.

Yu, T. C., Sinnhuber, R. O., and Hendricks, J. D. 1979. Effect of steroid hormones on the growth of juvenile coho salmon (*Oncorhynchus kisutch*). *Aquaculture*, 16 (4): 351–359. https://doi.org/10.1016/0044-8486(79)90074-7.

Zeitoun, I. H., Ullrey, D. E., Magee, W. T., Gill, J. L., Bergen, W. G., 1976. Quantifying nutrient requirements of fish. *J. Fish. Res. Board. Can.* 33:167-172. https://doi.org/10.1139/f76-019.