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Effect of fish meal, 11-ketotestoesterone and shrimp head protein hydrolysate on growth and haemato-immunological changes of juvenile *Oreochromis mossambicus* in saline environment

[1]Jahangeer Mian*, [1]Pir Zada Jamal Ahmed Siddiqui, [2]Arifa Savanur, [2] Habib Fatima, [3] Immink Anton & [4] Nani Goplal Das

[1] Centre of Excellence in Marine Biology, University of Karachi, Karachi 75270 Pakistan.
[2]Department of Physiology, University of Karachi, Karachi, 75270 Pakistan,
[3] Department of Aquaculture, Stirling University UK.
[4]Department of Reproductive Biology, Chittagong University, Bangladesh.

*[E-mail: maviamian@gmail.com]

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Present study consists the outcomes of fish meal protein (FMP), shrimp head protein hydrolysate (SHPH) and 11ketotestosterone (11KT) on biology of tilapia juvenile (*Oreochromis mossambicus*). 10 fish/ 50L were stocked in 4 treatments with 3 replicates with (mean weight, 10.03 ± 0.01 g) for 56 days. The 11KT was added to the feed (5mg/kg) by spray (+FMP, +SHPH) and was not added in control diets (- FMP, - SHPH). Specific growth rate and food conversion efficiency of fish fed on +11KT were significantly better than those fed on (-FMP or -SHPH) diets. Haemato-immunological parameters were improved in fish fed on +KT diets. Plasma T3 (triiodothyronine) levels were significantly progressed in males fish than females, whereas T4 (thyroxine) levels were increased in both sexes. Plasma 11KT levels were significantly ($P < 0.05$) higher in males than females. Plasma estrogen (E_2) levels were insignificant in female fish. The physical parameters of water maintained at satisfactory level. These findings obviously indicate that supplemented11KT to feeds motivated growth, simply moving the plasma levels of 11KT without producing any estrogenic activates.

[**Keywords:** Growth,11KT, Estrogen, haemato-immunology, Endocrine Status, SHPH]

Introduction

The tilapia species have been generally known as one of the protein sources around the world and for outstanding growth on variety of natural or formulated diets[1- 2- 3- 4]. Farming omnivores and carnivores fish species (e.g. tilapia, catfish, groupers etc.) puts supplementary require on marine fisheries because wild species remain constant principle ingredients of their feed [4]. Attempts have been made to replace fish meal by plant-based protein reduces growth performance of tilapia considerably [2-4-5-6].

According to Toguyeni et al.[8] that the males tilapia grow better than females this may be due to their obvious sex characters or due to male-female social interactions. Davis et al., Riley et al. [4-9] reported, that differential growth due to multipart interactions between growth hormones, and insulin like growth factor-I: IGF-I, gonadal steroid hormones for example, due to oral administration of 17α-methyltestosterone to the Mozambique tilapia produced the high frequency of males and faster grow than untreated combined-sex population during early developmental stages [4- 10- 11]. By contrast, when tilapia treated with ethynylestradiol (synthetic estrogen), created a greater numbers of females, grew more slowly than that of control group [12]. Davis et al., Riley et al. [4-9] also reported that significantly increased plasma growth hormone by treated with 17β-estradiol while tumbling insulin like growth factor-I and this phenomena vice versa when treaded with dihydrotestosteone. In general, in teleost species testosterone (TT) provides natural precursor to sex steroids like 11KT in males and 17β-estradiol (E_2) in females, which participates significant functions during ovarian and testicular development [4]. Lone and Matty [13] also noted that supplemented 11 KT enhanced the food adaptation competence in juvenile carp. Toguyeni et al. [8] stated that in mixed-sexed fishes, the male niloticus demonstrated the higher specific growth rate and lower feed conversion ratio than females, and suggested that raised plasma 11KT may engage in the higher metabolic activities in males.

Information of the haematological characteristics is an important approach that can be used to identify the health status (physiological and pathological changes) of fishes. Haematological indices also provide reliable knowledge on metabolic activities, deficiencies, chronic stress status and disorders before they are present in clinical setting [14]. In present cited literature seem to no information about the role of dietary11KT combined with SHPH and FMP on plasma levels of T_3, T_4, 11-KT, E_2, growth and immunity monitoring under experimental conditions.

The main object of this study was to evaluate growth through the oral administration of androgenic-steroid hormone for tilapia (*Oreochromis mossambicus*). From the available text, this study is also the first to inspect the effect of FazMP, SHPH and11KT on growth and haemato-immunological parameters.

Material and Methods

Two isocaloric (22.3 ± 0.1, KJg^{-1}) isonitrogenic (30.2%) diets were prepared according to Mian et al. [15]. The shrimp head protein (SHPH) produced according to Leal et al. [6] with inclusion level was 3.5% correspondingly 30% of total fish meal protein replaced in SHPH diet[15]. Formulated diet offered twice a day as described earlier [16] at 8-9 am and 16:00-17:00 pm. Fish were weighed, counted and measured weekly for growth assessments. Amount of ketotestosterone calculated in the diets by (Specific radioimmunoassay: SRA) as mentioned, and authenticated that 11-KT is equally allocated in the diet (9.63 ± 0.2) mg/ kg, *n*, 3).The 5 mg/kg of 11KT (11-ketotestoesterone: Sigma, St. Louis, Missouri, USA) added by spray according to Davis et al. [4] on fish meal protein diet (+FMP: 11K+FMP) and shrimp head protein hydrolysate diet (+SHPH: 11KT+SHPH) and without spray of 11KT (-FMP an -SHPH) [4].The sampling started 25 h after last feeding [4]. To remove the effect of crowding stress on growth rate, stocking densities were maintained at 5 g/L. Fish were also closely observed for any stress indication by behavioral changes or body color [4].

The fish were collected from Thatta Fish Hatchery (TFH) 720 fish, and considering around mean weight, 10 ± 0.01g, were labeled independently with (inert incorporate transponder labels: IIT labels; Biomark, Tokyo, Japan) and divided randomly into twelve tanks on behalf of 3 replicates of [4] experimental groups. Trials were carried out in accord with the rules and procedures endorsed by the Ethics Animal Care Committee (ECACU) Agha Khan University. At the beginning of the experiment, the males and females fish were at infertile gonadal stages. At the end of the study, the maturation stages were visually observed; vitellogenic oocytes were observed in most of the females, and some ovulating females were found, whereas spermiation was observed in the large males. The fish were separately weighed and their sex recognized by examination of genital papilla.

At the end of the study (after 56 days), plasma levels of ketotestosterone (11KT), thyroxine (T4), triiodothyronine (T3) and estrogen (E_2) were also measured in samples obtained 25 h post-feeding.

Biochemical analysis

The proximate analysis of the feeding ingredients are shown in (Table 1) were analyzed by using the standard methodology of AOAC [17]. Amino acid profiles of ingredients were estimated according to Wang and Parson [18]. Haematocrit (Hct %) as described (Papoutsoglou and Voutsions [19]. RBC and WBC counts were calculated [20]. The T3 and T4 were analyzed using ELISA immunoenzymatic kits (Monobind, USA). Plasma 11KT levels were determined by radioimmunoassay [21]. Plasma E_2 level was analyzed following previously described method by Davis et al.[4]. Alternative complementary pathway haemolytic activity (ACH) (20), serum albumin and globulin [4], liver glycogen [22], and haemoglobin (Beck man coulter, HMX) were also measured.

At weekly intervals 10 fish were measured randomly from the each tank. Final body weight and SGR values were calculated [23]. The food conversion efficiency was determined using method described earlier [13]. At the end of the 56 days, blood samples were collected from fish according to Steffens [24]. Hepatosomatic index (HSI) and visceral somatic index (VSI) was calculated [25]. Mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and alternative complement haemolytic activity pathway (ACH) were also calculated [26].

Two-way (ANOVA) and Duncan's multiple range test [27] were used to estimate the significant differences in the observed results for test groups. Significant level was set at ($P < 0.05$). Data are expressed as means ±SEM ($n = 3$).

Results

The composition of SHPH was formulated according to [14] (Table 1).The fish fed on +FMP and +SHPH had significantly higher growth rate and SGR

Table 1 — The % composition of experimental diets

Feed ingredients	FMP	SHPH
Fish meal Protein (56% CP)	24.2	15.4
Rice flour	12.6	17.8
Wheat flour	16.3	13.2
SHPH	0	3.5
Wheat bran	14.23	15.7
Tapioca flour	27.3	29.4
Calcium carbonate	1	1
Vitamin C	1	1
Antioxidant (BHT)	0.015	0.015
Binder	1	1
Vitamins/ Mineral premix	2	2
Gross energy (KJg^{-1})	22.2	22.1
Analytical Composition		
Crude Protein	31.3	30.3
Lipids	6.32	5.9
Ash	12.8	12.98
Fiber	5.8	5.9
Moisture	11	11.12
§NFE	34.1	30.7
Lysine	2.82	4.5
Arginine	1.9	3.97
Histidine	0.98	2.06
Threonine	1.94	3.9
Valine	1.7	3.3
Leucine	2.1	4.9
Isoleucine	1.63	2.96
Methionine	2.02	3.1
Cystine	1.65	2.4
Phenylalanine	1.09	3.84
Tyrosine	1.95	2.1
Tryptophan	1.3	1.21

§Nitrogen- free extract = 100 - (% protein + % fat + % ash + % fiber)

¶ Vitamins/minerals premix contained as (g) Riboflavin (0.78g): Folic acid (0.65g): Vitamin B6: (0.03): Vitamin B12 (0.05g): Vitamin E (0.3g), Vitamin D3 (0.4): Niacin (0.17g). Minerals premix: Iodine (0.015g), Iron (0.01g), Selenium (0.004g), Chromium (0.04g), SHPH (CP = 39.7), Soybean (SBM): (*Glycine max*): CP = 45.8%, Fish meal FMP: (CP= 56.6%): Tapioca flour: *Metroxylon sago* (CP=3.12%); wheat flour: *Triticum aestivum* (CP = 16.4).Vitamins/minerals premix were used as described by Mian et al. (23).

compared to the other groups (-FMP and -SHPH). The significantly ($P < 0.05$) higher FCE were found in fish fed +FMP and +SHPH diets. The HSI and VSI were significantly higher in fish fed on +FMP and +SHPH than the fish fed -FMP and -SHPH diets. The liver glycogen was improved in fish fed on +FMP and +SHPH (Table 2).

The haemato-immunological erythrocytes (RBC) white blood cells (WBC), haematocrit (Hct %), alternative complement hemolytic activity pathway (ACH), mean cell haemoglobin (MCH), mean cell haemoglobin concentration MCHC, mean cell volume (MCV) lymphocytes, neutrophils and albumin (AL) globulin (GL), AL/GL) associated with fish fed on the hormones supplemented diets (+FMP and +SHPH) had showed excellent results compare to other groups (Table 3). The physical parameters of water, such as temperature, salinity, ammonia (NH_3), nitride (NO_2), nitrate (NO_3) and dissolved oxygen (O_2) remained at satisfactory levels throughout the experiment (Table 4). The plasma T_3 levels were significantly ($P < 0.05$) higher in males than females (Fig.1 and 2). The plasma thyroxine levels were insignificant between males and females in all diet groups (Fig. 1 and 2)**.** Plasma levels of 11KT were significantly ($P < 0.05$) higher in males than in females in all diet groups (Fig. 1 and 2). The plasma E_2 levels were insignificant in females of all diet groups (Fig. 2).

Discussion

The effect of oral applications of androgenic-steroids supplemented fishmeal based diets have been investigated by a number of authors on growth behavior and physiological aspects [4-13]. In some recent studies, 11KT effects on teleost species have been investigated but information on its application at commercial level is not available [4- 28]. However, 11KT could play significant role in potential optimization of fish production and energy conservation in aquaculture sector [13]. The results of the present study clearly indicate that juvenile *Oreochromis mossambicus* fed on +FMP and +SHPH diets for 56 days grew significantly faster than other diet groups (- FMP and -SHPH). The supplemented growth by addition 11KT to FMP or SHPH diet is in agreement with some previous studies [4-9-13]. In our present finding, the SGR and feeding activities were increased with hormone supplementation. Evidences have been proved that 11KT increases growth rate in carp [13] and in *Oreochromis mossambicus* [4]. The results of the present study also indicate that fish fed on hormone supplemented diet (+ FMP and +SHPH) had higher FCE thus indicating a direct correlation between FCE and SGR.

It is generally known that plant-based formulated diets are poorer for growth performance due to presence of anti-nutritional factors [4-15]. [4] noted that commercial feeds usually contain up to 65% of fishmeal in diets. Pelissero and Sumpte[29] also observed that fishmeal often prepared from fish-by product or whole fish and may contain high levels of sex steroid hormones. It is also generally recognized that commercial diets contain phytoestrogens which

Table 2 — Effect of dietary FMP, 11KT and SHPH on growth and biological parameters of *Oreochromis mossambicus* (n = 3, and each n consist of 10 fish/ replicate, Mean ± S.E)

Biological parameters	FMP	SHPH	11KT+ FMP	11 KT+SHPH
Initial weight (g)	10.2 ± 0.2^{a}	10.3 ± 0.01^{a}	10.25 ± 0.1^{a}	10.24 ± 0.2^{a}
Final weight	21.4 ± 0.1^{a}	23.2 ± 0.1^{b}	25.75 ± 0.2^{c}	26.6 ± 0.1^{c}
FCE	0.56 ± 0.01^{a}	0.59 ± 0.01^{a}	0.68 ± 0.02^{b}	0.74 ± 0.01^{b}
SGR	1.31 ± 0.005^{a}	1.43 ± 0.001^{b}	1.7 ± 0.002^{c}	1.72 ± 0.004^{c}
VSI	7.43 ± 0.01^{a}	7.35 ± 0.01^{a}	8.4 ± 0.03^{b}	8.51 ± 0.02^{b}
H S I	2.2 ± 0.02^{a}	2.27 ± 0.02^{a}	2.32 ± 0.02^{a}	2.43 ± 0.03^{a}
Liver glycogen mg/g	2.36 ± 0.02^{a}	2.41 ± 0.03^{a}	2.38 ± 0.01^{a}	2.45 ± 0.01^{a}

Means that have same superscript letter within a row are not significantly different ($P > 0.05$). Shrimp head protein hydrolysate (SHPH), fish meal protein (FMP), ketotestosterone (11KT).Feed conversion efficiency (FCE) = [wet weight gain (g)/ food intake (g)]. SGR (% day^{-1}), specific growth rate: 100 × [ln terminal weight (g) – ln initial weight (g)/ Duration (days)]. Visceral somatic index (VSI) = 100 × [wet weight of visceral organs and associated with fat tissue (g)/ wet body weight (g)], Hepatosomatic index (HSI) = 100 × [wet weight of live (g)/ wet body weight (g)

Table 3 — Effect of fish meal protein (FMP), 11KT (ketotestosterone) and shrimp protein hydrolysate (SHPH) on hematological parameters of tilapia *(Oreochromis mossambicus)* (n = 3 and each n consist of 10/ replicate Mean ± S.E).

Haematological parameters	FMP (Control)	SHPH	11KT+SHPH	11KT+FMP	11KT+SHP
Hct%	39.2 ± 0.1^{a}	38.09 ± 0.1^{a}	40 ± 0.1^{a}	39.5 ± 0.01^{a}	39.4 ± 0.1^{a}
Hemoglobin (g/dl)	10.1 ± 0.1^{a}	10.12 ± 0.03^{a}	10.17 ± 0.01^{a}	10.12 ± 0.1^{a}	10.2 ± 0.02^{a}
RBC ($\times 10^{6}$ µl)	2.93 ± 0.02^{ab}	2.91 ± 0.03^{ab}	3.04 ± 0.01^{ab}	3.01 ± 0.01^{ab}	2.9 ± 0.02^{ab}
WBC ($\times 10^{3}$ µl)	22.1 ± 0.1^{a}	21.89 ± 0.3^{a}	22 ± 0.2^{a}	21.93 ± 0.1^{a}	21.2 ± 0.2^{a}
ACH (U/ ml)	243 ± 1.3^{a}	249 ± 1.1^{a}	257.3 ± 1.2^{a}	248.4 ± 2.4^{a}	240 ± 0.9^{a}
MCHC (mg /dl)	2.3 ± 0.01^{a}	2.4 ± 0.02^{a}	2.44 ± 0.01^{a}	2.36 ± 0.03^{a}	2.32 ± 0.01^{a}
MCH (Pg/ cell)	129 ± 2^{a}	140 ± 2^{a}	133 ± 0.1^{a}	133 ± 1^{a}	129.2 ± 1.1^{a}
MCV (nm^{3})	542 ± 3^{a}	537 ± 11^{a}	541 ± 2.3^{a}	531 ± 1.9^{a}	526 ± 3^{a}
Neutrophils (%)	13.1 ± 0.1^{a}	12.97 ± 0.03^{a}	12.89 ± 0.1^{a}	13.45 ± 0.2^{a}	13.03 ± 0.01^{a}
Lymphocytes (%)	78.2 ± 0.4^{a}	89.7 ± 0.5^{a}	80.2 ± 0.1^{a}	79.3 ± 0.2^{a}	76.2 ± 0.3^{a}
AL mg/ dl	0.87 ± 0.01^{a}	0.83 ± 0.005^{a}	0.84 ± 0.01^{a}	0.86 ± 0.002^{a}	0.81 ± 0.02^{a}
GL mg/ dl	0.95 ± 0.003^{a}	0.97 ± 0.004^{a}	0.92 ± 0.001^{a}	0.93 ± 0.001^{a}	0.91 ± 0.002^{a}
AL/GL	0.91 ± 0.001^{a}	0.9 ± 0.004^{a}	0.92 ± 0.001^{a}	0.93 ± 0.002^{a}	0.9 ± 0.01^{a}

Means that have same superscript letter within a row are not significantly different ($P > 0.05$) Shrimp head protein hydrolysate (SHPH) meal protein (FMP), ketotestosterone (11KT), haematocrit (Hct), [red blood cell (RBC) white blood cell (WBC) concentration], and alb (AL), globulin (GL). Mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) alternative complement hemolytic activity pathway (ACH). MCH (pg/ cell) = Hb (g/l)/ RBC (10^{6} ml) × 10 MCV (nm^{3}) = 10 × [Hct (%)/ (10^{6} ml)], MCHC (g/dl) = [Hb (g/ dl)/ Hct (%)]

Table 4 — Effect of fish meal protein (FMP), 11KT (ketotestosterone) and shrimp protein hydrolysate (SHPH) on physical parameters of water

Physico-chemical parameters	FMP (CON)	SHPH	11KT+ FM	11KT+SHPH
Temperature ˚C	27.2 ± 0.2	27.1 ± 0.1	27.2 ± 0.1	27.1 ± 0.01
Salinity mg/ l	33.4 ± 0.1	33.4 ± 0.02	33.3 ± 0.2	33.3 ± 0.02
P^{H}	7.1 ± 0.1	7.2 ± 0.01	7.1 ± 0.01	7.1 ± 0.02
NH_3 mg/ l	1 ± 0.03	1.04 ± 0.01	1.03 ± 0.01	1.02 ± 0.02
NO_2 mg/ l	2.4 ± 0.1	2.4 ± 0.01	2.44 ± 0.01	2.39 ± 0.002
NO_3 mg/ l	0.38 ± 0.002	0.39 ± 0.002	0.36 ± 0.004	0.4 ± 0.001
Dissolved O_2 mg/ l	6.47 ± 0.1	6.46 ± 0.01	6.45 ± 0.01	6.4 ± 0.002

may reduce the fish growth [4-30-31]. The sex steroids in the fishmeal or androgenic or estrogenic activities of other components in diets may potentiate the growth-promoting effects of 11TK [4]. Whilst, Davis et al. [4] fed the soybean-based diets without 11KT indicated that estrogenic activities or some other components in soybean-based diet potentially interfere with effect of 11KT on tilapia growth. In the present study, the growth performance of fish fed the SHPH diets without 11KT was equal to the fish fed on FMP diets and thus clearly indicates that there was no obvious affects of estrogenic activities on indigenous fish11KT which could have interfere in growth behavior.

Also, the fish fed on +FMP and +SHPH diets had higher values of VSI and HSI compared to (-FMP and –SHPH) diets. This indicates that, the lower index values of -FMP and -SHPH diets might be

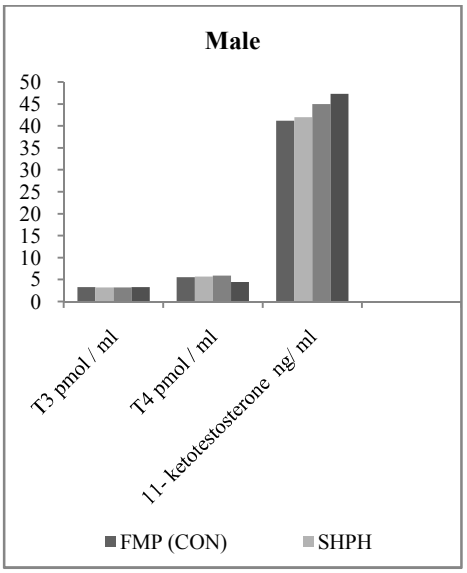

Fig. 1 — Effect of fish meal protein (FMP), shrimp head protein hydrolysate (SHPH) and dietary 11-KT (ketotestosterone) on plasma T3 (triiodothyronine), T4 (thyroxin) and 11-KT (ketotestosterone) in male *Oreochromis mossambicus.*

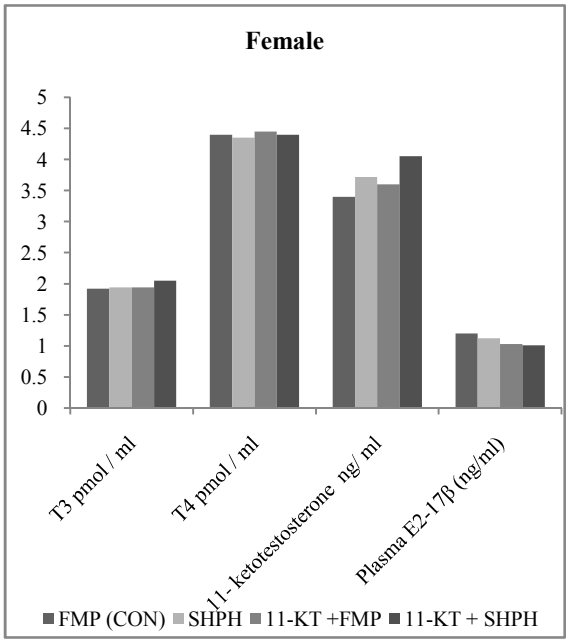

Fig.2 — Effect of Fish meal protein (FMP), shrimp head protein hydrolysate (SHPH) and dietary ketotestosterone (11-KT) on plasma T3 (triiodothyronine), T4 (thyroxin), 11-KT (ketotestosterone) and estrogen (E_2) in female *Oreochromis mossambicus*

safeguard [13,16]. The higher liver glycogen further supports the better growth. Garg [32] reported that the addition of dietary growth hormones may influence the increase the liver glycogen levels. In present findings, the liver glycogen was improved in those fish fed on + FMP and +SHPH diets.

Hematological parameters are implicated with the fish health and hence with the feeding performance, growth and resistant to toxicant [14]. In the present finding Hb, and red blood cells were not insignificant in all diet groups. These satisfactory findings presented that fish were healthy. Similar results were also reported Satheeshkumar et al. (15). Khlil et al. [33] noted that albumin (AL), globulin (GL) and AL/GL ratio decreased in fresh water fishes fed on diets lacking lysine and methionine. Kenari [34] also suggested that the fish oil replaced by the different vegetables oils improved the blood immunological parameters such as lysozomes activity, lymphocytes and Hb parameters, etc. In our findings all the immunological limits were improved in fish fed on +FMP and +SHPH diets. These results indicate that both diets with and without 11KT had no negative impact on haemato-immunological parameters.

Thyroid hormone shows the high capabilities in various physiological features in higher vertebrates as well as in fishes [13]. Our findings, the plasma thyroxine (T4) levels were comparable in all diet groups, whereas the plasma triiodothyronine (T3) levels were significantly higher in males than females. This supports better growth and feed utilization in males as has been previous reported Toguyeni et al. [8].

The 11KT in fish and testosterone in advanced vertebrates is required for spermatogenesis but its physiology has stayed incomprehensible [4]. In our present findings, the plasma 11KT levels were significantly higher in males than in females of all diet groups, which may be because of the presence of some components in FMP or SHPH diets that could regulate the 11KT levels in males but not in females. Higher plasma 11KT levels in males might be engaged in more metabolic activities Toguyeni et al. [8]. This has also been observed. Davis et al. [4] also reported similar results. In our present findings, the plasma estrogen E_2 levels in females were not significantly different. No, significant effect of FMP, 11KT, and SHPH on plasma levels of estrogen (E_2) was detected and no significant effect of FMP, SHPH, or +11KT was observed on plasma levels of 11KT in females. Davis et al. [4] proposed that gonadal differentiation in tilapia species arises as early as 30 to 35 days after hatching for males and 8 days for females. In the present study, 11KT was given to the fish (weighing 5 g/ kg), but this compound did not motivate sex modification in +SHPH and +FMP diet groups. The fish used in the current study were at a gonadal immaturity at the beginning of the study.

Though, no significant difference in body growth was noted between males and females across all diet groups. 11KT might be required for a long period and at a higher concentration to stimulate the sex change or sex-associated differentiations in growth rate. The aforementioned results clearly indicate that the no obvious effect of fish meal protein, SHPH or 11KT was observed on plasma levels of 11KT, T3, T4, E_2 and no sex alteration was examined. There was no oblivious estrogenic activity associated with fishmeal or SHPH in both sexes.

The results of the present study clearly demonstrate that the oral administration of 11KT improved the growth rate and food conversion efficiencies in fish. The results are also helpful feed management and high feed conversion efficiency by 11KT supplemented diets will also reduce the feed cost which is the main expenditure component in aquaculture.

Conclusion

In present study, the dietary ketotestosterone (11KT), and shrimp head protein hydrolysate (SHPH) had high capability for growth activity. Supplemented diets increased growth rate many folds in tilapia (*Oreochromis mossambicus*) fish. However, no obvious effect of fish meal protein, SHPH or 11KT was observed on plasma levels of 11KT, T_3, T_4, E_2 and no sex alteration was examined.

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