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Original Article

Effect of natural and synthetic androgen hormone on sex reversal of Nile Tilapia (*Oreochromis niloticus*)

Efeito do hormônio andrógeno natural e sintético na reversão sexual da Tilápia do Nilo (*Oreochromis niloticus*)

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Abstract

The present study aimed to produce a monosex population of all male Nile tilapia (*Oreochromis spp.*) using 17α-methyl testosterone and common carp testes (as a source of natural androgen). Trial was conducted into two consecutive phases, the first was fry (4-5 days old)administration with negative control (without hormone) and positive control (with hormone) feed viz., MT1:60mg/kg, MT2:70mg/kg (17α-MT), carp testis CT1:70% and CT2:80% for 30 days to reverse the sex of male fish and the second phase was nursing the fingerlings for two months on control diet (32% Crude protein).Results revealed a significant growth rate (P<0.05) in the control group where final weight (4.8±0.34ab) and weight gained was recorded as 0.66±0.03ac. In proximate chemical composition of body meat, CT2 treatment showed maximum retention of crude protein, crude fat, and ash whereas dry matter showed maximum retention in MT2 and CT1 treatments. Morphological and histological examination revealed significant difference (p<0.05) in phenotypic males of Nile tilapia fed with the highest percent in MT-treated diet (MT2) of 95±0.58a while MT1, CT2 and CT1 had males of 85±6.0b, 70±5.0b and 65±6.5b, respectively. It was concluded that synthetic androgen (17αMT) was more effective for masculinization but natural androgen scan be an alternative method to produce male tilapia population in an environment-friendly manner as they are inexpensive, eco-friendly, and radially available. These results suggested that synthetic and natural androgen supplementation in the diet plays a significant role in improving growth performance and body composition.

Keywords: common carp testes, exotic fish, growth performance, masculinization and 17α-methyl testosterone.

Resumo

O presente estudo teve como objetivo produzir uma população monossexuada de todos os machos de tilápia do Nilo (*Oreochromis spp.*) usando 17α-metil testosterona e testículos de carpa comum como fonte de andrógeno natural. O ensaio foi conduzido em duas fases consecutivas, a primeira foi a administração de alevinos (4-5 dias) com controle negativo (sem hormônio) e controle positivo (com hormônio), viz., MT1:60mg/kg, MT2:70mg/kg (17α -MT), testículos de carpa CT1:70% e CT2:80% por 30 dias para inverter o sexo dos peixes machos e a segunda fase foi amamentar os alevinos por dois meses com dieta controle (32% de proteína bruta), e analisar a taxa de crescimento (p < 0,05) no grupo controle, em que o peso final (4,8 ± 0,34 ab) e o peso ganho foram registrados como 0,66 ± 0,03 ac. Na composição química aproximada da carne corporal, o tratamento CT2 mostrou retenção máxima de proteína bruta, gordura bruta e cinzas, enquanto a matéria seca apresentou retenção máxima nos tratamentos MT2 e CT1. O exame morfológico e histológico revelou diferença significativa (p < 0,05) nos machos fenotípicos de tilápia do Nilo alimentados com o maior percentual na dieta tratada com MT (MT2) de 95 ± 0,58a enquanto MT1, CT2 e CT1 tiveram machos de 85 ± 6,0b, 70 ± 5,0 e 65 ± 6,5b, respectivamente. Concluiu-se que o andrógeno sintético (17αMT) foi mais eficaz para a masculinização, entretanto os andrógenos naturais podem ser um método alternativo para produzir população de tilápias machos de maneira ecológica, pois são baratos, ecológicos e estão disponíveis radialmente. Esses resultados sugerem que a suplementação de andrógenos sintéticos e naturais na dieta desempenha um papel significativo na melhoria do desempenho do crescimento e da composição corporal.

Palavras-chave: testículos de carpa comum, peixes exóticos, crescimento, masculinização, 17α-metil testosterona.

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1. Introduction

After carp, tilapia (*Oreochromis niloticus*) is a cultural fish native to Africa that can tolerate a wide range of environmental conditions. They are commonly referred to as “Aquatic Chicken” and “Poor man’s fish,” and they play an important role in the livelihood by providing low-cost protein. Over the last two decades, fish farming has grown enormously (Wokeh and Orose, 2021). Nile Tilapia (*Oreochromis niloticus*) is a commercially valuable fish food in aquaculture. In Pakistan, aquaculture was started during the 1970s. Pakistan is primarily an agricultural nation with abundant natural water resources and aquaculture provides 1 percent of the Gross Domestic Product (GDP). With substantial international trade between 2006 and 2019, the global production of Nile tilapia (*O. niloticus*) increased significantly from 1.74 to 4.59 million tons. Nile Tilapia is widely cultivated in tropical and subtropical regions around the world, and they are the world’s third largest finfish farm, with an annual growth rate of production of around 11.5 percent. As the aquaculture industry and technology grow, semi-intensive and intensive systems are changing tilapia culture (Ogola et al., 2020). Tilapias are widely raised in cages, ponds, raceways, concrete tanks, and rice fields in the majority of developing countries due to their traits that make them suitable for rearing (Christopher et al., 2021). Tilapia has several beneficial features that make it easier to grow such as an omnivorous diet, relatively disease resistant, faster growth rate, tolerant to a variety of environmental conditions such as poor water quality, and can tolerate salinity up to 36 ppt, Ph of 3.7 to 11, can survive well in between 8-24° C temperature, can withstand with low dissolved oxygen environment up to 0.1 mg/L, effective food conversion, easy reproduction and accepted by consumer makes them ideal for high-density aquaculture (Fregene et al., 2021). It reaches sexual maturity at a young age and reproduces in the pond every 4-6 weeks. Males of Nile tilapia and giant freshwater prawns grow faster than females (Indarjo et al., 2020).

Monosex stocks can be more beneficial to aquaculture because they limit undesired reproduction, reduce hostile interactions, and improve survival rates at harvest, in addition to potential growth advantages (Tan et al., 2020). In the aquaculture industry, hormonal sex reversal has been widely used to produce mono-sex populations. There are many options for hormone administration, but immersion or oral treatments are the most effective, and oral treatment is the most practical and effective technique. Testosterone is the most extensively researched natural androgen for fish growth enhancement. Methyl testosterone (MT) is a synthetic anabolic and androgenic steroid hormone that stimulates muscle growth as well as the development of male sexual characteristics (Suseno et al., 2020). Despite the many positive qualities of testosterone, it also has major drawbacks. Researchers have examined the use of hormones in aquaculture production because of their possible harm to human health (carcinogenic and endocrine disorders) as well as the risk they pose to the environment. When applying for commercial production, the availability and cost of 17-MT in developing countries must also be considered. This disagreement has prompted researchers to look for new ways to reverse sex in tilapia. Photochemical and other natural androgen sources with endocrine-disrupting activity may be used as alternatives.

2. Objective

The objective was to evaluate the effectiveness of synthetic (17α-methyl testosterone) and natural androgens (common carp testes) on sex reversion, carcass quality, and growth performance in *Oreochromis niloticus.*

3. Materials and Methods

This experiment decided the impact of synthetic androgens and natural androgens on masculinization, growth performance and body composition of *Oreochromis niloticus*. This trial was held in Fish Lab, Department of Zoology Government College University Faisalabad.

3.1. Collection of experimental fish

A total of 200 *Oreochromis niloticus* fries 4 to 5 days old were brought from Fish Seed Hatchery. 20 fries of equivalent size were randomly selected, weighed individually and evenly distributed to five aquariums each with one replicate.

3.2. Treatment design and protocol

The treatment design was “Complete Randomized Design” (CRD) with one control (without hormone+ 32% CP) and four experimental diets prepared with 17α-Methyl Testosterone at 60 mg/kg, 70 mg/kg and natural androgen with common carp testes at 70% and 80% in the diet. The water quality was monitored daily by standard procedures (APHA, 1998).

3.3. Feed preparation and feeding

Diets were prepared by mixing an appropriate amount of finely grounded ingredients at predetermined levels in an electric mixer for 10 minutes. Later, fish oil was gradually added while mixing continually for a further five minutes. Subsequently, to prepare the dough 10-15% water was added. Diet was prepared using a linear formulation process (Lovell, 1991). The methyl testosterone diet was prepared by dissolving 60 mg and 70 mg 17α-methyl testosterone (Sigma) in 500 ml of 95% ethanol which was later mixed with 1 kg of the prepared feed dough and then converted into pellets (3mm). Dried feed was kept in the refrigerator until use. Diets with natural and were prepared by using fresh common carp testes of more than one-year-old fish. Testes were separated from other muscle groups and blood vessels before being cut into small pieces and then placed in Petri dishes for sun drying. The dried testes were then ground with a grinder and sieved through a sieve with a mesh size of 60m. After that, 70 and 80% of powdered testes were mixed with feed and then placed in the refrigerator until use (Table 1).

3.4. Growth studies

Fish growth was calculated according to the method followed by (Brown, 1957).

Table 1. Proximate composition of control and treatment diets.

Feed Ingredients(g)	Control	MT1 (60mg/kg)	MT2 (70mg/kg)	CT1 (70%)	CT2 (80%)
Fish Meal	27	27	27	27	27
Rice Polish	16.14	16.14	16.14	16.14	16.14
Canola Meal	19.87	19.87	19.87	19.87	19.87
Wheat Bran	17.59	17.59	17.59	17.59	17.59
Rice Broken	9.57	9.57	9.57	9.57	9.57
Fish Oil	4.83	4.83	4.83	4.83	4.83
Vitamin Premix (1:1)	1	1	1	1	1
Carboxymethyl cellulose	4	4	4	4	4
17α- methyl testosterone (mg/kg)	-	60	70	-	-
Common carp testes (%)	-	-	-	70	80
Chemical composition of control and treatment diets.					
Moisture (%)	6	8	6	7	6
Ash (%)	83	85	88	84	82
Dry matters (%)	94	92	94	93	94
Crude protein (%)	30.18	31.25	31.81	32.16	32.73
Crude fat (%)	16	17	17	18	19
Nitrogen Free Extract	47.82	43.75	44.19	43.75	42.19
Gross energy (Kcal g^{-1})	517.8	516.4	527.1	525.9	537.9

Sigma Chemical, St. Louis, MO, USA., Composition of vitamins mineral mix (Quantity/Kg): Vitamin A, 15 M.I.U; Vitamin D3, Vitamin B1, 5000mg; Vitamin E, 6000 IU; Vitamin B2, 6000mg; Vitamin K3, 4000mg, Vitamin B6, 4000mg; Folic acid, 750 mg; Vitamin B12, 9000 mg; Calcium pantothenate, 10000mg; Vitamin C, 15000mg; Nicotinic acid, 25000mg.

Weight gain = average weight of current fortnight – average weight of previous fortnight

Length increment = average length of current fortnight – average length of the previous fortnight

Condition factor (K) (Formula 1)

$$K = W \times 10^5 \div L^3 \quad (1)$$

Protein efficiency ratio (PER) (Formula 2)

$$PER = \frac{Final\ body\ weight - Initial\ body\ weight}{Amount\ of\ protein\ fed} \quad (2)$$

3.5. Analytical methods

A representative sample of feed and body meat was treated with a motor and pestle and chemically analyzed: dry matter (DM) by oven drying at 105 °C, crude protein (CP) by microkjeldahl investigation, gross energy by formula and ash by electric furnace. While, lipid contents were determined by chloroform methanol extraction technique (Bligh and Dyer, 1959).

Gross energy (Formula 3)

$$GE\ (Kcal/g) = (5.64 \times protein\ \%) + (9.44 \times lipids\ \%) + (4.11 \times NFE\ \%) \quad (3)$$

Nitrogen free extracts (NFE) (Formula 4)

$$NFE\ \% = 100 - (crude\ protein + fat + moisture) \quad (4)$$

3.6. Sex determination

At the end of the trial, ten fish from the control and each treatment group were thoroughly dissected for gonad examination. The fish were weighed before being anaesthetized with clove oil (0.05ml/500ml of water). After dissection, gonads and liver weights were also recorded separately. The squash technique was used to prepare gonad slide of fish (Guerrero 3rd and Shelton, 1974). The gonad was analyzed under a microscope, ovarian tissue was characterized by oocytes, and the testicular tissue was identified by the presence of sperm cells. The Formula 5 is:

$$\mathbf{M} = Gm\ s^{-1} x\ 100\%,\ F = Gf\ s^{-1} x\ 100\%,\ I = Gis^{-1} x\ 100\% \quad (5)$$

M: Male, F: Female, I: Intersex, s: amount of sample, Gm: male fish, Gf: female fish, GI: Intersex fish

Gonosomatic Index (Formula 6)

$$GSI = \frac{Gonadal\ weight \times 100}{Body\ weight} \quad (6)$$

Hepatosomatic Index (Formula 7)

$$HSI = \frac{Liver\ weight}{Body\ weight - Gonad\ weight} \times 100 \quad (7)$$

3.7. Statistical analysis

Following the completion of the experimental trial, data was statistically analyzed through one-way ANOVA for treatment. DMRT was used to compare the means of significant results. The differences were considered statistically significant when P < 0.05.

4. Results

The growth performance of Nile Tilapia is shown in Table 2. Final body weight and body length gain results revealed a significant reduction in growth performance with natural and synthetic androgen inclusion levels (P < 0.05). Fish group that was fed with control diet showed the best results in terms of final weight (4.8±0.34) and weight gained (0.66±0.03). However, dietary administration of CT2 (80%) treatment yielded the lowest growth. Control group showed the best protein efficiency ratio (0.21±0.02) while CT2 (80%) treatment showed the lowest protein efficiency (0.07±0.15). Furthermore, the condition factor did not vary significantly (P>0.05).

Parameters of proximate chemical composition of body meat as well as feed composition and feeding rates, are significant determinants of fish physiological condition. Highest (P<0.05) moisture contents and ash value was recorded in CT2 treatment (8% and 86.5% respectively). Fish that were fed with CT1 diet showed the lowest moisture and ash contents in body meat. While fish treated with synthetic androgen, MT2 and CT1 treatment showed the highest dry matter retention (92.5%) and reduced in control and CT2 treatment (91.5%). Dietary administration of CT2 treatment revealed the highest crude protein and crude fat retention (36.73% and 25%, respectively) in fish body meat. Control group showed the lowest crude protein and crude fat (33.55 and 22.5%, respectively) in body meat. The highest nitrogen-free extract (35.45%) was observed in the control group whereas, CT2 treatment showed the lowest nitrogen-free extract (29.79%) in body meat. Fish fed with CT1 treatment showed the highest gross energy (566.8%) while the control group showed the lowest gross energy value (547.3%) Table 3.

The assessed sex of *Oreochromis niloticus* showed that increasing synthetic and natural androgen concentration with oral method increased the masculinization ratio of *Oreochromis niloticus* compared to the control group. However, the intersex population was not found in any treated and control group. Table 4 showed the efficacy of synthetic (17αMT) and natural (common carp testes)

Table 2. Effect of 17-αMethyl testosterone and natural androgen (common carp testes) on growth performance (mean + SE) and condition factors of *Oreochromis niloticus*.

Variable	CR (control)	MT1	MT2	CT1	CT2
	Control	17α M (60mg/kg)	17-α MT (70mg/kg)	Common carp Testes (70%)	Common carp Testes (80%)
Initial weight(g)	0.83±0.23ab	0.88±0.32abc	0.91±0.24a	0.87±0.45ac	0.9±0.01ac
Final weight(g)	4.80±0.34ab	4.40±0.02ac	4.31±0.41abc	3.81±0.25ab	3.37±0.23ac
Weight gain(g)	0.66±0.03ac	0.58±0.45abc	0.56±0.04ab	0.49±0.22ab	0.41±0.23ac
Body Weight(g)	2.64±0.40a	2.47±0.35ab	2.38±0.34bc	2.21±0.28cd	2.09±0.23d
Body length(cm)	5.27±0.35a	5.26±0.33ab	5.15±0.29bc	5.11±0.33cd	5.02±0.29d
Condition Factor	1.65±0.090a	1.61±0.09a	1.61±0.05a	1.64±0.13a	1.62±0.12a
PER	0.12±0.02ab	0.11±0.34abc	0.10±0.24ab	0.09±0.45a	0.07±0.15a

Means sharing a similar letter in a row or in a column are statistically non-significant (P>0.05).

Table 3. Effect of natural and synthetic androgens on proximate chemical composition (%; mean ± SE) of *Oreochromis niloticus*.

Variable	CR	MT1	MT2	CT1	CT2
	Control	17αMT (60mg/kg)	17αMT (70mg/kg)	Common carp Testes (70%)	Common carp Testes (80%)
Moisture	8.60 ± 0.30 a	8.00 ± 0.10 ab	7.50 ± 0.20 b	7.50 ± 0.10 b	8.70 ± 0.30 a
Ash	86.65 ± 0.35 a	84.50 ± 0.50 ab	86.00 ± 0.20 ab	83.25 ± 0.75 b	86.50 ± 0.50 a
Dry Matter	91.25 ± 0.25 b	92.00 ± 0.00 ab	92.90 ± 0.10 a	92.85 ± 0.15 a	91.30 ± 0.30 b
CrudeProtein	33.06 ± 0.24 b	35.15 ± 0.78 ab	35.94 ± 0.57 ab	36.72 ± 0.79 a	36.97 ± 0.04 b
Crude Fat	22.15 ± 0.15 b	24.00 ± 0.70 ab	23.50 ± 0.50 ab	24.50 ± 0.30 ab	25.60 ± 0.40 a
NFE (%)	35.58 ± 0.61 a	32.85 ± 0.78 ab	33.07 ± 1.17 ab	31.29 ± 0.79 ab	29.94 ± 0.47 b
Gross energy	539.80 ± 3.90 b	559.80 ± 6.20 ab	560.45 ± 2.25 ab	566.85 ± 3.55 a	570.45 ± 3.05 a

This means sharing a similar letter in a row or in a column are statistically non-significant (P>0.05).

Table 4. Effect of 17α Methyl testosterone and common carp testes on male & female percentage, GSI and HSI of *Oreochromis niloticus*.

Variable	CR		MT1		MT2		CT1		CT2	
	Control		17α MT (60mg/kg)		17α MT (70mg/kg)		Common carp Testes (70%)		Common carp Testes (80%)	
	M	F	M	F	M	F	M	F	Male	F
Sex ratio (%)	35±5.0c	65±5.0c	85±6.0b	15±6.0b	95±0.58a	5±0.03b	65±6.5a	35±6.5a	70±5.5b	30±5.b
Male gonad weight (g)	0.042± 0.01bc	0.097± 0.02a	0.046± 0.003bc	0.05± 0.015a	0.04± 0.003c	0.072± 0.018ab	0.038± 0.004ab	0.062± 0.018ab	0.030± 0.003a	0.036± 0.001a
GSI	1.400 ± 0.010 c		1.960 ± 0.130 ab		1.465 ± 0.085 bc		1.990 ± 0.110 a		2.060 ± 0.070 a	
HIS	0.110 ± 0.000 b		0.165 ± 0.015 ab		0.130 ± 0.010 b		0.155 ± 0.015 ab		0.210 ± 0.010 a	
Survival rate (%)	97.50±2.50 a		95.00±5.00 a		92.50±2.50 a		70.00±4.00 a		100.00±0.00 a	

Means sharing a similar letter in a row or in a column are statistically non-significant (P>0.05).

androgen levels on the male and female percentages of *Oreochromis niloticus* after an overall trial period (90 days). The results demonstrated a significant increase in the male percentage (90%) with a dietary administration of MT2 compared to the lowest male percentage in the control group (35%). In other treatments, low male percentage was recorded as 85%, 70%, and 65% in MT1, CT2, and CT1, respectively. However, significantly the highest ($P<0.05$) gonadosomatic index and hepatosomatic index was obtained in CT2 treatment indicating natural androgen resulted in the best masculinization that affects male percentage dose-dependently. As shown in Figure 1 presence of spermatogenic cells in male gonads while Figure 2 showed oocyte cells in female gonads. The overall survival rate at the end of the experiment fluctuated from 70-100%, which revealed that survival rate was not statistically different in the control and other treatment groups ($P<0.05$).

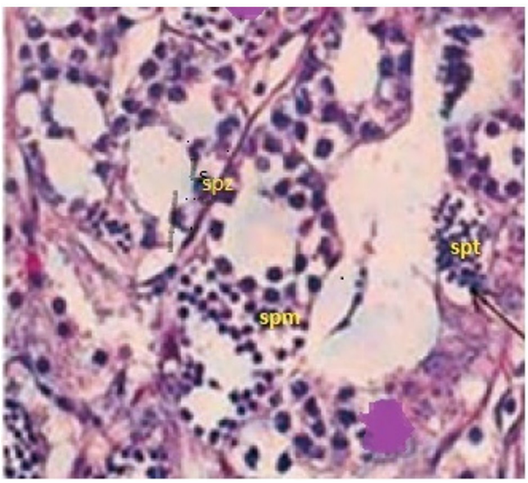

Figure 1. Testis histology of male tilapia, 3 months old (n = 3); group MT2-17α-Methyl testosterone (70mg/Kg) treated fish spt=spermatid; spz=spermatozoa; spm=spermatocytes.

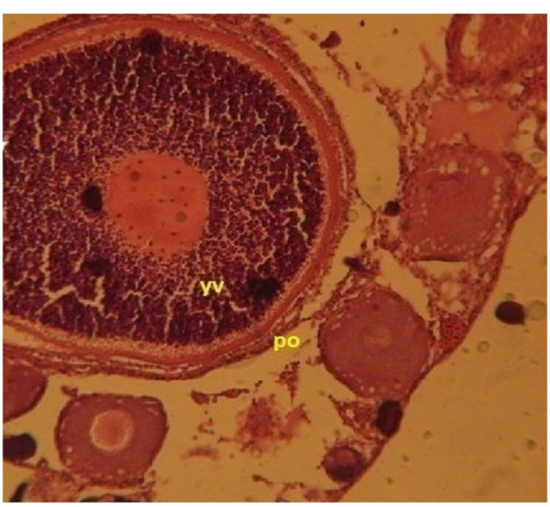

Figure 2. Ovarian histology of female tilapia, 3 months old (n = 3); group rohu testes CT1 (75%) treated fish, yv= yolk vesicles; po=primary oocyte.

5. Discussion

The findings revealed significant effects of synthetic and natural androgens on masculinization, survival and growth rate of *Oreochromis niloticus*. The maximum growth was observed in the group MT1 (60mg/kg) treatment, indicating a link between weight gain and synthetic androgen. In order for the fish to perform better, 17-alpha-methyltestosterone plays a significant role in accelerating growth performance (Chávez-García et al., 2020). Despite having the same initial weight, the synthetic hormone given treatments showed a significant variation in mean final weight. Safir et al. (2020) also made similar observations on the daily growth rate of *Pterapogon kauderni* that soaked with 17-Methyltestosterone. However, in other findings the 17 alpha MT hormone inclusion caused the lowest weight gain (M Zaki et al., 2021). Survival rate of *Oreochromis niloticus* was maximum in all treatments showing that supplementing treatment diet to Nile Tilapia had no effect on survival rate, which was not affected by feed type. Likewise, Safir et al. (2020) made observations that there was no significant difference in the survival rate between all treatments and control.

M Zaki et al. (2021) showed some contradictory findings that *T. terrestr is* and 17-alpha-methyltestosterone levels effected on the survival rate of Nile Tilapia. The results of proximate chemical composition of body meat demonstrated that hormone-induced masculinization also had a significant impact on the proximate body composition of fish. The maximum retention of moisture and ash contents were observed in control and CT2 treatment while the maximum value of dry matter was founded in MT2 and CT1 treatment. Similarly, maximum crude protein and fat retention in body meat was observed with CT2 treatment, which demonstrated that besides protein, the hormone had a considerable impact on body composition. Researchers found that 17α-methyl testosterone treatment was highly dependent on hormone concentrations, producing maximum phenotypic males of Nile tilapia populations, as evidenced by comparing males and females in each treatment group to the control group in order to determine its effectiveness. Results demonstrated that in all treatment groups the male and female ratio was different from the normal 1:1 ratio. High percentage of male fish was obtained after providing a high dose of 17αMT 70mg/kg in the diet and natural androgen (carp testes) while lowest percentage obtained in control group.

M Zaki et al. (2021) found similar results with red tilapia showed a significant increase in male percentage after administration of 60mg/kg 17-alpha-methyltestosterone in diet. Similarly Sreenivasa and Prabhadevi (2018) founded that the different doses of methyl testosterone diet showed variation in its effect on the percentage of male tilapia. Supplementing 60mg/kg 17αMT diet to red tilapia showed a higher percentage of males compared to 40mg/kg of 17αMT that showed the lowest percentage of males which was slightly higher than the control group. It found after supplementation of an increase of synthetic MT in the diet that MT is more efficient compared to other hormones for masculinization. Liu et al. (2021) found that there was no significant difference in survival rates between the MT-treated and control groups.

6. Conclusions and Recommendations

It was concluded that supplementation of synthetic and natural androgens in the diet for masculinization played a significant role in improving the growth and helped to achieve the faster growth rate in *Oreochromis niloticus*. Furthermore, synthetic androgen (17αMT) was found more effective for masculinization (sex reversal) as compared to natural androgen, while the use of synthetic androgen is harmful to human health and environment. However, the amount of natural androgen should be increased or other natural phytochemicals in fish diet must be recommended to increase better growth rate and masculinization in *Oreochromis niloticus* and to solve the issues related to human health and environment.

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